Journal of Photochemistry & Photobiology, B: Biology 156 (2016) 41–49

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Determination of UV action spectra affecting the infection process of *Oidium neolycopersici*, the cause of tomato powdery mildew

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ARTICLE INFO

Article history:
Received 14 August 2015
Accepted 13 January 2016
Available online 15 January 2016

Keywords:
Ultraviolet
Oidium neolycopersici
Powdery mildew
Action spectra

ABSTRACT

Oidium neolycopersici, the cause of powdery mildew in tomato, was exposed to UV radiation from 250 to 400 nm for 1, 12, or 24 min. Radiation ≤ 280 nm strongly reduced conidial germination, hyphal expansion, penetration attempt and infection of *O. neolycopersici*. From 290 to 310 nm the effect depended on duration of exposure, while there was no effect ≥310 nm. There were no significant differences within the effective UV range (250–280 nm). Conidial germination on a water agar surface was <20% or around 40%, respectively, if samples were exposed for 1 min within the effective UV range followed by 24 h or 48 h incubation. Twelve or 24 min exposure reduced germination to close to nil. A similar trend occurred for germination of conidia on leaf disks on water agar in Petri dishes. The effective UV range significantly reduced all subsequent developmental stages of *O. neolycopersici*. There was no cytoplasmic mitochondrial streaming in conidia exposed to the effective UV range, indicating that there may be a direct effect via cell cycle arrest. There was no indication of reactive oxygen species involvement in UV mediated inhibition of *O. neolycopersici*. Optical properties of *O. neolycopersici* indicated that the relative absorption of UV was high within the range of 250 to 320 nm, and very low within the range of 340 to 400 nm. Identification of UV wavelengths effective against *O. neolycopersici* provides a future basis for precise disease control.

1. Introduction

Tomato (*Solanum lycopersicum*) is a major crop plant worldwide, cultivated under both field and greenhouse conditions. The greenhouse environment is favorable to the growth of powdery mildew, and powdery mildew caused by *Oidium neolycopersici* is a significant threat to greenhouse-grown tomatoes [1]. Even though tomato fruits are not susceptible, powdery mildew infection of leaves and stems reduces photo assimilation and thus indirectly reduces yield [1]. Commonly used commercial tomato cultivars are susceptible to the pathogen [2]. Increasing concern about environmental pollution, risk of development of fungicide-resistant pathogen isolates associated with indiscriminate use of chemicals, and consumer concerns about pesticide residues demand effective and economically viable alternatives to control this disease.

UV is the short wavelength part of the electromagnetic spectrum, and it is subdivided into UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm) and vacuum UV (100–200 nm) [3]. The biological impact of UV depends on wavelength and irradiance level, as well as the complexity of the organisms exposed to UV. In its shorter wavelength range (<315 nm), UV can induce mutations and/or kill simple organisms once absorbed by DNA [4]. On the other hand, depending on dose and wavelength, UV can act as a signal for regulation of growth, development and defense responses in higher plants [5].

Previous studies have shown the potential of red light and UV in controlling severity and sporulation of a number of different powdery mildews [6–9], and indicate that both may be used as a powerful strategy in management of these economically important diseases. Nighttime application of UV-B irradiance of around 1 Wm^{-2} with a broad spectrum UV source (278–400 nm with peak at 313 nm) for ca. 5 min per night significantly reduced powdery mildew severity in roses [7], cucumber [6,10], strawberry, rosemary and tomato (Suthaparan et al., unpublished data) [11]. The efficacy of UV in controlling powdery mildew depends on the spectral composition of the background light. UV-B applied during darkness or in combination with red light gave the best control of powdery mildew, while UV-A or blue light applied together with UV-B reduced the efficacy [10]. Furthermore, UV with wavelengths above 290 nm had no suppressive effect on rose powdery mildew within the tested dose [7].

Determination of specific UV wavelengths effective against powdery mildew is a key factor in practical optimization of UV application. Without isolating the effect of specific wavelengths, their relative contribution

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http://dx.doi.org/10.1016/j.jphotobiol.2016.01.009
1011-1344/

(action spectra) to disease suppression cannot be assessed precisely. Action spectra, simply defined as the biological effect as a function of wavelength [12], play an important role in characterizing biological responses to electromagnetic radiation. In plants, different weighting functions have been developed to assess the effects of UV on various biological processes [13]. The most commonly used, Green's weighting function, indicates no effect of wavelengths within the UV spectrum greater than 313 nm on various plant processes [14]. Corresponding effects of various UV wavelengths on fungi, especially powdery mildew fungi, are not known. Our previous findings with rose powdery mildew [7] and those of other fungi [15] indicate that UV-B has no efficacy above 290 nm, which is a much shorter wavelength than the 313 nm threshold suggested for plants [16]. This illustrates a necessity to determine action spectra for UV radiation for various fungal responses. In this investigation, action spectra for various developmental responses, including conidial germination, hyphal length, penetration attempt, and successful infection of *O. neolycopersici*, were determined for the UV wavelength range of 250 to 400 nm.

2. Materials and Methods

2.1. Experimental Site

Experiments were conducted at the Okazaki Large Spectrography and Bioimaging facility at the National Institute for Basic Biology in Okazaki, Japan.

2.2. Plant Production

Seeds of the powdery mildew susceptible tomato cv. Espero were sown in 12 cm diameter plastic pots containing a standard peat growth medium (Jiffy, Nisshin, Japan). Plants were grown in a growth chamber with artificial lighting provided by 120 cm fluorescent lamps (32 W, Model FHF 32 Ex-N-H, Panasonic Inc., Osaka, Japan). A 16 h photoperiod was provided with a photosynthetic photon flux (PPF) (400–700 nm) of 160 ± 10 μmol m^{-2} s^{-1} at plant height. Temperature and relative humidity in the growth chamber were maintained at 22 ± 2 °C and 80 ± 5%, respectively. Plants were irrigated with a complete nutrient solution with a pH of 6.5 and an electrical conductivity of 1.5 mS cm^{-2}, prepared by mixing Kristalon Indigo and YaraLiva Calcinite (1:1[volume/volume]) (Yara International ASA, Oslo, Norway).

2.3. Pathogen Isolates

Clean colonies of *O. neolycopersici* (Isolate KTP-04, obtained from Kinki University, Osaka, Japan) were transferred to healthy powdery mildew susceptible tomato cv. Espero by spraying with a conidial suspension with a hand-held sprayer. For preparation of the conidial suspensions, tomato leaflets with 9-day-old colonies were placed in distilled water containing Tween 20 (20 μl per liter) and gently shaken to remove the conidia. Inoculated plants were maintained in isolated growth chambers at 22 ± 2 °C with 75 ± 5% RH. A 14 h photoperiod with an irradiance of 100 ± 20 μmol m^{-2} s^{-1} at plant height was provided by 60 cm fluorescent lamps of the same type as mentioned above. Eight to nine-day-old fresh inoculum was used for each experiment. To maintain fresh inoculum throughout the experiment, pathogen inoculum was renewed weekly by spraying conidial suspensions onto healthy tomato plants as described above.

2.4. Monochromatic UV Sources

Monochromatic UV with peak wavelengths of 250, 260, 270, 280, 290, 300, 310, 320, 350, or 400 nm with spectral half-width of <5 nm were generated by the Okazaki Large Spectrograph with Xenon arc lamps (30 kW, Ushio Inc., Tokyo, Japan) with double-blazed grating (Central Research Lab., Hitachi Ltd., Tokyo, Japan) as a radiation source, as described previously [16] with slight modifications. Three different photon fluences (doses) of each wavelength mentioned above were tested in all experiments. Samples were irradiated with the above-mentioned wavelengths with an irradiance of 1.04 ± 0.05 μmol m^{-2} s^{-1} 1 for 1, 12 or 24 min. The irradiance level was achieved by using combinations of filters with different transmission percentages (Neutral Density Filters, Hoya Ltd., Tokyo, Japan).

2.5. Conidial Germination and Hyphal Expansion on Water Agar

To understand the effect of different wavelengths and doses of UV on conidial germination and hyphal expansion, the following experiments were conducted. Conidia from eight to nine-day-old colonies of *O. neolycopersici* were deposited on the surface of water agar in 3.5 cm diameter Petri dishes by gently touching the diseased leaves to the agar surface. Immediately after conidial deposition, Petri dishes (without lids) containing conidia were exposed to the above-mentioned UV treatments. After UV exposure, the Petri dishes were sealed and incubated at 22 °C in complete darkness. Twenty four hours after the start of the experiments, a piece of water agar was taken from the center of each Petri dish, mounted on glass microscope slides and examined with an Olympus D72 microscope (200× magnification) connected to CellSens Dimension software (Olympus Soft Imaging solutions GmbH, Munster, Germany) for recording of conidial germination. Fifty conidia per sample were assessed for germination per treatment. Similarly, 10 germinated conidia per sample per treatment were assessed for germ tube (hyphal) length. In treatments where a lower germination percentage was achieved, approximately 100 conidia were examined to assess germ tube length of 10 conidia. In treatments where none of the conidia germinated (250–290 nm with 12 and 24 min of exposure), germ tube length was recorded as zero. Two independent experiments were conducted, with two replicate Petri dishes in each ($n = 4$).

To examine whether UV radiation inhibits or delays conidial germination, a similar experiment as described above was conducted, but here irradiated samples were incubated at 22 °C in darkness for 48 h instead of 24 h and then assessed for conidial germination.

2.6. Effect on Reactive Oxygen Species (ROS) Localization

To examine if UV induces accumulation of reactive oxygen species (ROS) in conidia/germlings of *O. neolycopersici*, experiments were conducted in a similar fashion as described above. Irradiated samples were incubated for 3 h in darkness at 22 °C and then flooded with 2.5 mM nitroblue tetrazolium chloride (NBT) (Roche Diagnostics GmbH, Mannheim, Germany) dissolved in 5 mM 3-(N-morpholino) propanesulfonic acid (MOPS) at pH 7.6 for 30 min in darkness. A piece of water agar cut from the center of each Petri dish was mounted on glass microscope slides and assessed with a light microscope at 200× magnification. Fifty conidia or germlings per sample per treatment were assessed for NBT staining pattern (blue-purple formazan precipitate in the presence of super oxide) as described previously with slight modifications [17], and categorized as follows: i) conidium with no germ tube and NBT staining observed in <25% of the area of the entire conidium; ii) conidium with no germ tube and NBT staining observed in ≥25% of the area of the entire conidium; iii) conidium with a germ tube <5 μm in length and NBT staining observed in <25% of the area of the entire germling; and iv) conidium with a germ tube <5 μm length in which NBT staining was observed in ≥25% of the area of the entire germling.

2.7. Effect on Cytoplasmic Mitochondrial Streaming

To examine the effect of UV treatment on cytoplasmic mitochondrial streaming of *O. neolycopersici*, experiment was conducted in a similar fashion as described above. One set of irradiated samples were stained