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Evolution of compatibility range in the rice-*Magnaporthe oryzae* system: an uneven distribution of R genes between rice subspecies

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Abstract

Efficient strategies for limiting the impact of pathogens on crops require a good understanding of the factors underlying the evolution of compatibility range for the pathogens and host plants, *i.e.* the set of host genotypes that a particular pathogen genotype can infect and the set of pathogen genotypes that can infect a particular host genotype. Until now, little is known about the evolutionary and ecological factors driving compatibility ranges in systems implicating crop plants. We studied the evolution of host and pathogen compatibility ranges for rice blast disease, which is caused by the ascomycete *Magnaporthe oryzae*. We challenged 61 rice varieties from three rice subspecies with 31 strains of *M. oryzae* collected worldwide from all major known genetic groups. We determined the compatibility range of each plant variety and pathogen genotype and the severity of each plant-pathogen interaction. Compatibility ranges differed between rice subspecies, with the most resistant subspecies selecting for pathogens with broader compatibility ranges and the least resistant subspecies selecting for pathogens with narrower compatibility ranges. These results are consistent with a nested distribution of R genes between rice subspecies.

Keywords

Rice, *Oryza sativa*, *Magnaporthe oryzae*, host range, compatibility range, resistance, R genes, specialization, nestedness, generalist/specialist trade-off, infection severity.

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Introduction

Plant pathogens pose a major threat to agriculture, and their emergence on new crop varieties may occur in two different ways (Parker & Gilbert, 2004). The first is an extension of host compatibility range (see definitions in the Material & methods section) for pathogens jumping from one host species (or genotype) to a new one (de Vienne *et al.*, 2009). The second operates at the intraspecific level and involves the accidental introduction of an allopatric pathogen genotype that occurs to be highly pathogenic on local plants (Barrès *et al.*, 2008; Fontaine *et al.*, 2013; Garbelotto *et al.*, 2013). Understanding the factors underpinning the evolution of compatibility range in host plants and pathogens could permit a better prediction and control of pathogen emergences.

The molecular (*i.e.* proximate) determinants of host-pathogen compatibility have been extensively studied. In addition to nonspecific host defense mechanisms (PAMP-triggered immunity), host-pathogen compatibility has been shown to be tightly related to host-pathogen recognition systems (Jones & Dangl, 2006), through the well known complete resistance mechanism. In this gene-for-gene model (Flor, 1956), the ability of a plant to prevent a pathogen from causing an infection is governed by Resistance (R) genes, which encode proteins recognizing pathogen effectors (encoded by “avirulence” genes). When the product of the R gene recognizes the pathogen effector, a hypersensitive response (HR) is triggered, blocking the entry of the pathogen into the plant. In addition to this complete resistance mechanism, the specificity of host-pathogen interactions is modulated by a second, less well understood mechanism of plant resistance known as partial or quantitative resistance (Poland *et al.*, 2009). Hosts displaying partial resistance are infected, but display less severe damage than susceptible plants. Partial resistance is probably determined by disparate molecular mechanisms, including nonspecific resistance mechanisms (e.g. thicker cell walls, the production of chitinase or detoxifying enzymes, see Fukuoka *et al.* (2009) and Parker and Gilbert (2004)) and partial breakdown of the resistance conferred by R genes (Nass *et al.*, 1981; Brodny *et al.*, 1986; Li *et al.*, 1999; Poland *et al.*, 2009). However, it remains unclear whether this last phenomenon is general or limited to a few R genes. If overcoming R genes is frequently involved in partial resistance in plants, then we would expect a positive correlation between complete and partial resistance. Plants with

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few R genes would show very low partial resistance and would display broad compatibility range, while plants with many R genes would be expected to have a narrow compatibility range and a high level of partial resistance. By contrast, if partial resistance is mediated principally by nonspecific resistance mechanisms (e.g. thicker cell walls, *etc.*), there should be no correlation unless there is a trade-off between these traits, but we are not aware of such a constraint in rice or other species. To our knowledge, such correlation has never been looked at in rice.

The eco-evolutionary (*i.e.* ultimate) determinants of host-pathogen compatibility have also been studied. Evolutionary factors, such as local adaptation trade-offs (Agudelo-Romero *et al.*, 2008; Wallis *et al.*, 2008) or the phylogenetic distance between plant species (Gilbert & Webb, 2007; de Vienne *et al.*, 2009; Ness *et al.*, 2011; Poulin *et al.*, 2011; Schulze-Lefert & Panstruga, 2011), and ecological factors, such as the spatial distribution of host populations and the rates of dispersal of the host and pathogen (Thrall & Burdon, 2002), have been shown to influence host-pathogen compatibility.

Despite the large body of work on both molecular and eco-evolutionary determinants of host-pathogen compatibility range, some results remain elusive. For instance, many cross-inoculation experiments reported that some pathogens could infect more plant genotypes than the number of genotypes to which they were exposed to, *i.e.* that their fundamental compatibility range was larger than their realized compatibility range (de Vienne 2009, and references therein). Some studies also observed particular local adaptation patterns (hereafter called the asymmetric pattern of compatibility range) in which highly resistant plant genotypes (specialists with narrow compatibility range) were associated with generalist pathogens (showing wide compatibility ranges), and reciprocally less resistant plant genotypes (generalists) were associated with pathogens showing narrow compatibility ranges (specialists) (Thrall & Burdon, 2003; de Vienne *et al.*, 2009). At the inter-specific level using several species of *Microbotryum* and Caryophyllaceae, De Vienne and colleagues (2009) found that both host and pathogen phylogenies were good predictors of compatibility range. They hypothesized that the asymmetric pattern of compatibility range they observed, was resulting from hot spots and cold spots of coevolution (de Vienne *et al.*, 2009). Thrall & Burdon (2003) made similar observations at the intra-specific level with the *Linum marginale*-

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Melampsora lini system. They showed that R genes were the main molecular driver of compatibility range, and that the gene-for-gene coevolutionary process between resistance genes in plants and avirulence genes in the pathogen was responsible of the asymmetry in compatibility range. Plant genotypes with many R genes (specialists) were selecting generalist fungal pathogens able to overcome all these resistances, while plant genotypes with few R genes (generalists) were selecting specialist pathogens. Whether a phylogenetic effect (de Vienne *et al.*, 2009) could explain this observation at the intraspecific level could not be verified because, (*i*) Thrall & Burdon (2003) work is anterior to de Vienne *et al.* (2009) and (*ii*) because Thrall & Burdon (2003) studied natural populations in which the genotypic composition was unknown. Thus, both of these studies provided key information to explain the origin of the asymmetry in compatibility range, even though the generality of this phenomenon remains to be proven by studying other plant-pathogen systems.

In this work, we aimed at establishing whether R genes could be responsible for the strong phylogenetic signal of compatibility ranges as observed by de Vienne and colleagues (2009). Specifically, we tested the impact of R gene distribution in rice varieties on the structure of the interaction network with various genotypes of the blast pathogen at the intraspecific level (Figure 1). Rice blast, caused by the ascomycete fungus *Magnaporthe oryzae,* is a good system to address the effect of R gene distribution on the evolution of host and pathogen compatibility ranges. First the rice species is composed of several subspecies, which phylogenetic history is well known (Huang *et al.*, 2012). Secondly the worldwide population genetic structure of *M. oryzae* has recently been described and its relation with the history of the rice-*M. oryzae* system has been established (Saleh *et al.*, 2014). Thirdly, the molecular determinants of rice compatibility range have been studied extensively (Ballini *et al.*, 2008; Vergne *et al.*, 2008), and differential varieties (varieties for which R gene contents are known) containing specific R genes are available, allowing testing the relationship between compatibility ranges and R gene contents.

We investigated the factors underlying the evolution of compatibility range with the rice-*M. oryzae* system, by characterizing rice-*M. oryzae* compatibility range fully, at the scale of the rice species. We carried out a large-scale cross-inoculation experiment, in which we assessed the ability of 31 fungal strains

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to infect 61 rice varieties. All fungal strains, and 38 varieties were chosen so as to represent the genetic clusters of *M. oryzae* that have spread around the world (*japonica* and *indica*) and the three main rice subspecies (*indica*, temperate *japonica*, tropical *japonica*). The other 23 varieties were differential varieties. We used these latter varieties to estimate the number of R genes that each fungal strain could overcome.

Materials and methods

Definitions

Compatibility range: For pathogens, compatibility range represents the proportion of host genotypes that a particular pathogen genotype can infect (*i.e.* the average infection success of the fungal strain). Reciprocally, host's compatibility range represents the proportion of pathogen genotypes that can infect a particular host genotype.

Severity score: This score evaluates the severity of infection, following the inoculation of rice plants with a *M. oryzae* strain. Infection severity was estimated on 20 to 25 plants, according to a scale extending from 1 to 6 (Silué *et al.*, 1992). A score of 1 indicates unsuccessful infection, and a score of 2 indicates HR-controlled infection (both of which are considered to correspond to complete resistance). Scores of 3 to 6 indicate successful infections of increasing severities (partial resistance). Each score describes a specific stage of lesion formation: 1 = no lesion (unsuccessful infection), 2 = black dots (infection controlled by the HR response), 3 = round lesions with differentiated centers less than 1 mm in diameter, 4 = round lesions with differentiated centers more than 1 mm in diameter, 5 = typical diamond-shaped susceptible lesions with a brown margin, 6 = typical diamond-shaped susceptible lesions with no brown margin (Figure S1). Severity score does not provide an estimate of aggressiveness, as it does not take into account the number of lesions on the plant. Severity score simply reflects the "immunological" state of the plant, and the extent to which it is able to control the infection. Scores from 3 to 6 provide an estimate of infection severity (*i.e.* of partial resistance).

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Studied rice varieties and pathogen strains

Two experiments were performed in this study. In the local adaptation experiment, the rice varieties and fungal strains were chosen so as to represent the three main rice subspecies (*indica*, temperate *japonica*, tropical *japonica*; Figure S2) and the two genetic clusters of *M. oryzae* that have spread worldwide (*i.e.* clusters B and C, referred to hereafter as the *japonica and indica* clusters, respectively) (Saleh *et al.*, 2014). Most of the rice accessions (other than differentials) were chosen to be, as much as possible, representative of the genetic diversity of the main rice subgroups. In a core collection containing mainly traditional varieties (Garris *et al.*, 2005), we selected randomly a given number of varieties in the three main subgroups. We used a sample of 31 fungal strains: 17 *indica* strains, collected from *indica* rice varieties; 14 *japonica* strains collected from *japonica* rice varieties (genetic cluster B), 10 of which were collected from temperate *japonica* rice varieties, the other four being collected from tropical *japonica* rice varieties (Saleh *et al.*, 2014). We used these strains to challenge 38 rice varieties: 22 *indica*, 8 temperate *japonica* and 8 tropical *japonica.* In a second experiment aiming at evaluating the number of R genes overcome by the fungal strains used in the first experiment, we used the 31 fungal strains described above to inoculate 23 differential varieties with known resistance genes (differentials; listed in Table S1).

Cross-inoculation experiments

Design: The two experiments were performed at the same time. All rice varieties were inoculated with all fungal strains, following the protocol described by Sallaud *et al.* (2003) and illustrated in figure S3. Briefly, rice seeds were sown in peaty soil in plastic trays and plants were grown in the greenhouse until the 4-5 leaf stage. The 61 varieties were grown in four trays, with 16 varieties per tray (16 x 4 = 64 but three varieties were excluded from the analysis because their origins were not clear). For each variety, we obtained 20 to 25 plants. Plants were inoculated by spraying 30 mL of spore suspension/tray. The suspension contained 25,000 spores/mL and was supplemented with 0.5% gelatin. At each date, six to eight independent inoculations were performed (an inoculation corresponding to the spraying of a particular strain on all 61 varieties). We always inoculated all 61 varieties together, to avoid complex “date x variety” effects. In this experiment, the experimental unit corresponds to a group of 20 to 25 plants of a specific

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variety, inoculated with a specific strain (*e.g. M. oryzae* A x rice variety 1, in figure S3). Each experimental unit was replicated two to three times, at different dates. The complete design required the inclusion of 20 inoculation dates. Symptoms were scored seven days after inoculation (one score per experimental unit, *i.e.* per group of 20 to 25 inoculated plants).

We measured the host and pathogen compatibility ranges respectively by determining the proportion of host varieties that could be infected with a particular fungal strain/group, and the proportion of fungal strains capable of infecting a particular plant variety/group of varieties. We initially pooled lesions in two categories: incompatible interactions (lesions with severity scores of 1 and 2, see the scale description above), and compatible interactions (lesion with severity scores of 3 to 6). We then calculated the proportion of compatible interactions by dividing the number of compatible interactions by the total number of crosses, for each rice or *M. oryzae* genotype. We also calculated the mean severity score for a given plant variety, for successful infections (severity scores of 3 to 6).

Statistics

Inoculation onto cultivated rice varieties

For the local adaptation analysis, the 38 plant varieties sampled worldwide were inoculated with the 31 fungal strains. The success of infection (used to estimate compatibility ranges), which is a binary response (infection or no infection) was analyzed using a generalized linear model with a binomial error structure and logit link function, including rice variety and fungal strain as main effects, and the variety x strain interaction. Contrasts were used to assess the specific degrees of freedom of main effects and interactions.

Inoculation onto differential rice varieties

We analyzed the number of R genes overcome by *M. oryzae* strains, by inoculating a subset of 23 differential varieties of known R gene content with the 31 fungal strains. We considered 19 R genes in this study (some varieties carried two resistance genes and some genes were present in several different varieties). When a given pathogen strain was able to infect the variety considered (*i.e.* a compatible interaction was observed), we considered the strain to be able to overcome all the R genes carried by the

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variety. After the inoculation of all differential varieties with a particular fungal strain, we could determine the total number of R genes overcome by this strain. In total, we used 1426 interactions (31 strains x 23 differential varieties x 2 replicates). We used a generalized linear model with a binomial error structure and logit link function, including fungal strain group as a main effect, to analyze these data.

Nestedness computation and significance

A network is considered as nested if the host or pathogen compatibility range of species/individuals is a subset of the host or pathogen compatibility range of more generalist species/individuals. Nestedness was estimated by calculating the nestedness metric based on overlap and decreasing fill (NODF) index developed by Almeida-Neto *et al.* (2008). This index relates to the proportion of interactions common to species pairs over the network and has been shown to have good statistical properties.

We determined whether the nestedness value obtained for our dataset was significant, by comparing it with those of interaction matrices generated under a null model (mgen null model in bipartite package of R software). In the null model used, the probability of an interaction between a pathogen strain and the rice variety tested is proportional to the product of compatibility range, such that strains/varieties with high compatibility range have a higher probability of being assigned to an interaction than strains/varieties with low compatibility range. Such null model thus conserves the heterogeneity of compatibility range observed in the original dataset.

Results

Local adaptation and nested model of interaction

We determined the compatibility range (proportion of compatible interactions) of the various groups of *M. oryzae* strains with the three rice subspecies (Figure 2). We found significant effects of plant variety group, fungal strain group and the interaction between these factors (Table 1). The plant variety group effect was due to temperate *japonica* varieties being susceptible to a larger number of strains (wider compatibility range) than the *indica* and tropical *japonica* varieties (contrast: temperate *japonica* vs. *indica* + tropical *japonica* z-value=10.894 $p<2 \times 10^{-16}$), for which compatibility ranges were similar (contrast: *indica*

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vs. tropical *japonica* *z*-value=-0.156 *p*=0.876). The strain group effect could be explained by two main observations: 1) the *indica* strains had the highest infection frequencies on hosts of any group, 2) the temperate *japonica* strains had the lowest infection frequencies on *indica* and tropical *japonica* plants. Finally, the host plant group x fungal strain group interaction could be explained by observations consistent with local adaptation patterns: temperate *japonica* strains mostly infected temperate *japonica* varieties, tropical *japonica* strains infected tropical *japonica* varieties more frequently than *indica* varieties and *indica* strains infected *indica* varieties more frequently than tropical *japonica* varieties (contrast *z*-value 2.839 *p*=0.005).

Most *indica* strains acted as generalists, infecting most varieties. Tropical *japonica* strains were able to infect *japonica* varieties (tropical and temperate), and temperate *japonica* strains were essentially specialists, with most strains able to infect only temperate *japonica* varieties. This pattern is consistent with a nested pattern of interaction (Figure 1C). We performed a nestedness analysis on this dataset (Figure 3) to test this hypothesis. The observed nestedness score was significantly higher than the one expected under the null model, demonstrating significant nestedness of the matrix (Figure 3B).

Importantly, the interaction matrix is overall nested, with narrow ranges being subsets of wider ranges. This nestedness was also true for strain groups with *indica,* tropical *japonica* and temperate *japonica* strains showing nested wide, intermediate and narrow compatibility ranges, respectively (Figure 3C). However, nestedness was not so clearly correlated with variety group. Temperate *japonica* varieties showed a significantly larger compatibility ranges than varieties from the two other subspecies (Figure 2 and 3C). But, compatibility ranges of *indica* and tropical *japonica* rice subspecies were highly variable among varieties and not significantly different (Figure 2 and 3C). It is to be noted that, as there is an interaction in our statistical analysis between the two factors “rice subspecies” and “strain group” (interaction observable on Figure 2), the statistical results shown on figure 3C should be taken with care. However it summarizes quite well the matrix shown on Figure 3A, and also allows a comparison of our results with those from Thrall & Burdon (2003).

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The implication of R genes

The compatibility range of *M. oryzae* strains and the proportion of R genes they could overcome were highly correlated (t = 2.8918, df = 29, p-value = 0.00719, Figure 4A). Fungal strain groups were able to overcome different numbers of R genes (df = 2, p = 0.002; Figure 4B). This result was mostly driven by the fact that temperate *japonica* strains were able to overcome fewer R genes than tropical *japonica* and *indica* strains (contrast *indica* and tropical *japonica* vs temperate *japonica*: t=3.059, p=0.0023, figure 4B). Surprisingly, *indica* and tropical *japonica* strains were able to overcome the same proportion of R genes, whereas these strains show significantly different compatibility ranges. This discrepancy could be due a lack of statistical power related to the fact that we used a limited number of strains. The fungal strain x R gene matrix (Figure S3) showed a similar pattern to that of the matrix shown in Figure 3, even though the ranking of fungal strains was modified, and the matrix not significantly nested.

Relationship between the pathogen compatibility range and infection severity

We investigated the relationship between the number of strains to which varieties were susceptible (the pathogen compatibility range of the host) and the ability of these varieties to control infections (mean severity score of successful infections). There was a positive correlation between the number of fungal strains capable of infecting a given variety and the severity of infection (t = 5.5562, df = 62, p = 6.17 x 10^{-7} - Figure 5). Thus, varieties able to protect themselves against infection by many different strains also seem to be able to limit the severity of the infection when it does occur, whereas varieties infected by many strains also displayed more severe infection.

Discussion

A nested pattern of compatibility

The confrontation of 31 *M. oryzae* strains representative of the worldwide diversity against a sample of 38 rice varieties resulted in a nested pattern of compatibility strongly structured by the pathogen strain group. *Indica*, tropical *japonica* and temperate *japonica* strains had respectively wide, intermediate and narrow compatibility ranges. Strains sampled on *indica* can thus be described as generalists able to

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infect most rice varieties, whatever their subspecies and geographical origin. Strains sampled on tropical *japonica* varieties were able to infect as many tropical and temperate *japonica* varieties but fewer *indica* varieties than strains isolated on *indica* varieties. Finally, strains from temperate *japonica* varieties were able to infect almost the same number of temperate *japonica* varieties but fewer *indica* and tropical *japonica* varieties than the others strains, making them specialists of temperate *japonica* varieties.

The nested pattern of compatibility range was also apparent for rice varieties, but less clearly. Because the compatibility ranges of strains was clearly structured by host subspecies, we would have expected varieties belonging to the *indica*, tropical and temperate *japonica* subspecies to have respectively narrow, intermediate, and wide compatibility ranges (like in Thrall & Burdon (2003)). We did observe that on average, temperate *japonica* varieties had wider compatibility ranges than the two other subspecies (Figure 3C). However, *indica* and tropical *japonica* varieties had similar average compatibility ranges and important variance (Figure 3C).

Yet it is quite clear that temperate *japonica* varieties can be infected by a greater number of strains than both *indica* and tropical *japonica* varieties, and this, independently of strain group. This pattern may result from the ecological constraints in the areas where the varieties are grown. Temperate *japonica* varieties are mainly cultivated in temperate areas and under irrigated conditions in which epidemics are usually less frequent and less severe than in tropical areas. Resistance to blast is therefore less of a concern to rice breeders that tend to prioritize improving yield and gustative characters over resistance during rice selection. Resistance to a wide range of strains could then be lost, either incidentally, *i.e.* because of the genetic drift inherent to breeding process, or because resistance mechanisms trade off with positively selected characteristics.

Under this “drifting” scenario, narrow compatibility ranges would be an ancestral state for rice varieties, and wide compatibility ranges would rather be a derived state. However compatibility range distribution is also highly variable within *indica* and tropical *japonica* subspecies. Both of these subspecies comprise varieties with very wide and very narrow compatibility ranges. In *indica* subspecies one finds both 10 out of the 12 varieties showing the narrowest compatibility range, and 5 out of the 10 varieties with

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widest compatibility ranges. Such variability suggests that compatibility range of varieties is not fully constrained by their phylogenetic history and that it may respond rapidly to environmental or agronomic (breeding) constraints. Both subspecies are cultivated over very large spatial scales in a diversity of eco-climatic conditions. It is thus possible that in some localities or under some conditions, resistance to pathogens is not prioritized in the breeding process, and varieties with wide compatibility ranges are selected for by rice breeders.

R genes are involved in compatibility range

Challenging *M. oryzae* strains with differential rice varieties for which R gene contents are known permitted us to observe a correlation between the compatibility range of fungal strains and their ability to overcome R genes (Figure 4). Such correlation is a clear indication that R genes are major drivers of compatibility range in the rice blast disease. This result confirms the implication of R genes in compatibility ranges already observed on rice (Hittalmani *et al.*, 2000; Divya *et al.*, 2014). This result also suggests that the nested pattern of compatibility ranges we observed can be interpreted in terms of R genes. First, the nested pattern implies that the network of compatibility between rice and *M. oryzae* is made of a single compartment (Figure 1C, and not like 1B where there are several independent compartments), *i.e.* that all rice subspecies share the same pool of R genes. In other words, the "phylogenetic" effect detected in our local adaptation experiment could be explained by the fact that temperate *japonica* varieties have fewer R genes than the other subspecies. Second, generalist pathogen strains (with a wider compatibility range) would be able to overcome more R genes than specialist strains only able to overcome a few R genes.

Interestingly, some results of the present study remain puzzling. First, we observed that even if the compatibility range of pathogens and their ability to overcome R gene correlate positively (Figure 4A), *indica* and tropical *japonica* strains have different compatibility ranges (Figure 3C) but could overcome the same proportion of R genes (Figure 4B). We hypothesize that this discrepancy could be due to the fact that rice varieties used in the two experiments (with differential and non differential varieties) did not contain the same sets of R genes. For instance, if some R genes often present in *indica* varieties were absent in the differential varieties, we could not observe that fungal strains sampled on *indica* plants could overcome

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more R genes than those sampled on tropical *japonica*. Another explanation would be that some R genes or R genes combinations are more frequent in some subspecies than others.

Second, *indica* and tropical *japonica* varieties have the same average compatibility range and share the same set of R genes (Figure 3A and C), a result that is not congruent with the local adaptation of fungal strains of the rice subspecies on which they were sampled (Figure 2). The fact that some R genes or R gene combinations were more frequent in some rice subspecies would be enough to create a local adaptation pattern. Another possibility is that fungal pathogens also adapt to other plant factors than R gene products. A firm demonstration of the present results will therefore require additional work to exhaustively describe the R gene content of varieties representative of the three rice subspecies.

The non-negligible relationship between R genes and infection severity

Severity scores allowed estimating both compatibility range and the severity of successful infections. This was a good opportunity to verify if the partial breakdown of R gene-mediated resistance is a general trend or simply restricted to a few R genes. More generally, it also permits study of the relationship between these two traits, which is equivalent to studying the relationship between complete (infection/no infection) and partial resistance (the ability to control fungal growth within the plant).

We found a positive correlation between susceptibility and pathogen compatibility range in rice varieties (Figure 5). The rice varieties attacked by the largest numbers of fungal strains and thus with the broadest compatibility ranges, were unable to control *M. oryzae* growth within the plant. Conversely, varieties with a narrow compatibility range were able to efficiently control the growth of the infecting pathogen. This correlation is consistent with the hypothesis that in rice, R genes are involved in the genetic control of both partial and complete resistance (Ballini *et al.*, 2008). This implication of R genes in partial resistance does not exclude the possibility of other partial resistance mechanisms occurring in rice.

The correlation between mean severity score and compatibility range also provides valuable information about the potential benefits of introducing more R genes into rice varieties. First, it shows that some varieties are resistant to most pathogen genotypes. This may be due to these rice varieties having

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large numbers of R genes, or to the presence of a few broad-spectrum R genes. Second, rice varieties resistant to most pathogen genotypes (*i.e.* with a narrow compatibility range) suffered only mild symptoms. This association between narrow pathogen compatibility range and low susceptibility indicates that, even if a *M. oryzae* strain can initiate infection in such plants, the plant is able to control its subsequent growth (and thus a potential outbreak). A trade-off between the ability to overcome R genes and spore production in *M. oryzae* may explain this pattern (Thrall & Burdon, 2003). A clear demonstration of the existence of such a trade-off in *M. oryzae* would confirm the value of introducing additional R genes into rice, provided that the addition of such genes is not costly to the plant. A few *M. oryzae* genotypes might still be able to infect such improved plants, but they would not thrive in these plants and would be unable to start an epidemic. Further investigations are required into the costs of accumulating R genes in plants and of the breakdown of resistance by *M. oryzae*.

Conclusion

In this work, we found that interactions between rice and *M. oryzae* are structured in a nested way. This pattern was consistent with a nested distribution of R genes among rice subspecies, which caused nested levels of compatibility ranges between rice subspecies and the asymmetric pattern of compatibility range. These results were mostly driven by the temperate *japonica* varieties having wider compatibility range than *indica* and tropical *japonica* varieties. We hypothesize that a nested distribution of R genes between plant species could explain why phylogeny has been shown to be a good predictor of infection success (de Vienne *et al.*, 2009).

Our results might also provide an explanation to the frequent observation of potential compatibility range of pathogens being larger than their observed compatibility range. Pathogens adapting to host plants carrying many R genes, will have a large compatibility range and therefore will be able to infect all plant genotypes that carry the same set (or a subset) of these R genes, regardless of a primary exposure of the pathogen to these plant genotypes. The host shifts of pathogen often observed in nature might actually not correspond to any sort of shift for the pathogen, if the R gene contents in the different plant species are the same.

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Tables

Table 1

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>χ^2)
NULL			2564	3526.7	
Rice	2	167.409	2562	3359.3	< 2.2E-16
Fungus	2	190.754	2560	3168.6	< 2.2E-16
Rice x Fungus	4	23.277	2556	3145.3	0.00011

Table 1: GLM analysis for the local adaptation experiment.

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Figure captions

Figure 1: Different interaction patterns between host variety groups (rice) and pathogen genetic groups (*M. oryzae*) in a gene-for-gene framework, under several hypotheses. Hypothesis A: All variety groups contain the same set of R genes. Under this hypothesis, for the pathogen, adapting to the local host is equivalent to adapting to all host variety groups. Hypothesis B: Each variety group contains a unique set of R genes (the sets of R genes present differ between variety groups). In this case, overcoming the resistance of the sympatric (local) host does not enable the pathogen to infect allopatric (foreign) hosts. Hypothesis C: The temperate *japonica* variety group has only some of the R genes carried by the tropical *japonica* variety group, and the tropical *japonica* variety group has only some of the R genes carried by the *indica* variety group. In this case, *indica* strains can overcome all R genes and can therefore infect all varieties, whereas tropical *japonica* strains can infect only *japonica* variety groups, and temperate *japonica* strains can infect only temperate *japonica* varieties. Columns correspond to hosts, and lines correspond to pathogens. Black squares represent compatible interactions (*i.e.* the pathogen can infect the host), whereas white squares represent incompatible interactions (no infection). The capital letters above each column represent R genes, and the lower case letters next to each line represent *avr* genes. Stars above avr genes represent mutations enabling the pathogen to escape recognition by the product of the R gene.

Figure 2: Proportion of compatible interactions between the various fungal strain groups and the different rice categories. The three lines on the graph represent the host compatibility ranges (proportion of compatible interactions) for the three *M. oryzae* categories (*indica*: red line and square symbols, tropical *japonica*: light green line and round symbols, temperate *japonica*: dark green line and triangular symbols) on the three categories of rice plants (*indica*, tropical *japonica*, and temperate *japonica*). Error bars were calculated with the GLM used to analyze the data and represent the 95% confidence intervals. The curves have been shifted laterally to improve readability.

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Figure 3: Matrix showing the interaction between rice and *M. oryzae*. Panel A shows the actual interaction matrix between rice varieties (column) and *M. oryzae* strains (lines) obtained in our experiments. Black squares indicate compatible infections. Individual strains and varieties are ordered according to their contribution to NODF. *Indica* rice varieties or fungal strains sampled from *indica* varieties are represented by red squares, tropical *japonica* varieties and the strains sampled from them are indicated by light green circles, and temperate *japonica* varieties and the strains sampled from them are represented by dark green triangles. Panel B shows the distribution of nestedness values (NODF) of 1000 matrices generated with the null model, and the nestedness value of the observed dataset (arrow). The observed interaction matrix is significantly nested, as its NODF value is greater than 97.5% of the null expectation (represented by a gray rectangle). Panel C shows both host and pathogen compatibility ranges for each group (*indica*, tropical *japonica* and temperate *japonica*). Error bars represent 95% confidence intervals, (but see discussion on this statistical analysis).

Figure 4: *M. oryzae* strains and R genes. Proportion of R genes overcome by *M. oryzae* strains, and the different strain groups of *M. oryzae*. The panel A shows the correlation between the compatibility range and the proportion of R genes overcome for each strain used in our local adaptation experiment. (n=31, R^2=0.22, t = 2.8918, df = 29, p-value = 0.00719). Panel B shows the proportion of the 19 R genes overcome by the strain groups, estimated with a generalized linear model. The error bars show the confidence intervals for each estimate. The numbers at the bottom of the graph show the numbers of data points used to obtain these estimates (two or three replicates were used).

Figure 5: Plot showing the pathogen compatibility range of the host against the mean severity score of compatible interactions for individual varieties. The *indica* varieties are represented as red squares, tropical *japonica* varieties are shown as light green circles, temperate *japonica* varieties as dark green triangles and

unassigned plant varieties are represented as blue crosses. The regression line is plotted. Pearson's correlation, calculated for the whole dataset, was significant (t=4.63, df=59, p=2.052 x 10^{-5}).

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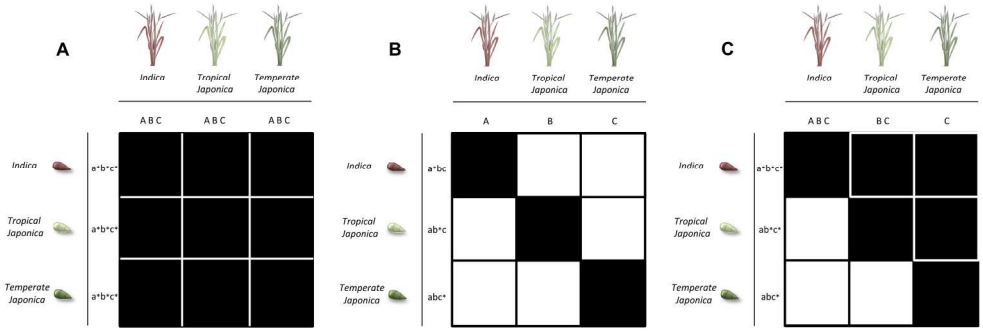

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310x109mm (200 x 200 DPI)

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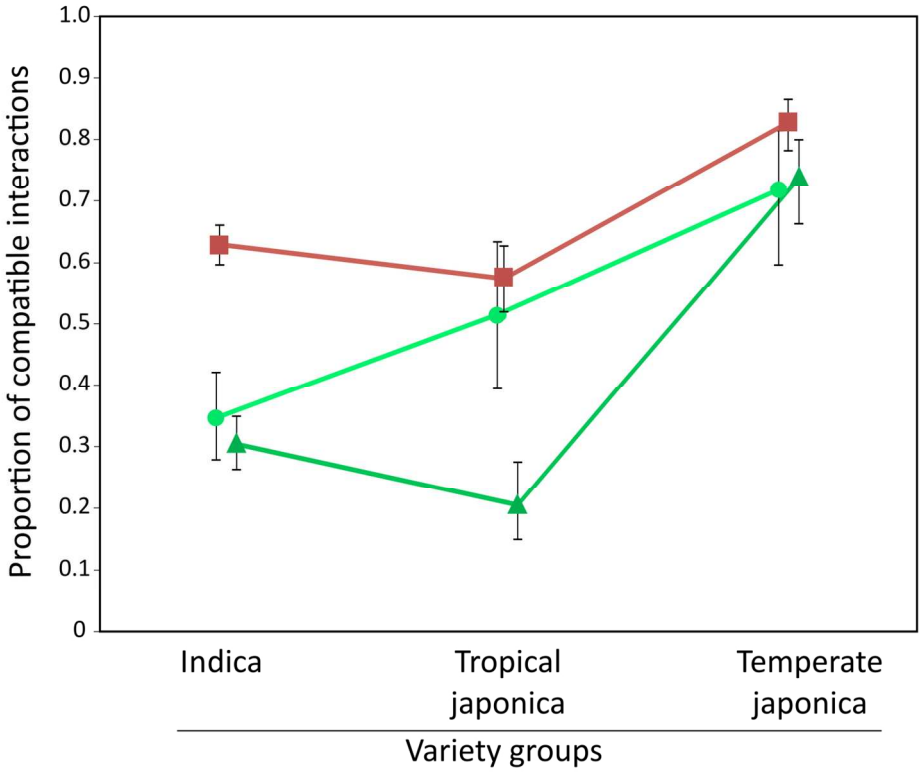

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135x108mm (300 x 300 DPI)

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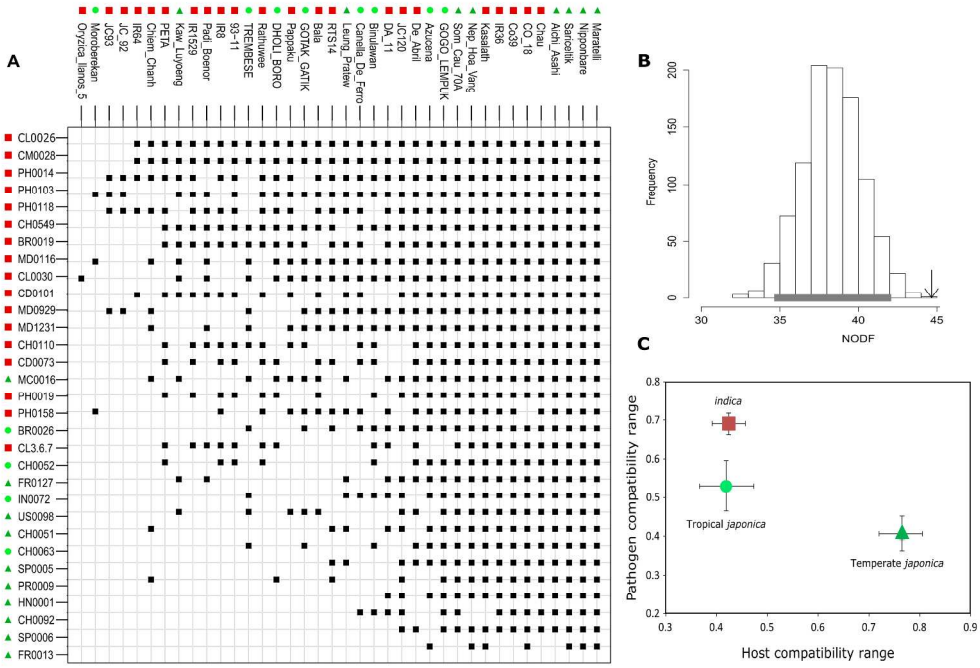

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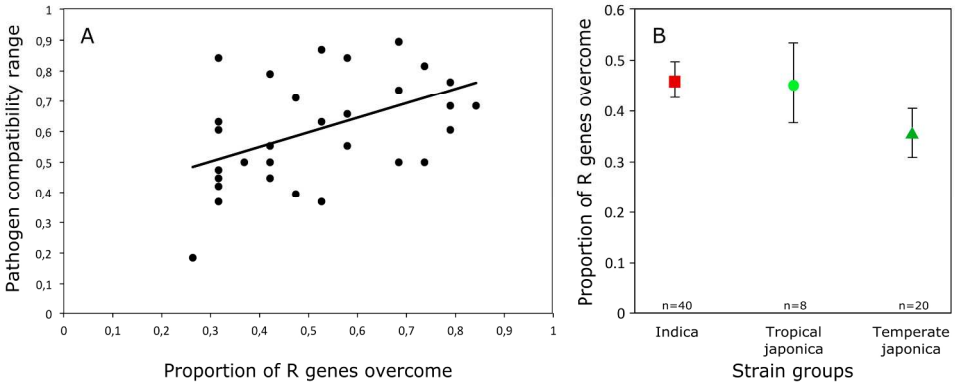

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222x91mm (300 x 300 DPI)

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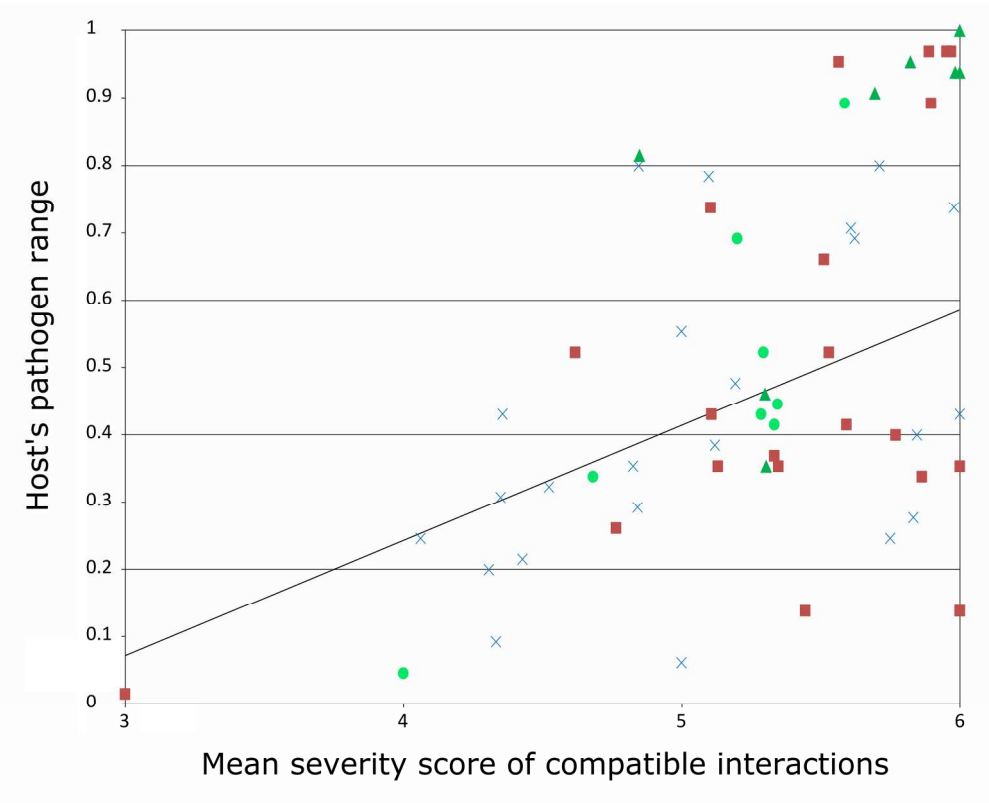

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192x154mm (300 x 300 DPI)

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Supplementary materials

Table S1: List of the rice varieties and *M. oryzae* strains used in this study. Strains and varieties of unknown group status (*i.e.* differential varieties or *nd*) were excluded from the local adaptation analysis.

Rice variety	Variety group	R genes
93 11	*indica*	
Bala	*Indica*	*Pi33*
Chau	*Indica*	
Chiem Chanh	*Indica*	
CO 18	*Indica*	
CO 39	*Indica*	
DA 11	*indica*	
De Abril	*indica*	
IR1529	*indica*	*Pi33*
IR36	*indica*	
IR64	*indica*	*Pi-a, Pi-30(t), Pi-33, Pi-ta, Pi-tq5, Pi-tq6*
IR8	*indica*	
JC 92	*indica*	
JC120	*indica*	
JC93	*indica*	
Kasalath	*indica*	
Oryzica llanos 5	*indica*	*Pi-2, Pi-33, Pi-b, Pi-sh, Pi-ta², Pi-z*
Padi Boenor	*Indica*	

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Pappaku	*indica*	
PETA	*indica*	
Rathuwee	*indica*	
RTS14	*indica*	
Aichi Asahi	temperate *japonica*	*Pi-a*
Kaw Luyoeng	temperate *japonica*	
Leung Pratew	temperate *japonica*	
Maratelli	temperate *japonica*	
Nep Hoa Vang	temperate *japonica*	
Nipponbare	temperate *japonica*	
Sariceltik	temperate *japonica*	
Som Cau 70A	temperate *japonica*	
Azucena	tropical *japonica*	*Pi-24(t), Pi-26(t), Pi-28(t)*
Binulawan	tropical *japonica*	
Canella_De_Ferro	tropical *japonica*	
DHOLI BORO	tropical *japonica*	
GOGO LEMPUK	tropical *japonica*	
GOTAK GATIK	tropical *japonica*	
Moroberekan	tropical *japonica*	*Pi-5(t), Pi-7(t)*
TREMBESE	tropical *japonica*	
75-1-127	Differential variety	*Pi-9*
BL1	Differential variety	*Pi-b*
C101 A51	Differential variety	*Pi-2*

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C101 LAC	Differential variety	*Pi-1, Pi-1b, Pi-33*
C104 LAC	Differential variety	*Pi-1*
C104 PKT	Differential variety	*Pi-3*
Fujisaka No.5	Differential variety	*Pi-i, Pi-ks*
Fukunishiki	Differential variety	*Pi-z, Pi-sh*
K1	Differential variety	*Pi-ta*
K2	Differential variety	*Pi-kp, Pi-a*
K3	Differential variety	*Pi-kh*
K59	Differential variety	*Pi-t*
K60	Differential variety	*Pi-kp*
Kanto 51	Differential variety	*Pi-k*
Kusabue	Differential variety	*Pi-k*
Nato	Differential variety	*Pi-i*
Pi n°4	Differential variety	*Pi-ta²*
Rico 1	Differential variety	*Pi-ks*
Shin 2	Differential variety	*Pi-ks, Pi-sh*
St 1	Differential variety	*Pi-f*
Toride 1	Differential variety	*Pi-zt*
Tsuyuake	Differential variety	*Pi-km*

Zenith	Differential variety	*Pi-a, Pi-z*

4

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M. oryzae strains	Previous names	Collection date	Country	Province or state	Place	Variety	Dominant rice type in the region or country	Strain group	Number of R genes overcome in differential varieties
BR0019		01/05/1986	Brazil	Goias	Goiana	Tetep	tropical *japonica*	*indica*	1
BR0026		10/09/1987	Brazil	Mato Grosso	Fazenda Progresso	IRAT177	tropical *japonica*	tropical *japonica*	6
CD0073		01/02/1981	Ivory Coast		Bouaké	BP 168	*indica*	*indica*	1
CD0101		01/04/1984	Ivory Coast		Odienne	BG 90-2	*indica*	*indica*	7
CH0051		??/??/????	China	Zhejiang		Causowdao	*indica*	temperate *japonica*	4
CH0052		22/10/1991	China	Hunan		B 40	tropical *japonica*	tropical *japonica*	5
CH0063		26/04/1985	China	Hunan			tropical *japonica*	tropical *japonica*	7
CH0092	PRC-9, 8-38	0?/0?/1983	China	Zhejiang		Xiushui 48	*indica*	temperate *japonica*	10
CH0110	ZJ-16, 7-3-2	0?/07/1991	China	Zhejiang	Ruian	Zhufengyuan	*indica*	*indica*	10
CH0549	95-12-3a	0?/0?/1995	China	Yunnan	Baoshan		tropical *japonica*	*indica*	12
CL0026	CICA 8-53	01/02/1989	Colombia	Méta	Santa Rosa	CICA 8	*indica*	*indica*	14
CL0030	Fanny 25-10	01/09/1989	Colombia	Méta	Altillanura	Fanny	tropical *japonica*	*indica*	10
CL3.6.7			Colombia	Méta	Santa Rosa		*indica*	*indica*	17
CM0028		01/02/1987	Cameroon		M'Bos	ITA 212	*indica*	*indica*	13
FR0013		01/09/1988	France			Rocca 1	temperate *japonica*	temperate *japonica*	4
FR0127		05/08/2004	France		Camargue	Faraman	temperate *japonica*	temperate *japonica*	7
HN0001		01/09/1993	Hungary		Szarvas	OKI-2	temperate *japonica*	temperate *japonica*	6
IN0072	105-1-1	02/01/1992	Indonesia			HR12	*indica*	tropical *japonica*	14
MC0016		22/09/1997	Morocco		Tazi	Sariceltik	temperate *japonica*	temperate *japonica*	5
MD0116		15/04/1992	Madagascar		Sambaina		nd	*indica*	11
MD0929		28/01/2005	Madagascar		Andranomanelatra	Fofifa161	nd	*indica*	14
MD1231		03/03/2006	Madagascar		Andranomanelatra	Fofifa154	nd	*indica*	14
PH0014	PO6-6	10/02/1986	Philippines		IRRI	Tetep	*indica*	*indica*	8
PH0019	IK81-25	10/02/1986	Philippines		IRRI, Los Baños	Milyang 49	*indica*	*indica*	13
PH0103	BN209	??/??/????	Philippines				*indica*	*indica*	7
PH0118	43	??/??/????	Philippines				*indica*	*indica*	4
PH0158	V86010	0?/0?/1986	Philippines		IRRI, Bo, Bula, Camarines Sur		*indica*	*indica*	3
PR0009		16/09/1991	Portugal		Sado Torre dos Cleri	Ringo	temperate *japonica*	temperate *japonica*	7
SP0005	ES5	15/09/1994	Spain	Tarragone	Amposta, Salats	Lido	temperate *japonica*	temperate *japonica*	3
SP0006	ES6	07/10/1994	Spain	Aragon		Huesca	temperate *japonica*	temperate *japonica*	4
US0098	A 125	0?/0?/1992	United States	Arkansas	Lonoke	Newbonnet	temperate *japonica*	temperate *japonica*	10

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Figure S1

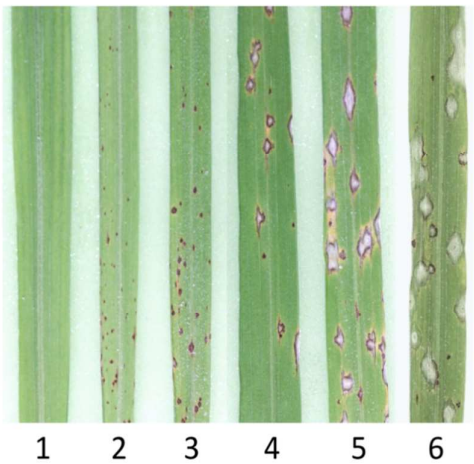

Figure S1: Infection severity score. This score can be used to estimate the ability of the plant to contain the infection caused by the pathogen inoculated.

Figure S2

Figure S2: Distribution of some of the rice varieties representative of the three main genetic sub-groups of rice. The tree was constructed by Garris et al. (2005) on the basis of microsatellite genotyping. Position of varieties in common between this study and the study by Garris et al. was marked by red dots for indicas, light green for tropical japonicas and dark green for temperate japonicas.

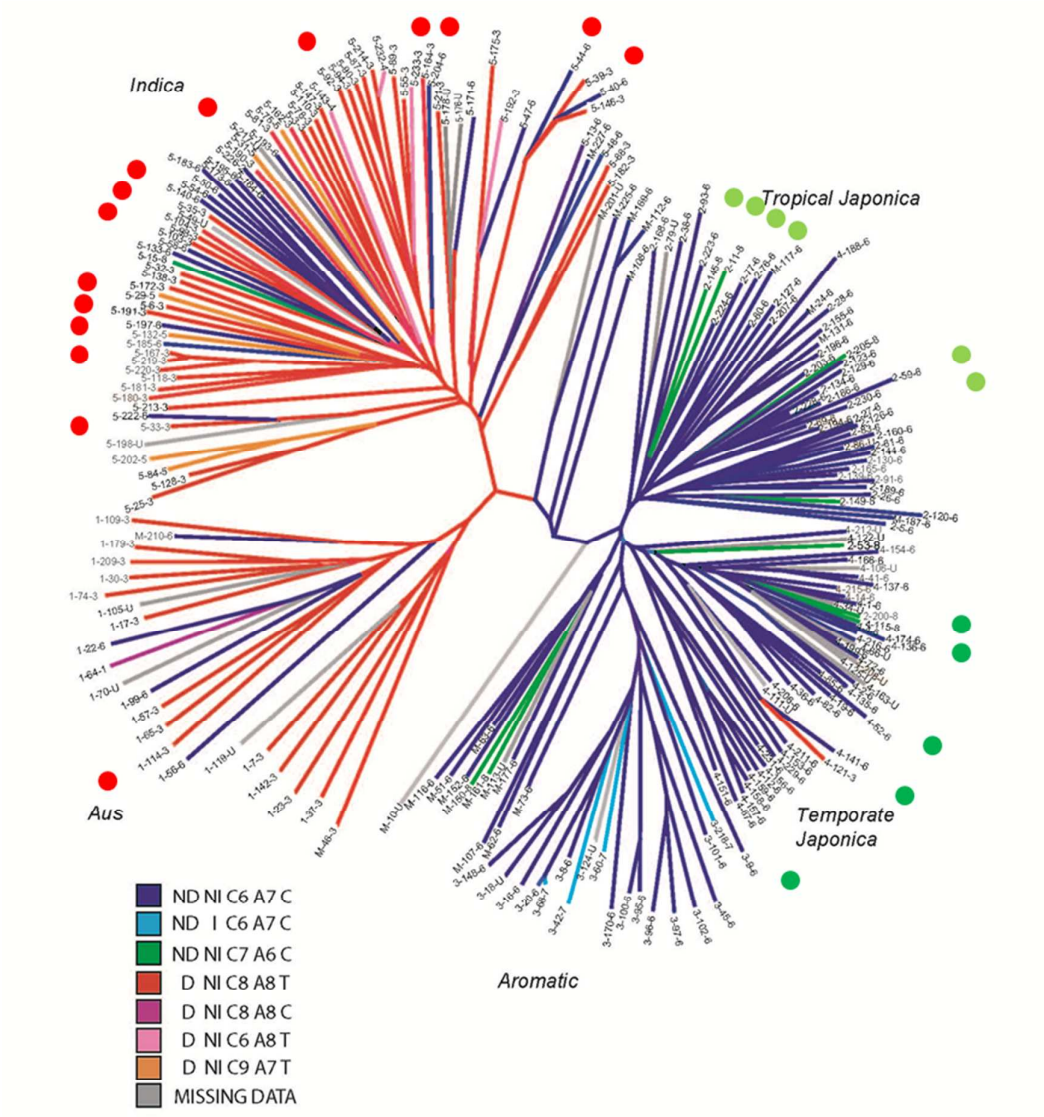

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Figure S3

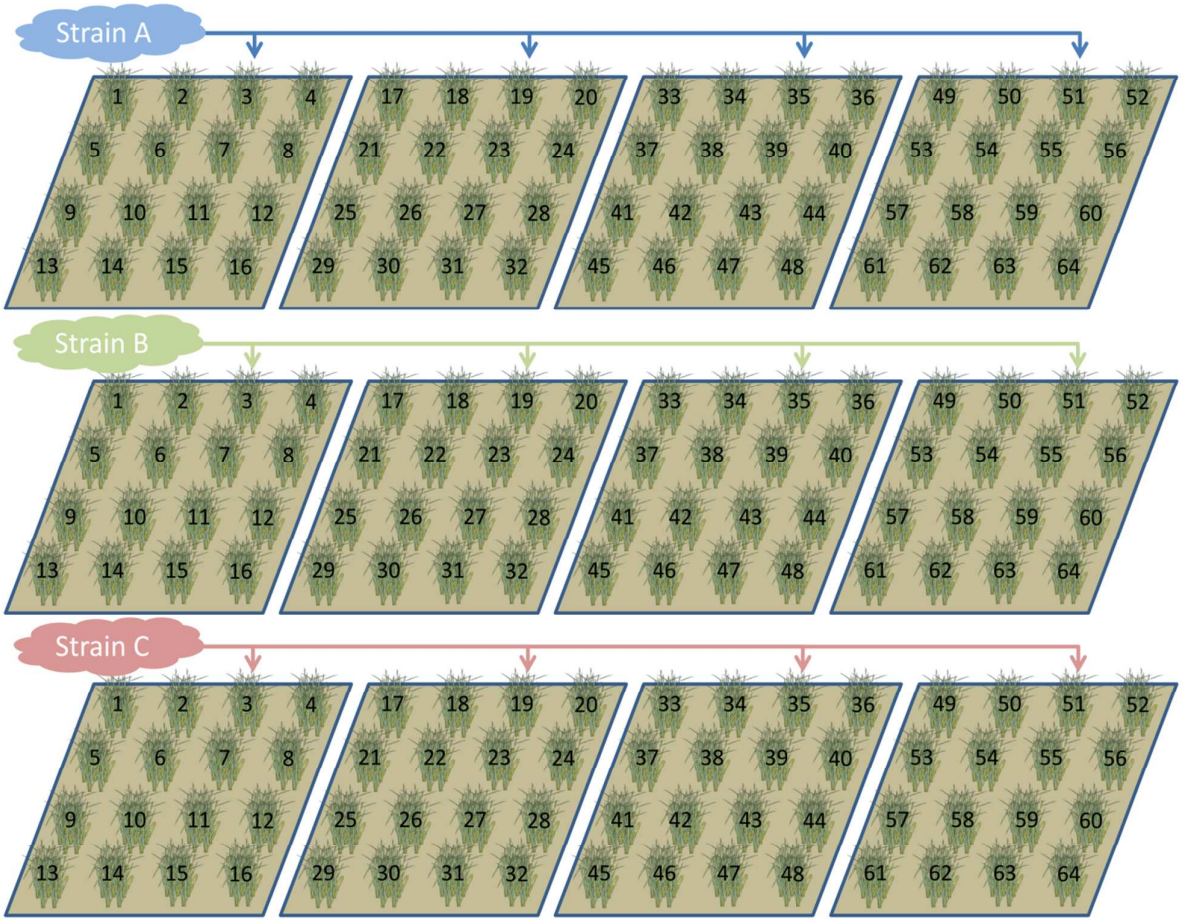

Figure S3: Inoculation protocol. The 61 varieties were grown in four trays (numbers represent different varieties – three varieties were excluded from the analysis). For each variety, 20-25 plants were obtained. Plants were inoculated by spraying with spore suspensions. Six to eight independent inoculations were performed at each date (only three inoculations are shown in this diagram). In total, inoculations were performed at 20 dates. The experimental unit was a group of 20-25 plants of a specific variety, inoculated with a specific strain (e.g. strain A x variety 1).

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Figure S4

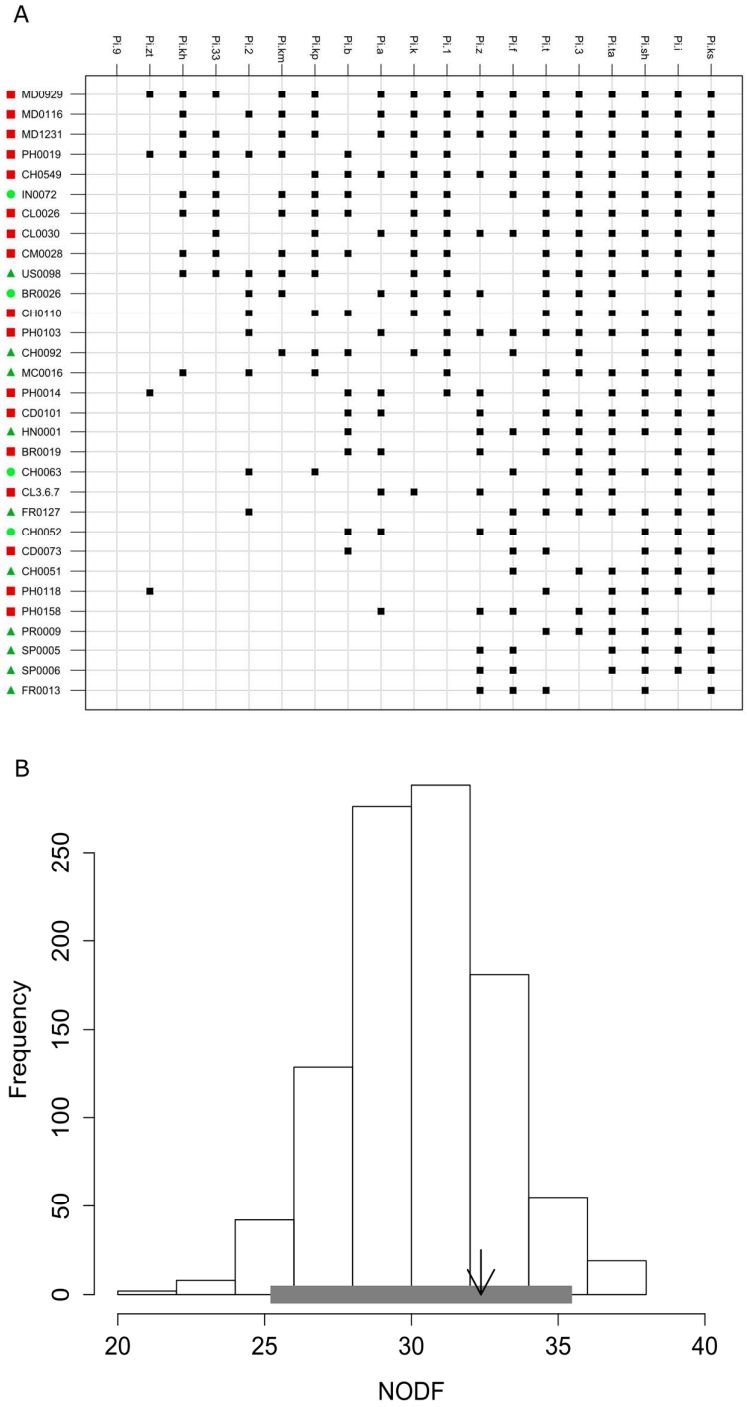

Figure S4: R genes present in the differential varieties and overcome by the different strain groups of *M. oryzae*. Panel A shows the interaction matrix, black squares symbolizing compatible infections. Individual strains and R genes were ordered according to their contribution to NODF. Fungal strains sampled from

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indica varieties were indicated by red squares, fungal strains sampled from tropical *japonica* varieties are indicated by light green circles, and strains sampled from temperate *japonica* varieties are indicated by dark green triangles. Panel B shows the distribution of nestedness values (NODF) of 1000 matrices generated with the null model, and the nestedness value for the observed dataset (arrow). The observed interaction matrix was not significantly nested as its NODF value was slightly lower than 97.5% of the null expectation (represented by a gray rectangle).

References

Garris A.J., Tai T.H., Coburn J., Kresovich S. & McCouch S. 2005.Genetic structure and diversity in *Oryza sativa* L. *Genetics* **169**:1631–1638.