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Light quality affects incidence of powdery mildew, expression of defence-related genes and associated metabolism in cucumber plants

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Abstract To determine whether light quality affects the incidence of disease, we exposed cucumber (*Cucumis sativus* L. cv. Jinyan No. 4) plants at the 4-leaf stage to white and other monochromatic lights and tested the effects on plant response to *Sphaerotheca fuliginea*, defence-related gene expression and metabolic changes. Exposure to red light resulted in higher levels of H_2O_2 and salicylic acid (SA), and stronger expression of defence genes such as *PR-1* than exposure to white or other monochromatic lights. In comparison, plants grown under purple and blue light had higher activities of phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) and higher level of flavonoids than plants grown under other lights. Furthermore, plants grown under red light were more resistant whilst plants grown under other monochromatic lights were less resistant to *Sphaerotheca fuliginea* than plants grown under white light. These results suggest a role of red light in light-enhanced resistance, which correlates with enhanced SA-dependent signaling pathway.

Keywords *Cucumis sativus* · Defence · Light quality · Salicylic acid (SA) · *Sphaerotheca fuliginea*

Abbreviations

APX	ascorbate peroxidase
CAD	*cinnamyl alcohol dehydrogenase*
CAT	catalase
CS	*callose synthase*
G-POD	guaiacol peroxidase
HR	hypersensitive response
LED	light emitting diode
PAL	phenylalanine ammonia-lyase
PPO	polyphenoloxidase
PR proteins	pathogenesis-related proteins
SA	salicylic acid
SAG	salicylic acid glucosidase
SAR	systemic acquired resistance
SOD	superoxide dismutase

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Introduction

Plants have developed a wide range of defence mechanisms to successfully adapt to a changing environment. In response to the attacking intruders,

plants can activate a multitude of defence mechanisms including rapid generation of reactive oxygen species (ROS), production of defence signals, such as salicylic acid (SA) and jasmonic acid (JA), biosynthesis of phytoalexins, increased expression of defence or defence-related genes including pathogenesis-related (PR) proteins, and hypersensitive response (HR). In certain plant species such as tobacco, Arabidopsis and cucumber, the defence responses could also be activated in the non-infected organs to establish systemic acquired resistance (SAR). Systemic increase in SA level is correlated with the development of SAR and is required for the induced expression of SA-dependent *PR* genes and systemic enhancement of disease resistance (Metraux 2002; Kumar and Klessig 2008).

Light regulates many aspects of plant growth and developmental processes in addition to being the source of energy for plant life. Light is also one of the important factors affecting disease development in plants. Lesion development in cucumber downy mildew is suppressed by limiting light exposure (Inaba and Kajiwara 1971). In rice and *Arabidopsis*, hypersensitive cell death during pathogen-induced HR is light dependent (Genoud et al. 2002; Guo et al. 1993; Zeier et al. 2004). Induction of disease resistance in plants against fungal pathogens by exposing plants to red light has been previously reported (Islam et al. 1998; Rahman et al. 2003). Most recently, Islam et al. (2008) found that systemic disease resistance to root-knot nematode *Meloidogyne javanica* and *Pseudomonas syringae* pv. *tomato* DC3000 was induced by red light. However, how light quality affects plant disease resistance, is still unclear.

Light is required not only for SA biosynthesis, but also for SA action. Mutants deficient or defective in phytochromes are compromised in SA-induced expression of the *PR-1* gene. Likewise, *Arabidopsis* plants grown in dim light or in the dark exhibit reduced induction of *PR-1* by exogenous SA (Genoud et al. 2002). *PR-1* expression is completely suppressed in dark-situated *Arabidopsis* plants after infection by *Pseudomonas syringae* pv. *maculicola* (Zeier et al. 2004). Although the suppressive effects of white and red light on disease development have been reported, the underlying mechanisms are not understood. Light signals are perceived and transduced into cellular responses by four kinds of photoreceptor families: the phototropins and cryptochromes, which both sense UV-A and blue light, the phytochromes, which absorb red/far-red light, and an unidentified ultraviolet B photoreceptor (Gyula et al. 2003). It is unclear whether these light-induced signaling pathways interact with defence pathways. Recently, several studies have shown that there exists cross-talk between phytochrome signaling, SA perception, HR development or SAR resistance in *Arabidopsis* upon inoculation with avirulent *Pseudomonas syringae* (Genoud et al. 2002; Griebel and Zeier 2008).

In this study, we examined the role of light quality in the resistance to powdery mildew (*Sphaerotheca fuliginea*) in cucumber. Powdery mildew is an obligate biotrophic fungus that causes yield losses worldwide. Chemical fungicides have been developed for the control of powdery mildew, the frequent application of the fungicides, however, has aroused public attention due to the negative effects of chemical fungicides on human health and the environment. Accordingly, there is growing interest on searching for the environmentally friendly technologies instead of chemical fungicides.

Reactive oxygen species play an important role as a signal for stress tolerance in many plants and transcription factors, such as *WRKY 6* and *WRKY 30* have been shown to be involved in the stress tolerance in cucumber (Xia et al. 2009). Meanwhile, significant changes in the activity of defence related enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) involved in secondary metabolism have been well observed (Ye et al. 2006; Han et al. 2009). We have previously reported the different responses of CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation to light quality in *Cucumis sativus* (Wang et al. 2009). The objective of this study was to study the interaction between *Cucumis sativus* and *Sphaerotheca fuliginea* under light of different wavelengths. Disease development after infection by *Sphaerotheca fuliginea*, accumulation of SA and H_2O_2, associated antioxidant and phenolic compounds, and the transcript levels of defence genes were analyzed in cucumber plants after exposure to light of different wavelengths. We show that red light-induced resis-

tance to *Sphaerotheca fuliginea* is correlated with SA-mediated defence mechanisms in cucumber plants.

Materials and methods

Plant material

Cucumber (*Cucumis sativus* L.cv.Jinyan No.4) plants were used throughout this study. Seeds were germinated in trays filled with a mixture of peat, vermiculite and perlite (6:3:1, V:V:V) in a glass house. When the first true leaf fully expanded, seedlings were transplanted into plastic pots (15 cm diameter and 15 cm deep, one seedling per pot) containing the same medium. The seedlings were watered daily with half-strength Enshi nutrient solution (Yu and Matsui 1997).

Light and pathogen inoculation treatments

Cucumber plants at 4–leaf stage were inoculated with a suspension of *S. fuliginea* (10^5 spores ml^{-1}) or distilled water. Powdery mildew spores were collected from naturally infected cucumber leaves in the glass house. After inoculation, plants were moved to a phytotron and exposed to incandescent reflector lamps as white light control (100 W, Nanjing Special Lamp Co., China), purple light (P) with a maximum intensity at 394.6 nm, blue light (B) with a maximum intensity at 452.5 nm, green light (G) with a maximum intensity at 522.5 nm, yellow light (Y) with a maximum intensity at 594.5 nm, or red light (R) with a maximum intensity at 628.6 nm for 72 h. P, B, G, Y and R were provided by light-emitting photodiodes (ZDL-100 W, Nichia, Japan). The environmental conditions were as follows: a 12 h photoperiod (light/dark), temperatures of 25/18°C (day/night), photosynthetic photon flux density (PPFD) of 350 μmol (photon) $m^{-2}s^{-1}$, and the relative humidity (RH) of 60%. The light intensity and spectral distributions of the LEDs were measured with a quantum sensor (Li-Cor, USA) and a STC 4000 spectrometer (Everfine Photo-E-Info Co., China) (Fig. 1), respectively. After 72 h exposure, leaves from the inoculated plant leaves were harvested, frozen quickly in liquid nitrogen and stored at −80°C for further analysis. Disease severity was assessed

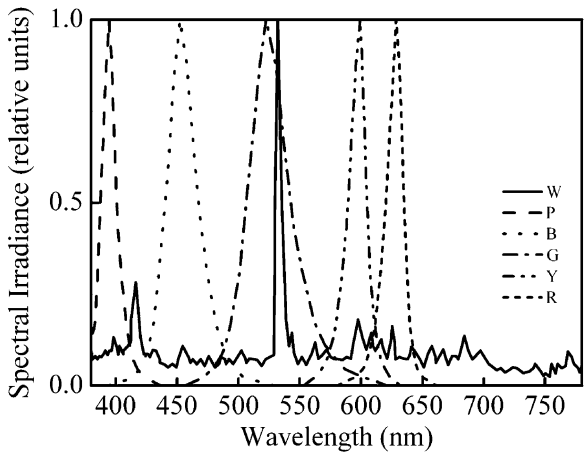

Fig. 1 Relative spectral distribution of the LEDs and white light used: W=white light; P=purple light; B=blue light; G=green light; Y=yellow light; R=red light

10 days after inoculation as numbers of powdery colonies from all leaves on each plant.

Salicylic acid measurement

The extraction and determination of free SA and conjugated SA were measured as described previously (Raskin et al. 1989) with some modification. Briefly, leaf samples (1 g) were ground with 3 ml of 90% methanol. After centrifugation, the pellet was re-extracted with 3 ml of 100% methanol and centrifuged again. The combined supernatants were dried in vacuum at 40°C and the obtained residue was dissolved in 3 ml distilled water at 80°C for 10 min. The supernatants were used to assess free SA and conjugated SA. The free SA was assayed as follows: 1 ml of supernatant was extracted with 2.5 ml of ethylacetate-cyclopentane (1:1, V/V) and 50 μl of 10 N HCl and then dried under nitrogen. The residues were dissolved with 1 ml of 20% (v/v) methanol dissolved in 20 mM sodium acetate buffer (pH 5.0), and then subjected to HPLC (LC-10AS; Shimazu Tokyo, Japan). SA in a 20 μl sample was determined with a HPLC spectrofluorescence detector (RF-10AXL; Shimazu Tokyo, Japan) at the excitation wavelength of 295 nm and emission wavelength of 370 nm with a flow rate of 1.0 ml min^{-1} of solvent (20% (v/v) methanol / 20 mM sodium acetate buffer; pH 5.0) at 35°C using a ODS column (C18, 4.6× 250 mm). The amount of SA glucosides (SAG) was

determined as follows: 1 ml of supernatant was incubated with 1 ml β-glucosidase (3U ml^{-1}) at 37°C for 6 h, and then analyzed as previously described. Conjugated SA was calculated based on the contents of free SA and SAG.

Measurement of antioxidant enzyme and H_2O_2

For the enzyme assays, leaves (0.3 g) were homogenized with 3 ml ice-cold 25 mM potassium phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2% PVP (adding 2 mM AsA for measuring ascorbate peroxidase). The homogenates were centrifuged at 12,000 g for 20 min at 4°C and the obtained supernatants were used as enzyme extract.

Superoxide dismutase (SOD) was measured using the method of Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the rate of nitroblue tetrazolium (NBT) reduction at 560 nm. The activity of guaiacol peroxidase (G-POD) was assayed according to the method of Cakmak and Marschner (1992). Catalase (CAT) activity was measured according to Patra et al. (1978) by measuring the decrease in absorbance at 240 nm. Ascorbate peroxidase (APX) was analyzed according to Nakano and Asada (1981) by estimating the rate of ascorbate oxidation at 290 nm. All spectrophotometric analyses were conducted on a SHIMADZU UV-2410PC spectrophotometer.

The content of H_2O_2 in leaves was assayed by monitoring the absorbance of the titanium-peroxide complex at 415 nm, using the method of Brennan and Frenkel (1977).

Determination of activities of PAL and PPO and contents of flavonoid and phenolics

PAL activity was estimated with L-phenylalanine as substrate (Zucker 1965). The activity of PPO was assayed by monitoring the increase in absorbance at 370 nm with caffeic acid as a substrate (Ruiz et al. 1999). Total soluble phenolics were extracted and assessed in the absorbance at 765 nm using caffeic acid as a standard after the addition of Folin–Ciocalteu reagent (Ruiz et al. 1999). Flavonoids were extracted and determined according to Tekel'ová et al. (2000). The soluble protein was measured by the method of Bradford (1976).

RNA extraction and real time RT-PCR for gene expression analysis

Total RNA was isolated from cucumber leaves after exposure to light of different wavelengths for 72 h. TRIZOL reagent (Sangon, China) was used during RNA isolation. Total RNA was dissolved in DEPC-treated water after extraction. First-strand cDNA was synthesized using a RevertAid^{TM} cDNA Synthesis Kit (Fermentas, USA) according to the manufacturer's instruction. Specific gene primers were designed for real time RT-PCR, as given in Table 1.

The iCycler iQ Multicolor real-time PCR Detection System (Bio-Rad, Hercules, CA) was used to perform with real time RT-PCR. PCR reaction included 10 µl iQ SYBR Green Supermix, 1 µl of diluted cDNA and 0.1 µM of each primer. PCR cycling was performed as follows: preliminary denaturation at 95°C for 3 min and 40 cycles of denaturation at 95°C for 10 s, annealing at 58°C for 45 s. During the 58°C step, the data were collected. The cucumber *actin* gene was used as an internal control. Relative gene expression was calculated according to Livak and Schmittgen (2001).

Statistical analysis

All treatments and assays were repeated three times. According to the experimental design, the variances between means were established using one-way or two-way ANOVA. Post-hoc comparisons for comparing all bars individually were performed using Duncan's multiple range test. When necessary, LSD comparisons were used to show the difference between infected- and not infected responses within a light treatment.

Results

Resistance to powdery mildew

The effect of light quality on resistance to powdery mildew was investigated by determining the numbers of powdery colonies from all leaves on each plant. After inoculation with *S. fuliginea* spores, plants were exposed to W, P, B, G, Y or R for 10 d (Fig. 2). On average, there were 12 powdery colonies per leaf in

Table 1 Primers used for real time RT-PCR assays

Gene	Accession No.	Primer pairs
PR-1	DQ641122	F: AACTCTGGCGGACCTTAC
		R:GACTTCCTCCACACTACT
WRKY30	FJ036895	F: CATCTTCACCCTTCTTCAT
		R:CGCATCTCTGCTTCTACTG
WRKY6	FJ036899	F: GAGGAGTTGATAGTGGTGG
		R:TTCTTGCTCTGATTTGGTT
PAL	DQ645596	F: ACGGTTTGCCTTCTAAT
		R:CATCCTGGTTGTGTTGC
CS	DQ124970	F: TGTTACCATTGGCCAGAGACTTC
		R: CCTATCGAACACGTCTGGATGTC
CAD	DQ178938	F: CAATCCCTCTATGTCGTTCG
		R: GCTTGAGATCACGGTGAAGA
actin	DQ641117	F: TGGACTCTGGTGATGGTGTTA
		R:CAATGAGGGATGGCTGGAAAA

W-grown plants. P, B, G, and Y exposure all significantly enhanced disease development when compared to W exposure. The number of powdery colonies per leaf were increased in P-, B-, G- and Y-grown plants. By contrast, disease development was markedly suppressed in R-grown plants.

Accumulation of salicylic acid (SA) and H_2O_2

We measured both free SA and conjugated SA contents in plants after 72-h exposure to different lights (Fig. 3). In uninoculated plants, free SA was significantly decreased in plants grown under P, B and G, relative to those grown under W. However, free SA content in R-grown plants was not significantly different from that of W-grown plants. Powdery mildew infection resulted in increases in both free and conjugated SA contents independent of the light quality applied. In powdery mildew-inoculated leaves both the contents of free and conjugated SA were markedly increased, compared to the healthy plants in each treatment. Among the plants exposed to the light of different wavelengths, those under R showed the most increase in free SA content whilst those under Y showed the most increase in conjugated SA content. Furthermore, Y-grown plants also had free SA level comparable to that of W.

The effects of light quality on H_2O_2 content both in inoculated and healthy plants were also investigated (Fig. 4). In healthy plants, H_2O_2 contents were higher in plants exposed to R compared to that of W-grown plants. On the other hand, H_2O_2 content was not significantly changed under other monochromatic lights. Inoculation of the powdery mildew pathogen resulted in an increase in the content of H_2O_2 except under Y.

Transcript of defence genes

We examined the expression of several defence-related genes in healthy and infected leaves grown after 72 h-exposure to light of different wavelengths (Fig. 5). Exposure to P, B, G and Y did not result in major increases in the transcript levels for *PR-1*, *WRKY30*, *WRKY6, PAL, CS* and *CAD*. However, the transcripts for *WRKY6* under P and Y and *CAD* under P and B were apparently higher than those of W-grown plants. Plants under R showed higher levels of *PR-1, WRKY30*, *WRKY6* and *PAL* transcripts. The mRNA levels of *PR-1*, *WRKY30* and *WRKY6* increased significantly in the inoculated leaves grown under W, Y and R. In addition, *PAL* and *CAD*, two genes in the phenylpropanoid pathway, were up-regulated in powdery mildew-infected leaves grown under W, P, and B. Interestingly, transcripts for *CS,* a gene involved in cell wall strengthening, significantly decreased in the infected leaves with exposure to W, Y and R but was up-regulated in plants exposed to P, B and G.

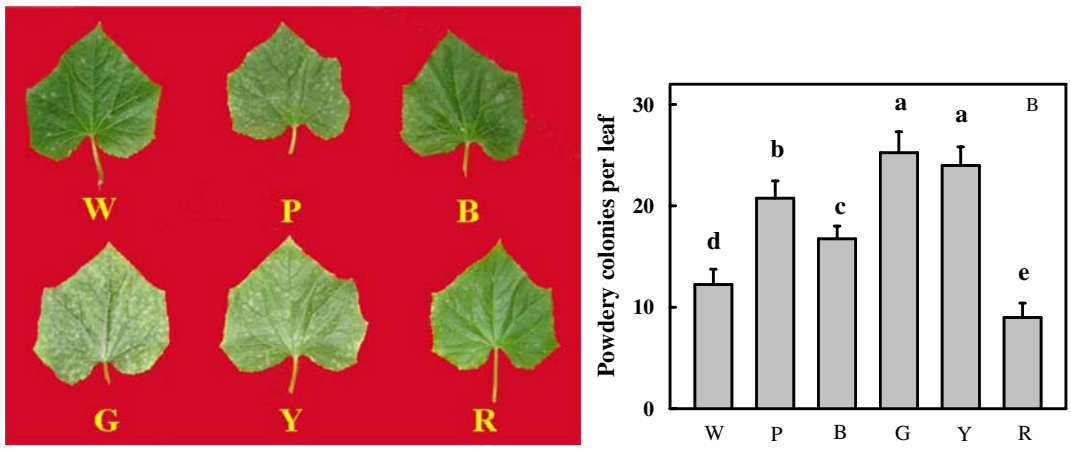

Fig. 2 Effects of light quality on the resistance to powdery mildew. Cucumber leaves were inoculated with *Sphaerotheca fuliginea* spores and then kept under different lights for 10 d. W, white light; P, purple light; B, blue light; G, green light; Y, yellow light; R, red light. Disease incidence was assessed as numbers of powdery colonies for all leaves on each plant and the mean for each treatment. The data were obtained from three independent experiments. Values are the means of three replicates with standard errors shown by vertical bars. Bars sharing the same letters are not significantly different by Duncan's multiple range test ($P \leq 0.05$)

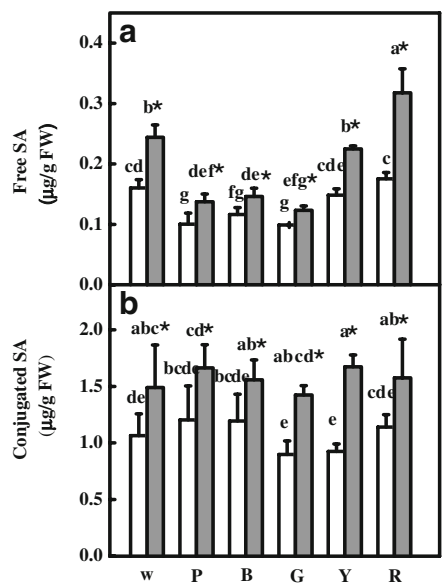

Fig. 3 Levels of free salicylic acid (**a**) and conjugated salicylic acid (**b**) in leaves of cucumber. Plants were kept in different quality light without (*open column*) or with the immediately inoculation of *Sphaerotheca fuliginea* spores (*gray column*) for 72 h. W, white light; P, purple light; B, blue light; G, green light; Y, yellow light; R, red light. Values are the means of three replicates with standard errors shown by vertical bars. Asterisks indicate values significantly different between infected and uninoculated leaves by LSD test ($P \leq 0.05$) whilst different letters indicate significant difference among all light treatments by Duncan's multiple range test ($P \leq 0.05$)

Activities of PAL and PPO and contents of flavonoid and phenolics

Generally speaking, light quality did not have significant effects on the activities of PAL and PPO. However, when measured 72 h after treatments plants grown under R showed higher PPO activity than

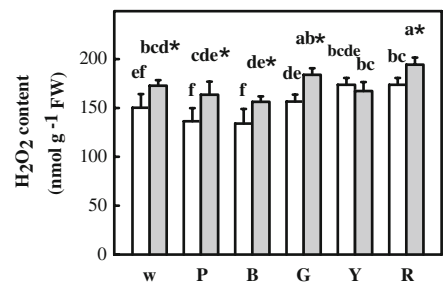

Fig. 4 H_2O_2 content in leaves of cucumber after exposure to different quality light for 72 h without (*open column*) or with the immediately inoculation of *Sphaerotheca fuliginea* spores (*gray column*). W, white light; P, purple light; B, blue light; G, green light; Y, yellow light; R, red light. Values are the means of three replicates with standard errors shown by vertical bars. Asterisks indicate values significantly different between infected and uninoculated leaves by LSD test ($P \leq 0.05$) whilst different letters indicate significant difference among all light treatments by Duncan's multiple range test ($P \leq 0.05$)

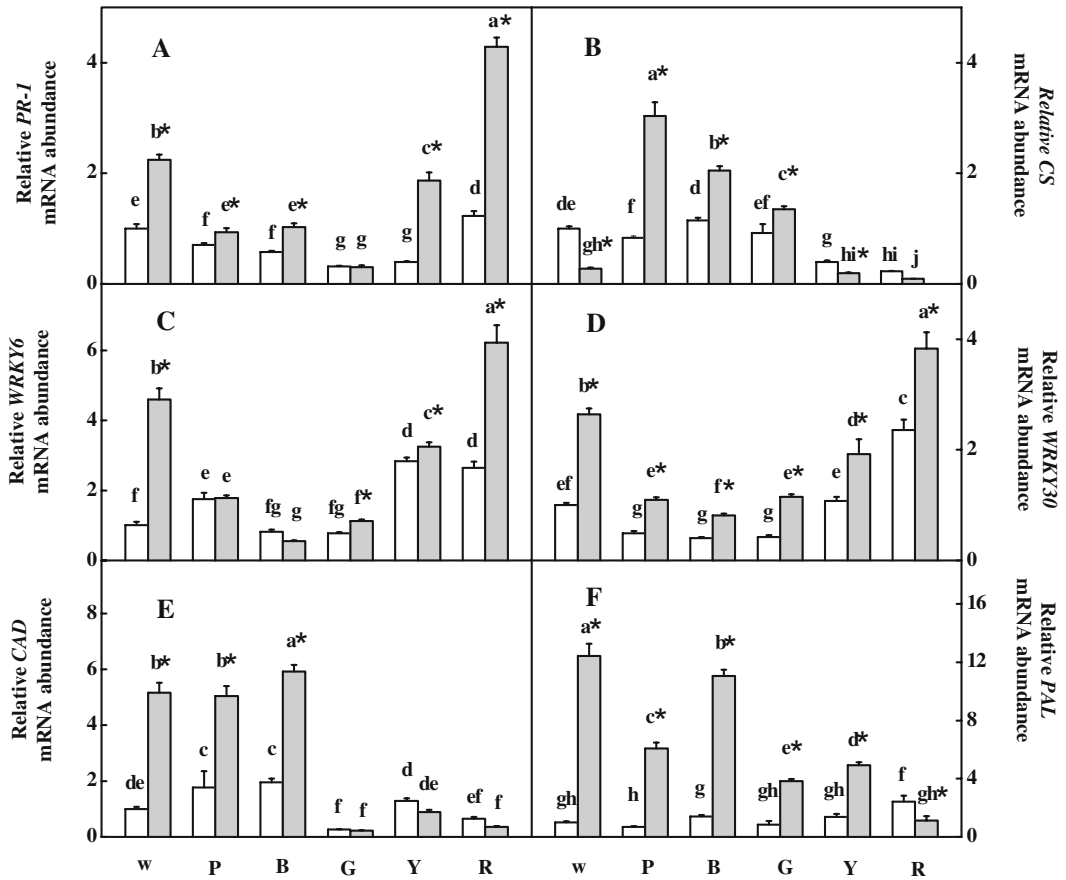

Fig. 5 Effects of different quality light on transcripts abundance in leaves of cucumber after exposure to different lights for 72 h without (*open column*) or with the immediately inoculation of *Sphaerotheca fuliginea* spores (*gray column*). W, white light; P, purple light; B, blue light; G, green light; Y, yellow light; R, red light. Values are the means of three replicates with standard errors shown by vertical bars. Asterisks indicate values significantly different between infected and uninoculated leaves by LSD test ($P \leq 0.05$) whilst different letters indicate significant difference among all light treatments by Duncan's multiple range test ($P \leq 0.05$). Expression levels produced by real time RT-PCR are expressed as a ratio to W, which was set at 1

plants exposed to light of other wavelengths (Fig. 6). Furthermore, inoculation of powdery mildew pathogen significantly increased the activities of PAL in W-, P- and B- grown plants and PPO in P-, B- and Y-grown plants. Similarly, flavonoid contents in healthy plants exposed to P, G, Y and R were similar to those under W, whilst plants exposed to B had higher flavonoid content than those of plants exposed to W. Inoculation with the powdery mildew pathogen resulted in significant increases in flavonoid contents under W, P, B and Y but not under G and R. There were significant decreases in the contents of phenolics in plants grown under P, B and G whereas powdery mildew infection increased the phenolic contents in P-, G- and Y-grown plants.

Activities of ROS scavenging enzymes

To examine whether resistance was correlated with changes in the activities of ROS scavenging enzymes, we measured the activities of SOD, G-POD, APX and CAT in plants exposed to different lights for 72 h with or without inoculation of the powdery mildew pathogen (Fig. 7). Plants under monochromatic lights xgenerally exhibited decreased SOD and APX activities but increased G-POD activity. However, plants exposed to B did not significantly change the SOD and APX activities compared with those exposed to W. CAT activity was increased only in plants exposed to B but decreased in plants exposed to other lights. Inoculation resulted in increased activities of many of these ROS-

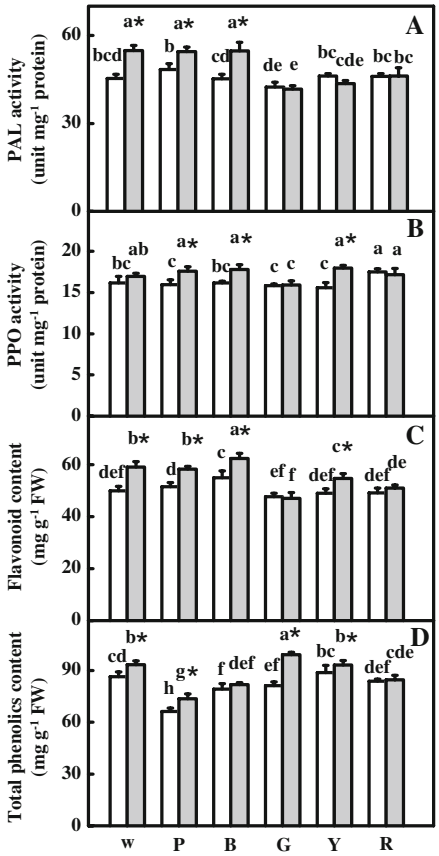

Fig. 6 Activities of phenylalanine ammonia-lyase (PAL) (**a**) and polyphenoloxidase (PPO) (**b**) and contents of flavonoids (**c**) and phenolics (**d**) in leaves of cucumber after exposure to different lights for 72 h without (*open column*) or with the immediately inoculation of *Sphaerotheca fuliginea* spores (*gray column*). W, white light; P, purple light; B, blue light; G, green light; Y, yellow light; R, red light. Values are the means of three replicates with standard errors shown by vertical bars. Asterisks indicate values significantly different between infected and uninoculated leaves by LSD test ($P \leq 0.05$) whilst different letters indicate significant difference among all light treatments by Duncan's multiple range test ($P \leq 0.05$)

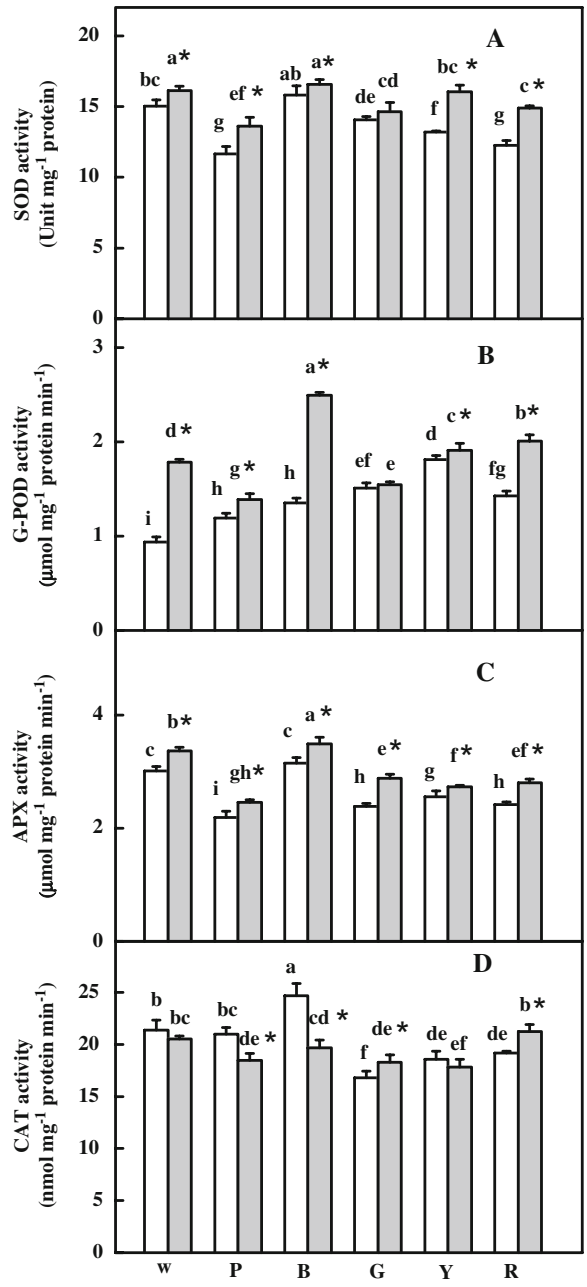

Fig. 7 Activities of superoxide dismutase (SOD) (**a**), guaiacol peroxidase (G-POD) (**b**), ascorbate peroxidase (APX) (**c**) and catalase (CAT) (**d**) in leaves of cucumber after exposure to different quality lights for 72 h without (*open column*) or with the immediately inoculation of *Sphaerotheca fuliginea* spores (*gray column*). W, white light; P, purple light; B, blue light; G, green light; Y, yellow light; R, red light. Values are the means of three replicates with standard errors shown by vertical bars. Asterisks indicate values significantly different between infected and uninoculated leaves by LSD test ($P \leq 0.05$) whilst different letters indicate significant difference among all light treatments by Duncan's multiple range test ($P \leq 0.05$)

scavenging enzymes. The increases were most significant in G-POD for W- and B-grown plants after inoculation.

Discussion

Light of a certain quality is required for induction of disease resistance in a broad range of plants. In this study, we found that incidence of powdery mildew in

cucumber plants was significantly reduced by red light but increased by other monochromatic lights (P, B, G and Y). The suppressive effects of red light on disease development in our study are in agreement with early studies in rice, *Arabidopsis* and broad bean (Guo et al. 1993; Islam et al. 1998; Zeier et al. 2004). Islam et al. (2002) have reported that red light induces resistance to *Phytophthora capsici* in pepper, pumpkin and tomato seedlings. Rahman et al. (2003) have found that red light induces resistance in broad bean to leaf spot disease caused by *Alternaria tenuissima*. Most recently, Islam et al. (2008) have reported that red light can induce systemic disease resistance against root-knot nematode *Meloidogyne javanica* and *Pseudomonas syringae* pv. *tomato* DC 3000. However, there have been few studies on the effects of light quality on disease development. Here, we found that red light significantly decreased whilst light of other wavelengths significantly increased the incidence of powdery mildew. Therefore, the suppressive effect of white light may be attributed to its red region.

SA is well known for its important roles in pathogen resistance and SAR. Mutants defective in SA synthesis show an enhanced susceptibility to powdery mildew (Achuo et al. 2004). Furthermore, Genoud et al. (2002) have also showed that mutants for phytochromes, plant red/far-red light photoreceptors, are compromised in SA induction and resistance to *P. syringae*. Recently, several mutants with altered resistance to powdery mildew have been isolated, including the *enhanced disease resistance1 (edr1)* mutant defective in salicylic acid signaling pathway (Vogel and Somerville 2000). Therefore, it is of interest to see whether SA is also involved in red light-induced resistance. In this study, we found that plants grown under R had SA levels similar to those under W but had a higher SA accumulation than those exposed to W if they were inoculated with the pathogen (Fig. 3). In comparison, plants grown under light of other wavelengths had reduced SA levels. These results suggest that SA-mediated defence responses may be involved in R-enhanced resistance to powdery mildew. SA-mediated defence is characterized by an early increase in the synthesis of SA and a concomitant activation of genes encoding PR proteins. Exposure to R up-regulated whereas exposure to P, B, G and Y down-regulated *PR-1* (a molecular marker of the SA-dependent SAR pathway), *WRKY6* and *WRKY30* (encoding two transcription factors involved in SAR pathway) (Fig. 5a,c,d). These results support early findings that SA and red light up-regulate transcript levels of *PR-1* (Genoud et al. 2002). Similarly, Griebel and Zeier (2008) have found that induction of SAR and SA-dependent systemic defence reactions are compromised in *phyAphyB* mutants defective in perception of red/far-red light. H_2O_2 participates in plant defence responses including those mediated by SA (Chen et al. 1993). In this study, H_2O_2 content was the highest in cucumber leaves grown under R and could be further elevated after powdery mildew infection (Fig. 4). This result is consistent with the early finding that SA treatment enhanced the endogenous H_2O_2, while inhibiting the accumulation of H_2O_2 may be compromised by the action of SA (Chen et al. 1993).

In spite of the increased disease incidence, plants under P and B had higher levels of transcripts for *CS*, *CAD* and *PAL* than those under R (Fig. 5). *CS* encodes callose synthases, which strengthen plant cell walls, forming a physical barrier during fungal infection. However, Nishimura et al. (2003) have found that a mutant lacking callose exhibits enhanced resistance to powdery mildew infection and that callose or callose synthase negatively regulated the SA pathway. Thus, down-regulated transcript levels of *CS* may lead to up-regulation of SA in plants grown under R after infection (Fig. 5b). Meanwhile, the enhanced resistance for plants grown under R did not appear to be associated with the increased activity of the secondary metabolism since transcript levels of *CAD* and *PAL,* activities of PAL and PPO and, the contents of flavonoids and total phenolics were not generally enhanced in these plants (Fig. 6). These results suggest that an enhanced physical barrier or accumulation of phytoalexins might not play a major role in the light-induced resistance. Similarly, Lin et al. (2009) have also found that phytoalexins are not involved in the acibenzolar-S-methyl-induced systemic resistance in cucumber plants.

PAL has been implicated in the response to a variety of biotic and abiotic stresses. Increased PAL activity and secondary metabolites, such as phenolic compounds and lignin were often observed in pathogen-infected plants (Ye et al. 2006). In the present study, powdery mildew infection of plants grown under P and B significantly increased the activities of PAL and PPO and the contents of flavonoids (Fig. 6), and up-regulated *PAL* and *CAD*,

two genes in the phenylpropanoid pathway (Fig. 5e,f). These results suggest that P and especially B may act as an important signal for the PAL pathway. Interestingly, although plants grown under P and B had increased activities of PAL and PPO and contents of flavonoids, they showed increased disease incidence when compared with that of W-grown plants (Fig. 6). This could be attributed to light-induced stomatal opening (data not shown). Several studies have shown that blue light was more effective than other lights in promoting stomatal opening (Shimazaki et al. 2007; Wang et al. 2009), which can lead to increased disease incidence.

Antioxidative metabolism has been implicated in the defence response. King (2008) has reported that foliar application of a mixture of riboflavin and methionine inhibits the powdery mildew development by inducing an oxidative stress in cucumber. Here, we found that plants under monochromatic lights exhibited decreased SOD and APX activities and powdery mildew infection caused only slight increases in the activities of these antioxidant enzymes (Fig. 7). Thus, antioxidative metabolism does not appear to be associated with differential resistance of plants exposed to light of different wavelengths.

Based on the results presented here, red light-enhanced resistance to powdery mildew is correlated with SA-mediated defence mechanisms. Exposure to R resulted in increased accumulation of SA and H_2O_2, and elevated expression of SA-regulated *PR-1* and *WRKY* genes. Our circumstantial evidence showed that these SA- mediated defence genes were not strongly induced in plants grown under other monochromatic lights. Further studies through genetic and molecular approaches could lead to identification of genes and associated biochemical and metabolic processes important for red light-enhanced disease resistance.

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