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Transposon-mediated NLR exile to the pollen allows rice blast resistance without yield penalty

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Introduction

Disease resistance (*R*) genes are of major importance in crop protection and breeding as they prevent potentially dramatic yield loss by plant pathogens. Most *R* genes encode immune receptors of the nucleotide-binding and leucine-rich repeat (LRR) domain protein (NLR) family¹. They act inside plant cells by recognizing virulence factors called effectors that pathogens introduce into host cells to promote disease.

Despite their importance in crop protection, NLRs are no silver bullets against pathogens and their use is bound by two principal factors. First, their durability is frequently limited. Due to high population sizes and particular genomic features that increase the mutation rate in virulence-related genes, pathogens can evolve extremely rapidly. As a consequence, pathogen populations adapt to the high selection pressure imposed by NLRs by losing or mutating recognized effector genes, thereby overcoming NLR-mediated resistance. In addition, NLRs can induce fitness costs to the plant that result in yield reduction. Such fitness costs are predicted by evolutionary theory and have been documented experimentally². However, the underlying mechanisms are not clearly understood. They might arise from residual auto-activity of NLRs inducing more or less strong and costly autoimmune responses³.

In crop breeding, the goal is to maximize yield and disease resistance. In this spotlight, we highlight an elegant case of NLR-mediated durable resistance in rice, which is effective against the devastating fungus *Magnaporthe oryzae*, but does not involve yield penalty. The genetic and molecular dissection of this broad-spectrum resistance has unraveled a fascinating epigenetic regulatory mechanism balancing blast resistance and yield that opens exciting new perspectives for crop improvement.

A cluster of duplicated NLRs confers durable resistance to rice blast

In their recent paper, Deng *et al.* show that one single NLR-coding gene, *PigmR*, confers Pigm blast resistance in the rice variety Gumei 4 (GM4)⁴. *PigmR* is located within a complex locus on rice chromosome 6 that carries, depending on the rice variety, up to 13 *NLRs* of up to 6 different classes, most of which are pseudogenized (Figure 1A). In other rice varieties, this locus harbors

the previously cloned blast *R* genes *Pi-2*, *Pi-9*, *Pi50* or *Piz-t* that are extremely similar to *PigmR*. Indeed, their products share ~95% identity with *PigmR*. Such complex *NLR* loci containing multiple copies of *NLRs*, many of which are inactivated by transposons, partial deletion or silencing, and possessing varying recognition specificities in different accessions of the same plant species, are quite common. They arise from the evolutionary arm race that is frequently engaged between plants and their pathogens and that makes *NLRs* the gene family with the highest diversity in plant genomes. The evolution of these loci is driven by multiple mechanisms such as duplication, recombination or transposon insertion.

Deng *et al.* demonstrate that *Pigm* resistance is effective against all tested *M. oryzae* isolates originating from very diverse Chinese and worldwide collections. Field data also support the durability and large spectrum of *PigmR* as it has been protecting rice from blast since more than 30 years in different varieties cultivated on large surfaces. This remarkable durability is unexpected and not understood since all other *NLR*-coding blast *R* genes and in particular the highly similar *Pi2*, *Pi9*, *Pi50* or *Piz-t*, are defeated. An explanation may reside in potential features and particularities of the uncharacterized AVR-*Pigm* effector recognized by *PigmR*. Indeed, AVR-*Pigm* might be widely distributed and particularly important for fungal fitness and/or could present limited possibilities for evolution. Alternatively, *PigmR* might recognize multiple effectors, either directly, or by surveillance of a crucial element of rice immunity targeted by these effectors.

Interestingly, the *Pigm* locus of GM4 carries two other *NLRs* highly homologous to *PigmR*, *R4* and *R8*. *R4* shares 99% identity with *PigmR* and they differ by only 4 residues in the LRR domain. The expression pattern of *R4* remains elusive but it seems neither to contribute nor to interfere with *Pigm* resistance. *R8* is slightly more diverse than *R4* and shares 96% identity with *PigmR*. Due to heavy methylation of its promoter that contains two tandem miniature transposons (MITEs), *R8* is not expressed in the leaves or any other plant tissue with the exception of pollen, where the level of promoter methylation is much lower than in the leaves. Ectopic expression of *R8* throughout the plant, either by inactivation of the RdDM (RNA-dependent DNA-Methylation) pathway or by expression under the *PigmR* promoter or a strong constitutive promoter, represses *PigmR*-mediated resistance. Therefore, *R8* was named *Pigm Susceptible (PigmS)*. This

negative interference was also observed in the heterologous *Nicotiana benthamiana* system where PigmR, but not PigmS, triggers an autonomous cell death response that is abolished by co-expression of PigmS. This repression could rely on the formation of PigmR-PigmS hetero-complexes that are detected upon co-expression of PigmS and PigmR and that involve association of the coiled-coiled domains. These hetero-complexes might prevent the formation of signaling-competent PigmR homo-complexes potentially required for cell death induction. Indeed, for several NLRs, formation of homo-complexes is a crucial step during activation and induction of downstream signaling⁵. Association of the CC domains is particularly important in this process and also documented for PigmR. Interestingly, constitutive expression of *PigmS* in rice, in the absence of PigmR, reduces basal resistance to the blast fungus. This suggests that PigmS does not only suppress PigmR but also affects other components of rice immunity. These components could be uncharacterized homologous NLRs, which might contribute to partial blast resistance, and would be inactivated by formation of inactive hetero-complexes with PigmS.

Similar negative interactions between highly homologous NLRs involving the formation of inactive hetero-complexes have previously been documented for the powdery mildew resistance NLR Pm3 from wheat^{6,7}. Indeed, the combination of *Pm3* alleles with different recognition specificities or of *Pm3* with *Pm8* from rye leads to loss of resistance. This highlights a fundamental problem with the co-expression of highly homologous NLRs in plants and has certainly tremendous importance for the evolution of complex *NLR* loci, where duplication and subsequent diversification of NLR-coding genes is a major evolutionary mechanism. In the case of *PigmR*, the conflict has been solved by restricting *PigmS* expression to the pollen. However, why has *PigmS* in that case not been completely inactivated like the other *NLRs* of the locus?

NLR regulation: balancing disease resistance with yield

Deng et al. found an answer to this question when they analyzed the yield penalty caused by *PigmR* and found that it is balanced by *PigmS* expression in the pollen. Indeed, *PigmR* expression, in the absence of PigmS, decreased yield through a reduction of grain weight and size (Figure 1B). The mechanism is unknown but it could be a consequence of weak autoimmune responses in the grain or the mother plant and influencing negatively grain

development and/or grain-filling. *PigmS* compensates this loss by increasing the number of seeds per plant and this occurs in the presence and in the absence of *PigmR* (Figure 1B). Given the pollen specific expression of *PigmS*, this may rely on increased vitality or fertilization potential of *PigmS* pollen resulting in higher fecundation and seed setting. Such an activity could again be related to weak autoimmune phenotypes caused by NLRs in pollen eventually related to activation of programmed cell death in the tapetum that surrounds the microsporocytes and partially repressed by the formation of hetero-complexes between *PigmS* and homologous NLRs. However such mechanistic interpretations remain speculative since a potential impact of *PigmS* on pollen development or vitality remains to be investigated.

Conclusion and outlook:

To ensure enough food for the ever growing human population that will have further increased by 25% in the next 30 years to reach 9 billion people in 2050, crop yield has to increase tremendously. The development of high yielding varieties, resilient to biotic and abiotic stresses, is a key issue for this and can only be achieved with a better understanding of the trade-offs between immunity, growth and yield. In the case of *PigmR*, blast resistance and balanced yield is achieved through epigenetic-mediated 'exile' of *PigmS* to the pollen. Further investigation is now required to understand at the cellular and molecular level how *PigmS* acts in the pollen. In addition, the elucidation of the reasons for the exceptional durability of *Pigm* resistance are a priority because this promises to teach us how an NLR can confer complete disease resistance without selecting resistance breakdown by rapid adaptation of pathogen populations.

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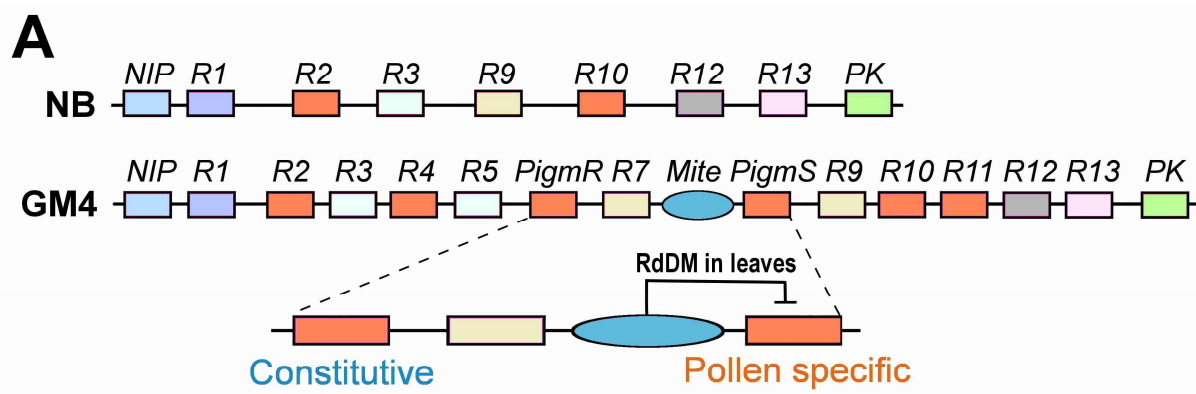
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Figure legend:

Figure 1: The *Pigm* locus confers rice blast resistance with no yield penalty. A) Genomic organization of the *Pigm* locus on chromosome 6 in the susceptible (*pigm*-) cultivar Nipponbare (NB) and in the *Pigm* resistant variety Gumei 4 (GM4). Identical colors indicate homology relationships between genes. *PigmR* is constitutively expressed in all rice tissues while *PigmS* expression is pollen specific due to RNA-directed DNA methylation (RdDM) of its promoter in other plant tissues. B) In the absence of *PigmS*, constitutively expressed *PigmR* (*C_PigmR*) carries yield penalty since *PigmR* plants produce smaller grains than the wild type. Constitutively expressed *PigmS* (*C_PigmS*) alone improves seed setting resulting in higher yield, but it also

increases susceptibility to rice blast leading to high yield loss under disease. In wild type *Pigm* resistant plants, *PigmR* is constitutively expressed and induces blast-resistance while *PigmS* expression is pollen specific (*PS_PigmS*) and compensates, by increasing seed setting, the yield penalty caused by *PigmR*. As a result, plants produce smaller seeds than susceptible *pigm*-plants but in larger number and therefore no yield loss is observed. When *PigmR* and *PigmS* are both expressed constitutively, *PigmS* partially suppresses *PigmR* resistance leading to blast susceptibility. It will be interesting to measure the productivity of these plants to see whether suppression of *PigmR* resistance relieves yield penalty. Overall, in terms of yield, the wild type *Pigm* resistant plants are optimal as indicated by a grey background.



B	NB (<i>pigm</i> -)	<i>C_PigmR</i>	<i>C_PigmS</i>	<i>C_PigmR</i> <i>PS_PigmS</i>	<i>C_PigmR</i> <i>C_PigmS</i>
	WT yield	Yield penalty	Yield ++	No yield loss	Yield + (?)
Non-infected					
	WT yield	Yield penalty	Yield ++	No yield loss	Yield + (?)
Infected					
	Susceptible	Resistant	Susceptible	Resistant	Susceptible