REVIEW

ARCHIPELAGO: A Dedicated Resource for Exploiting Past, Present, and Future Genomic Data on Disease Resistance Regulation in Rice

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Large amounts of expression data dealing with biotic stresses in rice have been produced in the past 5 years. Here, we extensively review approximately 70 publications and gather together information on more than 2,500 genes of the rice defense arsenal. This information was integrated into the OryGenesDB database. Several genes (e.g., metallothioneins and PBZ1) appear to be hallmarks of rice–pathogen interactions. Cross-referencing this information with the rice kinome highlighted some defense genes and kinases as possible central nodes of regulation. Cross referencing defense gene expression and quantitative trait loci (QTL) information identified some candidate genes for QTL. Overall, pathogenesis-related genes and disease regulators were found to be statistically associated with disease QTL. At the genomic level, we observed that some regions are richer than others and that some chromosomes (e.g., 11 and 12), which contain a lot of resistance gene analogs, have a low content of defense genes. Finally, we show that classical defense genes and defense-related genes such as resistance genes are preferentially organized in clusters. These clusters are not always coregulated and individual paralogs can show specific expression patterns. Thus, the rice defense arsenal has an ARCHIPELAGO-like genome structure at the macro and micro level. This resource opens new possibilities for marker-assisted selection and QTL cloning.

Plants are constantly exposed to microbial attacks and have developed sophisticated systems to counteract them. Besides the preformed defense systems such as cuticle (Skamnioti and Gurr 2007) and cell wall strengthening (Juge 2006), active resistance that requires both transcriptional (Dangl and Jones 2001) and post-transcriptional changes (Walling 2006) is usually mounted. This pathway can be divided into three steps. In the first step, the presence of the pathogen is detected by the plant throughout different recognition systems involving resistance (*R*) genes or other pattern recognition receptors (Bent and Mackey 2007; Jones and Dangl 2006). Whereas some *R* genes directly recognize pathogen molecules, other *R* genes indirectly

recognize metabolic perturbations triggered by the pathogen (guard hypothesis) (Jones and Dangl 2006). Depending of the presence or absence of *R* genes, the interaction will be incompatible (plant is resistant) or compatible (plant is susceptible). These *R* genes are usually expressed constitutively, although some regulation exists (Vergne et al. 2007). In a second step, signal transduction occurs and requires regulators such as mitogen-activated protein kinases (Mishra et al. 2006) and transcription factors (Eulgem 2005). Here, the genes that are not *R* genes are collectively called intracellular disease regulator (*IDR*) genes. Although usually first defined genetically by mutants affected in disease resistance, *IDR* genes are also often differentially expressed (Vergne et al. 2007). In a third step, defense responses are induced. These include production of antimicrobial secondary metabolites (phytoalexins) (Peters 2006), pathogenesis-related (*PR*) proteins (e.g., chitinases and glucanases) (Jwa et al. 2006), cell-wall strengthening (Hückelhoven 2007), and programmed cell death, leading to the hypersensitive response (*HR*) (Greenberg and Yao 2004). These genes that act downstream of the disease resistance signaling pathway are collectively called defense genes.

For the purpose of this review, the disease resistance pathway is thus divided into three steps, where *R* genes detect the pathogen and *IDR* genes transduce the signal generated by *R* genes to finally activate defense responses. In addition to these structural definitions, differential expression upon infection can be used to classify the genes. Here, we will use the term defense-related genes for genes that are differentially expressed upon infection. As mentioned above, there are examples of *R*, *IDR*, and defense genes (including *PR* genes) that belong to the class of defense-related genes.

Genetic and molecular analyses in *Arabidopsis thaliana* have demonstrated that most elements involved in these three steps (recognition, signal transduction, and induction of defense) are common to different types of pathogens and plants (Dangl and Jones 2001). At the genomic level, *R* genes are known to be organized in clusters (Ballini et al. 2008; Monosi et al. 2004). No information on the genomic organization of *IDR* and defense-related genes is currently available.

Some efforts have been made in the past to centralize available information on defense responses. For example, the DRASTIC database (Button et al. 2006) referenced a large amount of data on several plant–pathogen interactions. With the genomic era, transcriptomic and proteomic approaches have generated large sets of data that are often dispersed and require dedicated tools for their integration. For example, in

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Arabidopsis this has been completed for most of the available Affymetrix transcriptomic data (Zimmermann et al. 2004). This was made possible by the fact that a limited number of types of techniques and DNA chips were used.

In the past decade, rice has emerged as a central plant model for monocots (Shimamoto and Kyozyuka 2002). Rice has important advantages compared with other monocots, including reverse genetic databases (An et al. 2005; Droc et al. 2006; Miyao et al. 2007; Wu et al. 2005; Zhang et al. 2006) now containing more than 160,000 insertion lines, easy transformation protocols (Sallaud et al. 2004; Toki et al. 2006), and full-genome sequences of indica and japonica cultivars (Goff et al. 2002; Yu et al. 2002). In addition, the genomes of three major rice pathogens—*Magnaporthe oryzae* (Dean et al. 2005), *Xanthomonas oryzae* pv. *oryzae* (Lee et al. 2005), and *Rice yellow mottle virus* (RYMV) (Yassi et al. 1994)—have been fully sequenced and are genetically accessible. These naturally occurring pathogens cause important field losses (e.g., blast disease caused by *M. oryzae* is a major cause of crop loss worldwide) (Valent 2004). Rice feeds more than half of the world's population (Khush 2005) and extensive quantitative trait loci (QTL) studies have been conducted to identify genetic determinants of disease resistance and improve rice tolerance through marker-assisted breeding. Some of these QTL to various rice diseases were recently anchored in the rice genome (Wisser et al. 2005). This led to the identification of chromosomal segments associated with broad-spectrum quantitative disease resistance. An extensive analysis of blast disease QTL is presented in an accompanying article (Ballini et al. 2008).

Wisser and associates (2005) noted that some gene families associated with QTL were found in clusters of several paralogs; however, this observation was not further studied and only limited examples of gene expression in such clusters are available in rice (Chittoor et al. 1997). Recently, it was demonstrated that genes automatically annotated as involved in biotic stress are found in clusters in *A. thaliana* and rice (Rizzon et al. 2006). This is a common observation for *R* genes (Monosi et al. 2004). However, no global comparison between expression data and genomic structure of the defense arsenal in rice has been made.

Wisser and colleagues also observed that some gene families, such as glutathione-*S* transferases and some proteases, were significantly associated with disease QTL (Wisser et al. 2005). Two major models for quantitative resistance are usually proposed. In the first model, quantitative resistance is the result of defective *R* genes that recognize pathogens with low efficiency. This is supported by the observation that QTL, *R* genes, and resistance gene analogs (RGA) seem to colocalize (Wisser et al. 2005), although this conclusion may suffer from technical drawbacks (Ballini et al. 2008), including a significant probability of colocalization only by chance. An example of such a case is provided by the RFO1 gene in *Arabidopsis*.

This gene confers quantitative resistance to *Fusarium oxysporum* (Diener and Ausubel 2005). A second model, which may not be mutually exclusive, suggests that defense-related genes (including defense genes such as *PR* genes), as the final executors of resistance, explain significant parts of quantitative resistance. This is supported by the work of Ramalingam and associates (2003) and Liu and associates (2004), which shows that marker-assisted selection using defense-related genes improves blast resistance in rice. However, there is no global demonstration of statistically significant colocalization of defense-related genes and QTL (Ballini et al. 2008). An alternative model that has not yet been thoroughly explored is the possibility that *IDR* genes could explain some QTL. Rice is a good system to examine this possibility because this approach requires extensive QTL knowledge and *IDR* identification. Thus, a prerequisite for such a study is a complete inventory of both QTL and *IDR* genes.

In the past 5 years, there has been an exponential increase in the amount of available information on rice genes and proteins that are differentially expressed upon infection by pathogens and pathogen-related stresses. As the volume of this type of data continues to grow, there is a clear need for an integrated resource that would allow new datasets to be compared with previous results. Thus, we conducted an extensive review of such studies and integrated the resulting resource, called ARCHIPELAGO, into the web-based database OryGenesDB.

Building the ARCHIPELAGO resource.

All types of rice–pathogen interaction were considered, including experiments where elicitors and signaling molecules such as jasmonic acid were used to induce defense (see Supplementary Table 1 for the complete list of publications included). Some data originated from experiments where expressed sequence tag libraries were used to perform in silico expression; this represents 1,415 genes of the “Expressed” category (Table 1). The majority of the genes originated from experiments where differential information was directly available (e.g., microarray, differential-display, and protein two-dimensional electrophoresis data) and were grouped in the category “Differentially expressed” (Table 1). This category represents more than 2,500 genes that are hereafter referred to as defense-related genes. Finally, genes from the kinome database (Dardick et al. 2007), which were identified by yeast-two-hybrid and tandem affinity purification tag (TAP-TAG) experiments, were included because they represent proteins that interact with putative *R* genes and signal transduction regulators. Disease regulators (*IDR* genes) such as *NPR1* were also included because some have been proven to be differentially expressed upon infection (Vergne et al. 2007). Overall, this represents a total of approximately 4,500 genes (approximately 10% of the gene pool) from approximately 70 publications. More than 65% of the “differentially expressed” cate-

Table 1. Gene content and origin of the ARCHIPELAGO resource

Category	Additional information	Publications	Gene number
Expressed upon infection	Bacteria, elicitor, <i>Magnaporthe grisea</i>	6	1,415
Differentially expressed	<i>Magnaporthe oryzae</i> infection	31	1,676
	Bacteria infection	4	273
	Elicitor treatment	17	323
	Insect (BPH) attack	2	115
	Lesion mimic mutant	3	117
	Virus (<i>Rice yellow mottle virus</i>) infection	1	22
Disease regulators	...	1	35
	Total for differentially expressed gene	59	2,562
Kinome data	Yeast two-hybrid data	1	127
	Tandem affinity purification tag (TAP-TAG) data	1	384
	Total	67	4,488

gory was obtained from *M. oryzae*, suggesting that other types of plant–pathogen interactions are probably under-represented.

This wealth of data was integrated into the OryGenesDB database (Droc et al. 2006) together with new data on blast QTL, metaQTL, *R* genes, and RGA (Ballini et al. 2008). Classical search and display tools associated with OryGenesDB are available (Fig. 1; Supplementary Fig. 3) (e.g., pop-up windows that display, for a given feature, the functional category, such as differentially expressed, and additional links, including PubMed links). Anchoring this information on the rice physical genome map allowed several different series of analyses.

Identification of ubiquitous markers of defense.

For defense-related genes identified in interactions with *M. oryzae*, there were sufficient data to establish comparisons of global biological function (Supplementary Fig. 1). Two functional categories are over-represented when compared with a

random pool of genes. These include genes annotated as involved in cell death (MYB transcription factors and MLO proteins) and transcription factors. This confirms previous hypotheses that intense reprogramming occurs during disease resistance (Dangl and Jones 2001). In contrast, *R* gene analogs did not seem to be massively differentially regulated upon infection. For example, only 5.5% of the 959 RGA listed in ARCHIPELAGO (Ballini et al. 2008) were differentially expressed in interactions between rice and *M. oryzae*. In contrast, we observed that 63% (22/35) of the *IDR* genes (Table 1, disease regulators) were differentially expressed upon *M. oryzae* infection (Supplementary Table 3).

Any potential overlaps between different types of interactions with *M. oryzae*, *X. oryzae*, RYMV, and insects were evaluated (Supplementary Fig. 2). Approximately 30% of the genes induced by bacteria, virus, and insects were also induced by *M. oryzae*. There were no genes found in common between

Fig. 1. The ARCHIPELAGO resource display in OrygenesDB illustrated by the *PBZ1* cluster on chromosome 12. Only eight annotation layers are shown but more are available on OrygenesDB. The panels on the right provide examples of the pop-up windows attached to each feature. Links to more detailed files or positions on the genome are underlined.

Table 2. Genes found differentially expressed in interactions with *Magnaporthe oryzae* and at least two other interactions

Tigr name	Putative function	Bacteria	Elicitor	Insect (BPH)	<i>M. oryzae</i>	Mutant	Virus (RYMV) ^a
Os01g10400	Expressed protein	1	1	1	1	1	0
Os01g21250	Protein induced upon tuberization	1	0	1	1	0	0
Os02g52390	Protein hypA	1	0	1	1	0	0
Os05g11320	Metallothionein-like protein type 3	1	1	1	1	0	0
Os10g32550	Chaperonin CPN60-1, mitochondrial precursor	0	1	0	1	1	1
Os11g26850	Adenosylhomocysteinase	1	1	0	1	1	0
Os12g17600	Ribulose biphosphate carboxylase small chain C	1	0	1	1	1	0
Os12g36880	Major pollen allergen Bet v 1-D/H	0	1	0	1	1	1

^a RYMV = Rice yellow mottle virus.

insects and RYMV; however, this may be due to under-representation of these biological conditions in our database. The limited overlaps (81 genes) found between *M. oryzae* and bacterial infection are consistent with previous observations. For example, using macroarrays, Li and associates (2006) showed that approximately 15% of the genes differentially expressed

were common to both pathogens. Seven genes were often found associated with response to different infection situations (Table 2). Two classes of genes could be distinguished. The first represents genes involved in cellular stress (e.g., metallothionein and PBZ1). The Os01g10400 gene was one of two metallothioneins found in almost all situations of biotic stress

Table 3. Rice genes differentially expressed upon *Magnaporthe oryzae* infection and interacting with the rice kinome

TIGR	Annotation ^a
Gene expressed ^b	
Os03g52840	Serine hydroxymethyltransferase, mitochondrial precursor
Os08g43640	26S proteasome non-ATPase regulatory subunit 3
Os09g02710	Cystathionin β synthase protein
Os05g25210	Expressed protein
Os03g56280	Malate dehydrogenase, glyoxysomal precursor
Os01g04650	PB1 domain-containing protein
Os04g53350	Expressed protein
Os03g59360	Remorin
Os04g40850	26S proteasome non-ATPase regulatory subunit 6
Os01g74020	ARR1 protein-like
Os08g26870	Wound-responsive protein
Os01g58890	Cystatin-1 precursor
Os04g56160	Plasma membrane ATPase
Os08g37490	14-3-3-like protein A
Os06g11980	Expressed protein
Os09g25070	OsWRKY62—superfamily of rice TFs having WRKY and zinc finger domains
Os01g22900	β -Fructofuranosidase
Os05g04630	Retrotransposon protein
Os03g51600	Tubulin α -3 chain
Os07g36570	K1 domain-interacting kinase 1
Os01g10400	Expressed protein
Os05g34070	Nucleic acid binding protein
Os06g06090	OsMPK6—putative MAPK based on amino acid sequence homology
Os05g11320	Metallothionein-like protein type 3
Os04g38870	14-3-3-like protein GF14-6
Os04g38480	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor
Os03g14590	Calcium ion binding protein
Os07g37250	Chloroplast-localized Ptr ToxA-binding protein1
Os12g36880	Major pollen allergen Bet v 1-D/H
Os12g19394	Ribulose biphosphate carboxylase small chain C, chloroplast precursor
TAP-TAG ^c	
Os01g02580	Receptor kinase ORK10, putative
Os01g02580	Receptor kinase ORK10, putative
Os01g10450	Protein kinase, putative, expressed
Os01g19160	Receptor-like protein kinase 5 precursor, putative, expressed
Os01g32660	OsMKK6—putative MAPKK based on amino acid sequence homology, expressed
Os01g47530	OsMPK20-4—putative MAPK based on amino acid sequence homology, expressed
Os01g47530	OsMPK20-4—putative MAPK based on amino acid sequence homology, expressed
Os01g60910	CBL-interacting serine/threonine-protein kinase 15, putative, expressed
Os01g67160	Cell division control protein 2 homolog C, putative, expressed
Os02g40240	Leucine-rich repeat receptor protein kinase EXS precursor, putative, expressed
Os03g12470	OsWAK25—OsWAK receptor-like protein kinase, expressed
Os03g61060	Receptor protein kinase TMK1 precursor, putative, expressed
Os03g61060	Receptor protein kinase TMK1 precursor, putative, expressed
Os03g61060	Receptor protein kinase TMK1 precursor, putative, expressed
Os05g45420	SNF1-related protein kinase catalytic α subunit KIN10, putative, expressed
Os05g45420	SNF1-related protein kinase catalytic α subunit KIN10, putative, expressed
Os09g19700	Protein kinase APK1A, chloroplast precursor, putative, expressed
Os10g10130	OsWAK112d—OsWAK receptor-like protein kinase, expressed
Os11g46950	Wall-associated kinase 1, putative, expressed
Os12g44090	ATP-binding protein, putative, expressed
Os01g18800	CBL-interacting serine/threonine-protein kinase 1, putative, expressed
Os01g32660	OsMKK6—putative MAPKK based on amino acid sequence homology, expressed
Os01g32660	OsMKK6—putative MAPKK based on amino acid sequence homology, expressed
Os02g06930	Serine/threonine-protein kinase receptor precursor, putative, expressed
Os05g05160	Extracellular signal-regulated kinase 1, putative, expressed
Os06g10160	Protein kinase APK1A, chloroplast precursor, putative, expressed
Os07g42940	Serine/threonine-protein kinase SAPK2, putative, expressed
Os02g05950	Phytosulfokine receptor precursor, putative, expressed
Os10g25090	Leucine-rich repeat transmembrane protein kinase 1, putative, expressed
Os12g01200	Transposon protein, putative, unclassified, expressed

^a MAPK = mitogen-activated protein kinase; MAPKK = MAPK-kinase.

^b Gene differentially expressed upon *M. grisea* infection.

^c Tandem affinity purification tag (TAP-TAG) interactor.

reviewed here. Overexpression of metallothionein *OsMT2b* has been shown to affect disease resistance (Wong et al. 2004). However, the *OsMT2b* gene (Os05g02070; Supplementary Table 2) was not the most common copy of this gene family in our database. The *PBZ1* gene, which belongs to the *PR10* family (Jwa et al. 2006) was also found to be differentially expressed under most conditions tested. This gene belongs to a large gene family in rice, members of which are often found in genomic clusters (Fig. 1 and discussed below). In *Medicago truncatula*, recent evidence suggests that silencing of some members of the *PR10* gene family reduces susceptibility to a root-colonizing oomycete (Colditz et al. 2007). However, there is no direct evidence for the role of this gene in disease resistance in rice. The second class of genes (e.g., late embryogenesis proteins [LEA], peptidase, chaperonin, and Rubisco) likely represents functions indirectly affected by general cellular stress. Expression of LEA is usually linked to the acquisition of desiccation tolerance in seed, and many LEA proteins are induced by cold, osmotic stress or exogenous abscisic acid (Wise and Tunnacliffe 2004). The observation that one LEA is commonly found in our database supports cross-talk between biotic and abiotic stresses—a phenomenon also documented in rice (Xiong and Yang 2003). The roles of peptidase M16, chaperonin, and Rubisco in this list of genes are more enigmatic, although protein degradation (Vergne et al. 2007), protein folding (Arthur et al. 2007), and photosynthesis (Vergne et al. 2007) are all likely to be involved in disease resistance.

Interactions between the kinome and the rice defense arsenal.

Protein kinases have recently been used in TAP-TAG and yeast two-hybrid experiments to build a kinome interaction network (Dardick et al. 2007 and data downloaded from the Rice Kinase Database). The corresponding genes, which are included in ARCHIPELAGO, represent 95 original kinases used for TAP-TAG and yeast two-hybrid baits as well as the 291 identified targets (preys). A significant number of these targets (30 proteins) (Table 3) happened to be differentially regulated in interactions with *Magnaporthe oryzae*, corre-

sponding to 11 of the 95 original kinases. Thus, a large portion (at least 10%) of the genes that associate with kinases are defense-related genes. Interestingly, three proteins found to be ubiquitous markers (Table 2) also interacted with kinases: two metallothioneins (Os01g10400 and Os05g11320) and *PBZ1* (Os12g36880).

Five of the kinases used as baits (Os01g47530, Os02g40240, Os10g10130, Os02g06930, and Os05g05160) that interact with defense-related genes were also described as differentially regulated upon infection. Like most kinases in Table 3, these genes belong to the RD kinases, a class of kinase not originally associated with disease resistance (Dardick et al. 2007). Five other kinases used as baits (Os01g02580, Os01g32660, Os01g47530, Os03g61060, and Os05g45420) were found associated with two or three defense-related genes, suggesting that large protein complexes could be transcriptionally regulated upon infection.

Archipelago-like structure of the rice defense arsenal.

In the course of building the ARCHIPELAGO resource, we often observed small regions of the genome where several paralogous copies of defense-related genes were concentrated in groups or clusters. Although this is well established for RGA (Monosi et al. 2004), this was quite unexpected for defense-related genes. Thus, we extensively scanned the rice genome using the dedicated software SCANBLOCK (J.-B. Morel and C. Perin, *unpublished data*) and identified the regions where such clusters occurred. The biological processes of the corresponding genes as well as a random control pool of genes were established using automated annotation tools (Ouyang et al. 2007; Yuan et al. 2005; data not shown) and are shown Figure 2. As expected, RGA are found organized in clusters (100 to 140 clusters detected). Some families, such as UDP-gulcosyl transferases (Fig. 2) and cytochrome P450 (included in secondary metabolism), were previously observed by Wisser and associates (2005). More surprisingly, all the biological processes that were organized in statistically significant clusters in this analysis can be attributed to defense-related genes or cell-wall modification enzymes. For example, Dirigent

Fig. 2. Clusters of paralogs represent genes with functions related to disease resistance. Biological processes of the most statistically significant ($P < 0.05$, Bonferroni correction) clusters of paralogs in the rice genome are shown. A gene ontology (biological process) was attributed to each of the 1,848 clusters detected in the genome (black bars) and to a random pool of 1,500 genes not found in clusters (gray bars). Classes of functions were introduced for simplicity (data not shown) and χ^2 tests performed to compare clusters and random genes. A Bonferroni correction was included to take into account the number of tests. Only those classes for which the difference is significant are shown. LRR = leucine-rich repeat, WAK = wall-associated kinase, UGT = UDP-gulcosyl transferase, and LOX = lipoxygenase.

proteins (Davin and Lewis 2005), expansins (Li et al. 2003), and wall-associated kinases (Kohorn 2001) are proteins involved in cell-wall modification. Thus, genes organized in clusters tend to be defense-related genes and genes participating in cell-wall integrity.

Reciprocally, using the ARCHIPELAGO resource, we tested whether defense-related genes were preferentially organized in clusters. We found that 15% of the 2,562 defense-related genes were found in clusters, compared with 8.6% of a random pool of genes (Supplementary Fig. 4). Thus, the kind of clusters found (e.g., for *PBZ1*) (Fig. 1) are common to a large portion of the genes annotated as involved in defense. Therefore, the rice defense arsenal, like the *R* gene battery (Monosi et al. 2004), is organized in clusters of repeated copies of defense-related genes.

The observation that only individual members of the *PBZ1* cluster on chromosome 12 seemed to be differentially expressed (Fig. 1) prompted further testing to determine whether there was a differential use of each copy of a given cluster. Using quantitative reverse-transcriptase polymerase chain reaction, we measured gene expression of individual members of three clusters upon *M. oryzae* infection (Fig. 3; Supplementary Table 4). These experiments showed that there was coexpression of the

paralogous copies in some cases (Fig. 3A) but no apparent co-expression in others (Fig. 3B). Thus, more extensive genome-wide transcription analysis is required to establish the rules for the expression of such gene clusters.

Finally, we examined defense-related gene density across rice chromosomes (Fig. 4). The defense-related gene density varied from 4.4 on chromosome 11 to 10.5 genes/500 kb on chromosome 3. Thus, some chromosomes appear to be richer than others in the presence of defense-related genes. Surprisingly, chromosomes 11 and 12 are among the poorest in defense-related genes whereas they are among the richest for RGA (The Rice Chromosomes 11 and 12 Sequencing Consortia 2005).

It also became apparent from this analysis that some regions of the genome accumulate large numbers of defense-related genes from different functional categories. For example, a hot-spot of defense-related genes at the telomeric position of chromosome 12 contains 10 defense-related genes within 350 kb (Supplementary Fig. 5): one peroxidase, a cluster of five LTP (two of which are differentially expressed), and a cluster of four WRKY (three of which are differentially expressed). Thus, given this heterogeneity at the megabase level and the microstructure observed in clusters, the rice defense arsenal displays an archipelago-like structure.

Fig. 3. Gene induction for two clusters of defense genes upon infection by *Magnaporthe oryzae*. Rice Nipponbare plants were infected with the avirulent isolate CL3.6.7. **A**, Four out of five members of a Subtilase cluster show coregulation; **B**, four out of seven members of a Bowman birch cluster are not coregulated. Mean ratios (infected versus noninfected) from three independent experiments are shown. Gene expression was measured by quantitative reverse-transcriptase polymerase chain reaction according to Vergne and associates (2007). hpi = hours postinoculation.

Blast metaQTL colocalize with *IDR* genes and *PR* genes.

Ballini and associates (2008) detected no significant association between defense-related genes and QTL with disease resistance to blast. However, this could be due to the fact that defense-related genes are defined by their expression pattern, thus including genes that are indirectly differentially expressed upon infection. Therefore, these genes are not expected to directly participate to disease resistance. In their pioneering work, Wisser and associates (2005) did not detect statistical colocalization between disease resistance QTL and some candidate defense-related gene families known as putative regulators (WRKY, MYB, ERF, and bZIP). However, they observed that these families (except ERF) were more abundant in disease resistance QTL. Using the orthology database GreenPhylDB (Conte et al. 2008), we gathered a list of 377 *PR* genes from rice (Supplementary Table 5). We then asked for possible colocalizations between these *PR* genes and blast metaQTL. We found significant associations between partial resistance metaQTL (Ballini et al. 2008) and PR1, chitinases (PR3, PR4, PR8, and PR11), PR6 (protease inhibitors), and PR13 (thionins). We also observed a significant colocalization of metaQTL and *IDR* genes ($P < 0.05$), despite the limited number of identified *IDR* or putative *IDR* genes in rice (35 in this study). Thus, *IDR* genes and *PR* genes seem to explain QTL better than defense-related genes.

Although there is no global colocalization of defense-related genes and metaQTL, it remains possible that defense-related genes are responsible for some QTL in some cases. We identified four QTL smaller than 500 kb in which defense-related gene density is high (Table 4) and where there is no RGA. Interestingly, one of these QTL (q6G12) (Ballini et al. 2008) contains two endochitinases. One of the latter is one of a small number of genes under the control of CeBIP receptor for chitin (Kaku et al. 2006).

Concluding remarks.

The ARCHIPELAGO resource, combined with QTL data (Ballini et al. 2008), provides an important source of informa-

tion on genomic regions and markers that can be used for marker-assisted selection. For example, the genomic localization of disease regulators showed that, in some cases, several regulators (e.g., *OsRacB*, *EDS5*, and *OsBI-1* on chromosome 2) are concentrated in small chromosomal regions (Fig. 4). This may have important implications for marker-assisted selection.

This resource should be further expanded, particularly to include pathogens other than *Magnaporthe* spp., and future work should focus on such pathogens to establish more accurately possible overlaps between different plant-pathogen interactions. We are currently developing a user-friendly web-based tool (G. Droc, *unpublished*) to allow the rapid and easy integration of new datasets into the ARCHIPELAGO resource by the rice community.

The question of possible colocalization of defense-related genes and QTL remains unanswered. Whereas Wisser and associates (2005) suggested possible colocalization, exhaustive analysis by Ballini and associates (2008) using the ARCHIPELAGO data set indicates that, so far, there is no statistically significant link between defense-related genes and QTL. This apparent discrepancy can be explained by a combination of several factors. First, QTL studied by Wisser and associates (2005) included QTL against several diseases (mostly rice blast and bacterial blight), under the assumption that there should be broad-spectrum resistance loci. In their accompanying article, Ballini and associates (2008) show that, even in the case of one disease (rice blast), very few broad-spectrum QTL are described in the literature. Thus, it is unlikely that the hypothesis made by Wisser and colleagues was fulfilled. Second, we showed that there is limited overlap between defense-related genes induced by rice blast and bacterial blight. Thus, the mechanisms underlying resistance to these diseases may in fact be different, as also suggested by Li and associates (2006). However, testing *PR* genes separately for colocalization with blast metaQTL indicates that some *PR* families are more often found in blast metaQTL than elsewhere in the genome. Thus, *PR* genes represent potential candidates for QTL. The cloning of more disease QTL is now required to demonstrate globally

Table 4. Small meta-quantitative trait loci (QTL) rich in defense genes and devoid of resistance gene analogues

MetaQTL name ^a	Chr. ^b	CI (kb) ^c	Gene ^d	Defense gene content	
				Tigr name	Putative function ^e
q2G9	2	340	35	Os02g56700	Dihydroflavonol-4-reductase
				Os02g56810	Phloem-specific lectin
				Os02g56850	Glutathione reductase, cytosolic
				Os02g57160	ABC-1
				Os02g57260	3-Ketoacyl-CoA thiolase 2, peroxisomal precursor
q5G7	5	430	73	Os05g50560	OsMPK20-2—putative MAPK based on amino acid sequence homology
				Os05g50610	OsWRKY8v2—superfamily of rice TFs having WRKY and zinc finger domains
				Os05g50624	Transposon protein, putative, unclassified
				Os05g50750	ATP-binding protein
				Os05g50770	C4-dicarboxylate transporter/malic acid transport protein
q3G9	3	310	42	Os05g50890	Indole-3-acetic acid-amido synthetase GH3.5
				Os03g26970	Proteasome subunit α type 2
				Os03g27019	Expressed protein
				Os03g27030	Splicing factor, arginine/serine-rich 2
				Os03g27080	Calmodulin-binding transcription activator 2
q6G12	6	90	11	Os03g27230	Phospho-2-dehydro-3-deoxyheptonate aldolase 2, chloroplast precursor
				Os03g27250	Transferase, transferring glycosyl groups
				Os03g27310	Histone H3
				Os06g51029	OsFtsH1— <i>Oryza sativa</i> FtsH protease, homologue of AtFtsH1/5
				Os06g51050	Basic endochitinase 1 precursor
				Os06g51060	Basic endochitinase 1 precursor

^a Information from Ballini and associates (2008).

^b Chromosome.

^c Confidence interval size.

^d Gene content. Pseudogenes have been eliminated.

^e MAPK = mitogen-activated protein kinase.

Fig. 4. Defense genes and meta-quantitative trait loci (QTL) against *Magnaporthe oryzae*. Chromosomes were drawn according to genome physical data (scale bar). Intracellular disease regulator (*IDR*) genes are indicated to the right of each chromosome. MetaQTL are indicated as bars to the left of the chromosomes. Black bars represent “partial resistance metaQTL” and white bars represent other metaQTL (Ballini et al. 2008). The histograms attached to the chromosomes represent the defense gene density, as calculated in a sliding window of 500 kb. In QTL names, “F” represent field resistance metaQTL, “G” represent greenhouse resistance metaQTL, and “P” represent partial resistance metaQTL.

whether defense-related genes, *IDR* genes, or *PR* genes are behind QTL.

With the availability of the rice genome and metaQTL on the one hand and of insertion libraries and deletion mutants on the other, cloning metaQTL in rice is now feasible. Candidate gene approaches using reverse genetics strategies (Droc et al. 2006) can now be applied to small metaQTL to complete our understanding of quantitative resistance in rice.

The observation that the rice defense arsenal is massively organized in clusters adds a new layer of complexity to the disease resistance pathway. That *R* genes are clustered in the genomes of plants is well known. This likely favors unequal crossing-over and subsequent production of new alleles to face constantly changing pathogen populations (Michelmore and Meyers 1998). The most evolutionarily successful tandem du-

plication events are most likely to target genes at the end of biochemical pathways or genes representing flexible steps in a pathway, such as *PR* genes, where fluctuation in copy number is unlikely to affect downstream genes. It may not be surprising that clustered genes are enriched for duplication of biotic response genes, because these may provide a rich source of environmental responses, often without substantial damage to critical steps in genetic networks.

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AUTHOR-RECOMMENDED INTERNET RESOURCES

University of California-Davis Rice Kinase database: rkd.ucdavis.edu
 OryGenesDB database: orygenesdb.cirad.fr/
 GreenPhylDB database: greenphyl.cines.fr/