The rate of entry of Magnaporthe oryzae into pen2 mpk6 plants was higher than that into pen2 plants. The infection hyphae in the pen2 mpk6 plants were longer than those in the pen2 plants. The proportion of branched hyphae development in the pen2 mpk6 plants was higher than that in the pen2 plants. These results suggest that MPK6 functions in both penetration and post-penetration resistance to M. oryzae in Arabidopsis thaliana.

Key words: non-host resistance; penetration; post-penetration; Magnaporthe oryzae

Rice blast, caused by Magnaporthe oryzae, is a devastating disease of rice. The mechanisms of rice resistance to blast have been studied extensively, and the rice-M. oryzae pathosystem has become a model system in plant-microbe interaction studies, but the mechanisms of non-host resistance (NHR) to blast in other plants remain poorly understood.

Arabidopsis mutants with altered non-host interactions following Blumeria graminis hordei (Bgh) infection were described recently, and three genes were identified: PENETRATION 1 (PEN1), PEN2, and PEN3. PEN1 encodes a plasma membrane–anchored syntaxin with a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) domain. PEN2 encodes an atypical myrosinase involved in glucosinolate metabolism in defense responses. PEN3 encodes a pleiotropic drug resistance (PDR) ATP-binding cassette (ABC) transporter. Collectively, these studies demonstrate that Arabidopsis NHR to non-adapted biotrophic powdery mildews has two successive multicomponent defense layers: penetration and post-penetration resistance. We have found that PEN2, PMR5, AGB1, and MLO2 are involved in both penetration and post-penetration resistance to M. oryzae in A. thaliana.

Plants have evolved effective basal defense systems to detect and limit the growth of pathogens. Pathogens can be recognized by the host via the perception of conserved microbial structures called pathogen-associated molecular patterns (PAMPs). PAMPs are recognized by transmembrane pattern recognition receptors (PRRs) that bind specific PAMPs and initiate intracellular immune responses. These PAMP-triggered immunity (PTI) responses include the generation of reactive oxygen species (ROS) and protein phosphorylation with associated gene regulation, which ultimately restricts the growth of the pathogens.

Mitogen-activated protein (MAP) kinase cascades play important roles in plant immunity. A MAP kinase cascade consists of a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPK), and a MAP kinase (MPK). Signals from upstream receptors are transduced and amplified through the MAP kinase cascade. The best-characterized MPKs are MPK3, MPK4, and MPK6, each of which is activated by a diversity of stimuli, including abiotic stresses, pathogens, and oxidative stress in A. thaliana. While MPK4 negatively regulates biotic stress signaling, MPK3 and MPK6 act as positive mediators of defense responses. However, it remains to be clarified whether MPK4 and MPK3/MPK6 are involved in NHR to M. oryzae in A. thaliana.

In this study, we examined the function of MPKs in NHR to penetration and hyphal growth of M. oryzae in A. thaliana.

Arabidopsis plants were grown under short-day conditions (9:15 L:D) at 22°C in a growth room, as previously described. We used the following mutants: pen2-1, mpk4-2 (SALK_056245), and mpk6-3 (SALK_127507) (all with the Col-0 background). These mutants were used for crosses, and double mutants were identified as previously described. Fungal inoculation was conducted as previously described. Quantification of cell entry and fungal growth were analyzed as previously described. The significance of differences between mean values of cell entry and fungal growth were assessed by one-way analysis of variance.

To determine whether MPK4 and MPK6 cascades would affect NHR to M. oryzae in A. thaliana, mpk4 and mpk6 mutants were inoculated with M. oryzae and monitored by microscopy. Analysis of the mpk4 and mpk6 mutants following M. oryzae challenge revealed that both mutants exhibited levels of penetration resistance similar to that of the wild-type plants (data not shown). At the next step, double mutants were generated between the pen2 and mpk mutants to evaluate the roles of MPKs in the NHR to M. oryzae. We found that pen2 mpk4 was lethal under our growth conditions. Our analysis was therefore restricted to the pen2 mpk6 mutant. We harvested leaves of infected plants at 48 h post-inoculation (hpi) and examined them microscopically. Consistently with our previous observations, the entry rate into pen2 plants was higher than the rate into wild-type plants (Fig. 1A). The entry rate into pen2 mpk6 plants was higher than the rate into pen2
plants (Fig. 1A). This suggests that MPK6 had a function in penetration resistance against *M. oryzae* in *pen2* background.

We investigated the role of MPK6 in post-penetration resistance further by measuring the lengths of the longest infection hyphae in the double mutant at 48 hpi (Fig. 1B). The infection hyphae in the *pen2 mpk6* plants were longer than those in the *pen2* plants (Fig. 1B). Subsequently, we examined the penetration process in the *pen2 mpk6* double mutant. We divided the process into four events (i–iv) as previously described: 1) cell-wall penetration, 2) the establishment of infection hyphae, 3) elongation of the infection hyphae, and 4) branch formation on the infection hyphae (Fig. 2). Short (<10 μm) infection hyphae (type ii) were produced much less frequently in the *pen2 mpk6* plants (Fig. 2). In contrast, the proportion of branched hyphae development (type iv) in the *pen2 mpk6* plants was significantly (p < 0.05) higher than that in the *pen2* plants (Fig. 2 and Fig. 3). These results indicate that MPK6 played a role in post-penetration resistance against *M. oryzae* in *pen2* background.

In conclusion, MPK6 was probably involved in penetration and post-penetration resistance as a positive regulator to *M. oryzae* in *A. thaliana*.

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**Fig. 1.** Quantitative Analysis of Non-Host Resistance to *M. oryzae* in Arabidopsis Mutants.

**A.** Mean frequency of *M. oryzae* penetration into Arabidopsis mutants 48 h post-inoculation (hpi) expressed as a percentage of the total number of infection sites. **B.** Mean length of infection hyphae was measured at 48 hpi. Values are means ± standard errors (n = 6) using three biological replicates. Bars sharing the same lowercase letters are not significantly different (p = 0.05).

**Fig. 2.** Quantitative Analysis of Post-Penetration Resistance to *M. oryzae* in Arabidopsis Mutants.

Mean frequencies of infection site types on Arabidopsis mutants at 48 hpi, expressed as percentage of penetrated cells. The penetration process was divided into four events (i–iv): i, successful penetration without infection hyphae; ii, successful penetration with short (<10 μm) infection hyphae; iii, successful penetration with long (>10 μm) infection hyphae; and iv, successful penetration with branched hyphae. Values are means ± standard errors (n = 6) using three biological replicates. Within each type, bars sharing the same lowercase letters are not significantly different (p = 0.05) as between the *pen2* and the *pen2 mpk6* plants.

**Fig. 3.** Microscopic View of Infection Sites in Arabidopsis Mutants.

**A.** Light microscopic view of infection sites of the *pen2* plants at 48 hpi. **B.** Cell death-associated autofluorescence at infection site of (A) was viewed by fluorescence microscopy. **C.** Light microscopic view of the infection sites of *pen2 mpk6* plants at 48 hpi. **D.** Cell death-associated autofluorescence at infection site of (C) was viewed by fluorescence microscopy. ap, appressorium; ih, infection hyphae. Bars = 50 μm.

**References**


