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# Nonhost resistance to *Magnaporthe oryzae* in *Arabidopsis thaliana*

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**Rice blast, caused by *Magnaporthe oryzae*, is a devastating disease of rice (*Oryza sativa*). The mechanisms involved in resistance of rice to blast have been studied extensively and the rice—*M. oryzae* pathosystem has become a model for plant—microbe interaction studies. However, the mechanisms involved in nonhost resistance (NHR) of other plants to rice blast are still poorly understood. We have recently demonstrated that AGB1 and PMR5 contribute to PEN2-mediated preinvasion resistance to *M. oryzae* in *Arabidopsis thaliana,* suggesting a complex genetic network regulating the resistance. To determine whether other defense factors: RAR1, SGT1 and NHO1, affected the *A. thaliana-M. oryzae* interactions, double mutants were generated between *pen2* and these defense-related mutants. All these double mutants exhibited a level of penetration resistance similar to that of the *pen2* mutant, suggesting that none of these mutants significantly compromised resistance to *M. oryzae* in a *pen2* background.**

**Key words:** nonhost resistance, PEN2, RAR1, SGT1, NHO1

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Plants face microbial attacks and have evolved innate immunity systems to defend against these threats. The initial step of the immunity signaling pathway is recognition of intra- or extracellular pathogen-derived molecules. Externally oriented transmembrane-type proteins containing leucine-rich repeat (LRR) domains detect extracellular molecules, whereas cytoplasmic sensors possess nucleotide-binding (NB) and LRR domains (NLR).[1,2] The LRR domain serves as a pattern-recognition receptor to detect pathogen-derived molecules or host proteins that are targeted by pathogen peptides that have entered the cell, effectors.[3] NLR-type sensors are the substrates of a structurally and functionally conserved chaperone complex that consists of HEAT SHOCK PROTEIN 90 (HSP90) and its cochaperone SUPPRESSOR OF THE G2 ALLELE OF SKP1 (SGT1). REQUIRED FOR MLA12 RESISTANCE 1 (RAR1) regulated the HSP90-SGT1 complex, resulting in the stabilization of NLR proteins. Thus, SGT1 and RAR1 are required for the function of multiple and distinct R genes that encode NLR immune sensors in plants.[4] Experiments in RAR1-silenced transgenic rice lines showed that RAR1 is not essential for *Pib*, which encodes an NLR against rice blast fungus.[5] In contrast, basal resistance to normally virulent races of rice blast fungus or bacterial blight is significantly reduced in RAR1-silenced lines. This result is consistent with earlier reports that RAR1 is involved in basal resistance to virulent Pseudomonas bacteria in Arabidopsis or blast fungus in barley.[6,7] The requirement of SGT1 for immunity in plants is shown mostly by transient silencing of a number of NLR proteins.[8,9] In addition, SGT1 is also required for immune responses triggered by non-NLR-type sensors.[10] This requirement indicates that either SGT1 function is not limited to the NLR sensors, or some unknown SGT1-dependent NLR proteins also operate downstream of non NLR-type sensors. Furthermore, SGT1 is involved in nonhost resistance, indicating that SGT1 may be a general factor of disease resistance.[10] An Arabidopsis mutant, *nho1* (*nonhost resistance 1*), has been isolated on which *Pseudomonas syringae* pv. *phaseolicola* grows and causes disease symptoms.[11,12] It is significant that this mutant is also compromised in

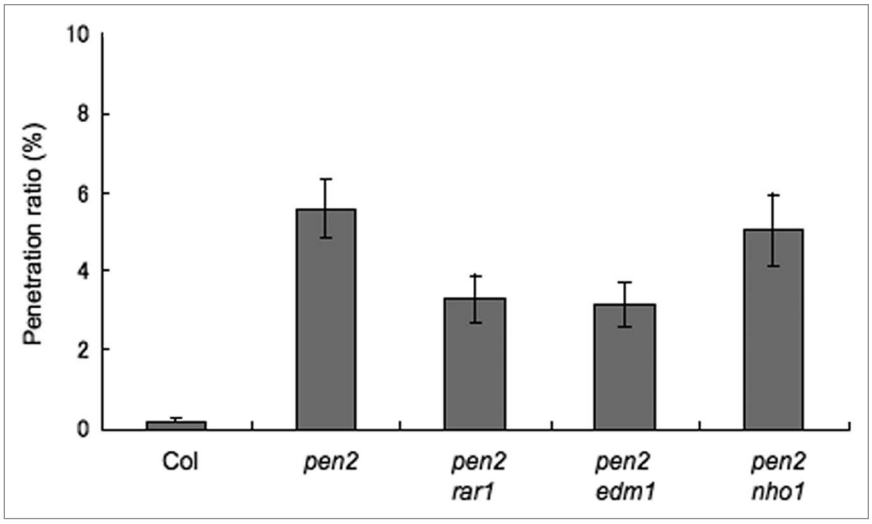


**Figure 1.** Double mutant analysis to evaluate the role of the defense related genes on resistance to *Magnaporthe oryzae* in *Arabidopsis thaliana*. The frequency of *M. oryzae* penetration on double mutants at 3 days post-inoculation was expressed as a percentage of total appressoria. Data were collected from six independent plants per line. A minimum of 100 infection sites was inspected per leaf. Results represent mean ± standard error of three independent experiments.

R-gene-mediated resistance to *P. syringae*.[11] Although NHO1 is the flagellin-induced glycerol kinase, whose exact function in NHR remains elusive.[12,13] A possible explanation might be that altered plant glycerol pools either directly or indirectly affect nutrient availability for *P. syringae*. NHO1 is also required for resistance to the fungal pathogen *Botrytis cinerea*, indicating that NHO1 is not limited to bacterial resistance.[12] However, these contributions to NHR to *M. oryzae* in *A. thaliana* have not been understood.

To determine whether these factors were necessary for the resistance to *M. oryzae* in *A. thaliana*, the following *A. thaliana* mutants were inoculated with *M. oryzae* and monitored by microscopy: *rar1-21*;[14] *edm1-1*;[15] *nho1-1*,[11] (all Col-0 background). All these mutants exhibited a level of penetration resistance similar to that of the wild-type plants (data not shown), suggesting that none of these mutants significantly compromised resistance to *M. oryzae*. We have recently shown that among the *penetration* (*pen*) mutants, only the *pen2*,[16] mutant allowed increased penetration into epidermal cells by *M. oryzae*.[17] Thus, double mutants were generated between *pen2* and these mutants to determine whether these factors were necessary for the resistance to *M. oryzae* in a *pen2* background: *pen2 rar1-21*; *pen2 edm1-1*; *pen2 nho1-1*. All these double mutants exhibited a level of penetration resistance similar to that of the *pen2* mutant (**Fig. 1**), suggesting that none of these mutants significantly compromised resistance to *M. oryzae* in a *pen2* background. This might indicate that NHR against *M. oryzae* may not be conferred by RAR1- and SGT1-dependent NLR immune sensors. Alternatively, since there has been no report that RAR1 is required for any known transmembrane sensors, such as FLS2, EFR or Xa21, RAR1- and SGT1-independent transmembrane-type immune sensors may be required for NHR against *M. oryzae*. Future studies will be required to reveal the genetic and mechanistic requirements for NHR in *A. thaliana-M. oryzae* interactions.

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