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Physiological and Molecular Plant Pathology 66 (2005) 144–159

PMPP

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Silicon influences cytological and molecular events in compatible and incompatible rice-*Magnaporthe grisea* interactions

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Accepted 3 June 2005

Abstract

Many monocot plants grown in soils amended with silicon (Si) exhibit increased levels of resistance to fungal diseases. Silicon-mediated resistance to rice blast disease, caused by *Magnaporthe grisea*, has been hypothesized to be the result of a mechanical barrier produced from Si polymerization *in planta*. The present study examined the cytological and molecular influences of Si amendment during genetically defined incompatible (resistant) and compatible (susceptible with no major resistance genes) interactions of rice with *M. grisea*. Differential accumulation of glucanase, peroxidase, and PR-1 transcripts were associated with limited colonization by *M. grisea* in epidermal cells of Si^+ plants of the susceptible cultivar M201. Katy, a resistant cultivar, responded to an avirulent race of *M. grisea* through the development of a hypersensitive response along with a strong induction of PR-1 and peroxidase transcripts independent of Si amendment. These findings support an active participation, distinct from single-gene-defined resistance, for Si in the defense of rice against *M. grisea*. While not discounting a physical barrier role for Si, new insights into a potential active role for this ubiquitous element during non-genetically defined rice-blast resistance is suggested.

Keywords: Silicon; Autofluorescence; Lignin; Soluble phenolics; Cytoplasmic granulation; PR-proteins; *Oryza sativa*; *Magnaporthe grisea*

1. Introduction

Rice (*Oryza sativa* L.) is one of the worlds most - important food crops and blast disease, caused by the hemibiotrophic fungus *Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udagawa) Barr (anamorph *Pyricularia grisea* (Cooke) Sacc.), is a major constraint to its production worldwide [44]. Rice blast disease follows a gene-for-gene interaction as proposed by Flor [15], in which a race of *M. grisea* expressing an avirulence gene (AVR) triggers the corresponding resistance (R) gene-mediated defense [7,33,54]. Two of the major blast R genes, *Pi-ta* and *Pi-b*, have been cloned and characterized at the molecular level. These genes are predicted to encode cytoplasmic proteins with a centrally located nucleotide binding site and a carboxy terminal leucine-rich repeat (LRR) region [2,61]. The cultivar Katy contains the *Pi-ta*^{2} R gene or both *Pi-ta* and *Pi-ta*^{2} R genes [38] while the cultivar M201 has no known major or minor gene(s) for resistance to race IB-49 of *M. grisea* [53].

The hypersensitive response (HR), defined as a rapid and localized death of host cells to restrict pathogen colonization [17], is a well known hallmark for race-specific resistance of rice cultivars to *M. grisea* [21]. The HR is characterized by plasma membrane depolarization, detachment of plasma membrane from the epidermal cell wall, alteration of ion channel

Abbreviations: Si, Silicon; AVR, avirulence gene; R, resistance; LRR, leucine-rich repeat; HR, hypersensitive response; *PAL*, phenylalanine ammonia-lyase; hai, hours after inoculation; kb, kilo base pair; bp, base pairs; *CHS*, chalcone synthase; FDI, fungal development index; TSP, total soluble phenolics; LTGA, lignin-thioglycolic acid; *chit*, chitinases; *Glu*, β-1,3-glucanase; *POX*, peroxidase; *l.w.*, lyophilized weight.

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doi:10.1016/j.pmpp.2005.06.002

activities, change in calcium homeostasis, degeneration of the nucleus, lack of cytoplasmic streaming, cytoplasmic granulation and protoplast collapse [17,31,60]. In addition to the above cellular events, other mechanisms of the rice defense response such as oxidative burst; formation of papillae; fortification of cell walls by phenolics and lignin deposition; and induction of phytoalexins and pathogenesis-related proteins [3,16,39,45,52] are also triggered in the *M. grisea* AVR-Pi-ta-rice interaction [24,43].

Several defense-related PR-like genes encoding PR-1, β-1,3-glucanases (PR-2), chitinases (PR-3), peroxidases (PR-9), and phenylalanine ammonia-lyase (*PAL*) have been cloned from rice [39,56]. PR-1 is a reliable marker for resistance in many pathosystems including rice-*M. grisea* [34]. Chitinases and β-1,3-glucanases are capable of degrading chitin and β-1,3-glucan, respectively, in the hyphae of *M. grisea* [13]. Phenylalanine ammonia-lyase is the key enzyme in determining the rate of phenolic production through the phenylpropanoid pathway [25] while peroxidases participate in the biosynthesis of lignin [18]. The flavonoid biosynthetic gene, chalcone synthase, is involved in the production of many flavonoids such as the rice phytoalexin sakuranetin [30].

Autofluorescence of epidermal and mesophyll cells is another marker of rice defense against *M. grisea* attack. Koga [31] induced death of rice leaf cells by heat shock and sodium arsenite, a fatty acid synthesis inhibitor, and observed that the growth of *M. grisea* within the penetrated cell was not affected unless the dead cells exhibited strong autofluorescence. Several physical and biochemical studies have demonstrated that compounds accumulating in autofluorescent epidermal cells of barley and oat plants attacked by *Erysiphe graminis* f.sp. *hordei* have ultraviolet absorption and emission characteristics of phenolics [5,6,36]. Although the temporal and spatial production of these autofluorogen compounds appears to be affected by Si [5], its positive or negative effect in the production of phenolics depends on each pathosystem. Carver et al. [4] observed a strong autofluorescence of epidermal cells in contact with germ tubes of *Blumeria graminis* and an increase in phenylalanine ammonia-lyase activity. Although autofluorescence of oat epidermal cells infected by *B. graminis* appeared to be independent of Si accumulation, these two events coincided spatially [4]. The massive accumulation of Si within autofluorescent barley epidermal cells that responded to an avirulent race of *B. graminis* suggests that monosilicic acid forms complexes with organic hydroxy compounds present in those cells [31]. The objectives of this study were to investigate the effects of silicon amendment on these dynamic cytological processes and determine if they correlate with specific molecular processes in compatible and incompatible rice-*M. grisea* interactions.

2. Materials and methods

2.1. Growth of rice plants, silicon amendment, and inoculation with M. grisea

Plastic pots (12 cm in diameter) were filled with 2 kg of peat Fafard No. 2 (soilless medium) (Conrad Fafard Inc.; Agawan, MA) and amended with calcium silicate slag (22% available soluble Si; Calcium Silicate Corp., Lake Harbor, FL) at the rates of 0 and 10 g pot^{-1} five days before sowing [49,50]. The native Si concentration in the substrate was 4 mg Si l^{-1}. Rice seeds were sown at the rate of eight seeds per pot and, at three days after emergence, each pot was thinned to two seedlings. Seedlings were then fertilized by adding 100 ml of a nutrient solution to each pot containing (in milligrams per kilogram of peat Fafard No. 2): 100 N, 300 P, 150 K, 85 Ca, 70 Mg, 40 S, 0.81 B, 1.33 Cu, 3.66 Mn, 0.15 Mo, and 4.00 Zn [49]. The nutrient solution was prepared using silicon-free water. A second application of this nutrient solution was made 25 days later. Iron deficiency was avoided by adding 10 ml of a solution containing 300 g of $FeSO_4$ l^{-1} per pot after thinning. Plants were kept under flooded conditions until the end of the experiments.

Plants from rice cultivars of Katy (P.I. 527707) and M201 (C.I. 9980) were inoculated at the time of emergence of the seventh leaf from the main tiller [35] with a suspension of 4×10^5 conidia ml^{-1} of race IB-49 of *M. grisea* (isolate 793). The rice cultivar M201 has no known major or minor genes for resistance to race IB-49 of *M. grisea* resulting in a compatible interaction [53]. Race IB-49 is avirulent on Katy because it carries the *Pi-ta*^{2} R gene, or both *Pi-ta* and *Pi-ta*^{2} R genes [38]. Katy also contains a tightly linked cluster of at least seven R genes that map in the same region as *Pi-ta* and *Pi-ta*^{2} [8,38]. The suspension of conidia was applied as a fine mist to the adaxial leaf blades of two plants per pot until runoff using an aerosol sprayer (Crown Spra-Tool; Fisher Scientific Co., Pittsburgh, PA). Gelatin (1%, w/v) was added to the sterile water used to prepare the conidial suspension to aid conidial adhesion to the leaf blades. The non-inoculated plants from each cultivar were sprayed with a solution of gelatin-sterile water (1%, w/v) until runoff. Inoculated and non-inoculated plants were covered with plastic bags and transferred to a growth chamber configured to the following conditions: constant temperature of 25 °C and photoperiod of 12 h dark and 12 h light with approximately 162 μE m^{-2} s^{-1} provided by cool-white fluorescent lamps. Pots were placed in water-filled plastic trays to maintain the relative humidity inside the growth chamber at approximately 85% for the remainder of the experiment. Inoculated and non-inoculated plants were submitted to an initial 24 h dark period and 48 h after inoculation the plastic bags were removed from the plants.