FUNGAL BIOLOGY 118 (2014) 743–753

British Mycological Society promoting fungal science

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

Pre-illumination of rice blast conidia induces tolerance to subsequent oxidative stress

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ARTICLE INFO

Article history:
Received 12 December 2012
Received in revised form
3 April 2014
Accepted 10 June 2014
Available online 20 June 2014
Corresponding Editor: Katja Sterflinger

Keywords:
Antioxidant capacity
Fungitoxicity
Light
Oxidative stress
Rice blast spores

ABSTRACT

Many environmental factors, alone or combined, affect organisms by changing a pro-/antioxidant balance. Here we tested rice blast fungus (*Magnaporthe oryzae*) for possible cross-adaptations caused by relatively intense light and protecting from artificially formed reactive oxygen species (ROS) and ROS-dependent fungitoxic response of the host plant. Spore germination was found to be suppressed under 4-h and, to larger extent, 5-h illumination. The effect was diminished by antioxidants and, therefore, suggests involvement of ROS. One-hour of light did not affect spore germination, but stimulated their chemically assayed superoxide production. The illuminated spores were more tolerant (than non-illuminated ones) to artificially generated H_2O_2, O_2^-, or $^{\cdot}OH$ or to toxic diffusate of rice leaf. They also caused more severe disease symptoms if applied to leaves of the susceptible rice cultivar at low concentration. Spore diffusates decomposed hydrogen peroxide. They detoxified exogenous H_2O_2 and superoxide radical as well as leaf diffusates. Spore illumination increased some of these protective effects. It is suggested that short-term light led to mild oxidative stress, which induced spore antioxidant capacity, enhancing spore tolerance to subsequent stronger oxidative stress and its aggressiveness *in planta*. Such tolerance depends partly on the antidotal action of spore extracellular compounds, which may also be light-stimulated. Therefore, a certain ROS-related environmental factor may adapt a fungus to other factors and so modulate its pathogenic properties.

Introduction

Success in plant colonization by pathogens is determined to large extent by their ability to survive under the harsh conditions accompanying a parasitic lifestyle. The hostile factors comprise reactive oxygen species (ROS), produced by plants to cope with many pathogens, at least, biotrophic (Doke *et al.* 1987; Baker & Orlandi 1995; Lamb & Dixon 1997).

One of ROS anti-infectional effects is their cytotoxicity, killing pathogenic fungi and oomycetes or retarding their development (Peng & Kuc 1992; Shetty *et al.* 2008). Such impact from plant shoots starts in the infection droplet. Elimination

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http://dx.doi.org/10.1016/j.funbio.2014.06.003
1878-6146/

of ROS, e.g., by exogenous catalase, promotes penetrations of fungi, causing rust, anthracnose, or mildew in leaves of their host and nonhost plants (Mellersh et al. 2002). On wheat leaf treated with catalase, *Septoria tritici* produces earlier symptoms, while infiltration of H_2O_2, by contrast, delays their appearance (Shetty et al. 2008). Diffusates of rice leaves of some blast-resistant cultivars suppress spore germination of blast fungus *Magnaporthe oryzae*, to higher extent in spore-inoculated than healthy leaves and than both healthy and infected leaves of susceptible cultivars. This fungitoxicity involves ROS, since it is diminished by antioxidants scavenging hydrogen peroxide, superoxide (O_2^-), and hydroxyl ($^{\cdot}OH$) free radicals (Aver'yanov & Lapikova 1988; Pasechnik et al. 1998).

As do any aerobes, pathogenic fungi (Heller & Tudzynski 2011) including *M. oryzae* (Aver'yanov et al. 2007; Egan et al. 2007) yield ROS by themselves. An extreme environment may stimulate the process and suppress, *via* ROS, parasite's development. One such factor is daylight. It affects interacting host and pathogen both as a signal and damage to either partner (Roberts & Paul 2006). Many photoreactions involve ROS. For instance, active oxygen participates in photoinhibition of spore germination of fungi and other organisms (Nikolaev et al. 1988; Imbert & Blondeau 1999). Therefore, in the infection droplet, the pathogen may suffer from either its own or its host's oxidative bursts.

Naturally, pathogenic microbes may adapt to extreme factors. Spores of *M. oryzae* derived from a culture 12-h daily illuminated for 10–14 d at 2 klux are more tolerant to light at 2–10 klux, rice leaf diffusates, or ROS-generating chemicals than those from a darkness-incubated culture (Nikolaev & Aver'yanov 1991). Possible effects of such short-term light do not appear to be probed.

Antioxidants play key roles in fungal adaptation to oxidative stress (Heller & Tudzynski 2011), principally intracellularly. Additionally, melanin pigments tightly bound to fungal cell walls are antioxidants (Butler & Day 1998). Melanin of *M. oryzae* renders spores tolerant to superoxide and hydroxyl radicals, hydrogen peroxide (Aver'yanov et al. 1987), and singlet oxygen (Aver'yanov et al. 1986). Some fungal antioxidants are extracellular; copper–zinc superoxide dismutase (SOD) of *Claviceps purpurea* is at least partially secreted (Moore et al. 2002). Extracellular catalases are known in *Blumeria graminis* (Zhang et al. 2004), *Botrytis cinerea* (Schouten et al. 2002), *C. purpurea* (Garre et al. 1998), and *M. oryzae* (Skamnioti et al. 2007). *Alternaria alternata* secretes mannitol, a hydroxyl radical scavenger (Jennings et al. 1998). Similarly, *Sclerotinia sclerotiorum* and *B. cinerea* secrete oxalate, which suppresses oxidative bursts in infected tobacco or soybean cultures (Cessna et al. 2000) and *Arabidopsis* leaves, respectively (L'Haridon et al. 2011). The self-suppression of *M. oryzae* spore germination in overly dilute suspensions is restored by the addition of not only known antioxidants but also of diffusates of spores germinated at optimal concentration (Aver'yanov & Lapikova 1990).

The phenomenon studied here is the suppression of *M. oryzae* spore germination by visible light, artificially produced ROS, or rice leaf diffusates. The purpose was to determine whether a preliminary illumination of spores, equally intensive but short-term, might prevent these oxidative damages and change the pathogen's aggressiveness. We also attempted to reveal the protective properties of spore diffusates under these conditions.

Materials and methods

Fungus material

Magnaporthe oryzae strains Ina 168 (race 101, virulence genes *Av-a*$^+$, *Av-ta*$^+$) and Kyu 82-395A were obtained from CIRAD-CA (France). The strain H5-3 (race 007, *Av-k*$^{s+}$, *Av-a*$^+$, *Av-i*$^+$) first isolated from the far-eastern region of Russia was taken from the collection of Research Institute of Phytopathology (Russia). The cultures were maintained on carrot broth agar at 28 °C in darkness. Rice leaf fragments of the cv. Sha-tiao-tsao, to which these strains are compatible, were added to the broth (Aver'yanov et al., 2007). To maintain virulence, plants were inoculated twice a year; fungus was re-isolated from lesions and transferred to carrot medium.

Suspensions of spores (conidia) were washed off from 10-day-old cultures with distilled water, 15 mL per Petri dish; mycelium fragments were removed by a stainless steel sieve. Spores were washed twice with 50 mL of distilled water followed by centrifuged for 10 min, at 8000 ×g and 4 °C. Supernatants were discarded. Distilled water was added to the spores to adjust the concentration, which was determined using a haemocytometer.

Plant material

Rice cultivar Zenith (resistance genes *Pi-a*, *Pi-i*, *Pi-z*) was obtained from Agricultural Research Service USDA. Cvs. Tadukan (*Pi-k*, *Pi-ta*, *Pi-ta*^{2}), Raminad Str 3, and Sha-tiao-tsao (*Pi-k*^{s}) were from the collection of the Russian Research Institute of Phytopathology. Susceptibility of Sha-tiao-tsao and complete resistance of Zenith and Tadukan to the strain H5-3 has been reported (Pasechnik et al. 1998); such resistance of Raminad Str 3 was revealed in preliminary experiments.

Plants were grown for 21–28 d (to the stage of four leaves) in plastic pots (0.5 L natural soil per 8 plants) in a growth chamber at 28–29/21–23 °C (day/night), at 70–85 % RH. Light at 150–240 μmol m^{-2} s^{-1} was provided by two DRI-2000 lamps, 2 kW each, for 12 h daily. The lamp emission started from 350 nm and covered the whole visible range (with peaks at 450, 540, 600, and 670 nm) but not IR range. The 2-week-old plants were fertilized with 0.3 % ammonium nitrate.

Effect of spore illumination on their germination

To test the effects of light on spore germination, 50 μL water suspension of 3.5 × 10^4 spores mL^{-1} was incubated in 96-well plate (Greiner Bio-One, Germany) in the growth chamber for 5 h. Three exposure times were used, 1, 4, or 5 h. To protect against heat from the lamp, a glass Koch dish with 2-cm water level and paper filter, to diffuse light, was mounted above the plate resulting in light energy of 180 μmol m^{-2} s^{-1} (measured directly with Quantum Sensor). Once the light exposure period was over, the 96-well plate was placed in an opaque box for the remainder of the 5-h incubation period. Control spore