Magnaporthe oryzae Genetic Diversity and Its Outcomes on the Search for Durable Resistance

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1. Introduction

Rice is one of the most important cereal crops, feeding more than 50% of the world population. In some places in Asia, this cereal is responsible for more than half of the total calories intake. Also in Brazil this crop is essential to supply food and is one of the crops responsible for great economic incomes for the Brazilian southern states.

If we consider that the world populations will continue to raise for approximately another 30-40 years (until 2040-2050), so the absolute demand for food will also increase and it would be necessary for the agriculture in all parts of the world to make improvements in order to support the demand.

This required increase in crop production will occur in a context of mounting water scarcity, decreasing area and environmental degradation of arable land (that is partially caused by agriculture), increasing pollution, inevitable emergence of new races and biotypes of pathogens and pests, and possible adverse effects of climate change (Collard & Mackill, 2008).

Plant breeding will play a key role in this coordinated effort for increasing food production. Given the context of current yield trends, predicted population growth and pressure on the environment, traits related to yield stability and sustainability should be a major focus of plant breeding efforts (Collard & Mackill, 2008).

Meanwhile, if this requirement must be attended under environmental conditions of sustainability, this means that this increase in food production must be done under conditions of the best use of environmental goods and services while not damaging these resources (Pretty, 2008), and this represents an unprecedented challenge to scientists and farmers.

In front of this, studies about genetic background are essential for rice culture, to surpass actual productivity levels and mainly for adaptation of modern cultivars to actual environmental conditions.

Rice blast disease is the most important and destructive disease for rice crop, and is caused by ascomycete Magnaporthe oryzae. The disease is spread worldwide occurring in almost all
rice-producing areas and can be very destructive when environmental conditions are favorable. Disease occurrence and severity vary by year, location and even within a field depending on environmental conditions and crop management practices. Yield loss estimates from other areas of the world have ranged from 1-50%. It is estimated that each year this disease destroys enough rice to feed more than 60 million people. The economic losses are nearly unmeasurable, but some data suggests values over $70 billion of dollars.

The fungus *M. oryzae* presents genetic diversity and is able to recombine by parasexual reproduction, and this causes difficulties on develop resistant cultivars, being also responsible for the short living resistance of released cultivars.

Because of the narrow genetic basis of the elite lineages used for crosses, one of the main troubles in rice breeding is the search for resistance gene sources and how to use them in order to prevent great losses in the crop. Obviously it is necessary to apply a correct culture management in order to use obtained resistance as long as possible.

In this way, we present a shortly state-of-the-art about the pathogen and its epidemiology, what is reported about resistance to the disease, and something about our experience of breeding and management of these disease. We also explain that in our view, the most promising alternative is the use of cultivars with stacking resistance genes allied to a constant follow up on about pathogen genotype frequency by molecular tools. So, emerging of new pathogen races able to overcome resistance could be identified early and some decisions can be made in order to avoid the cultivars to lose their resistance to the disease over the time.

Knowledge about the pathogen population genetic structure could also be useful in order to guide breeding actions related to which and how many resistance genes to exploit for develop the new rice cultivars.

2. The pathogen and the disease

2.1 Rice blast

The first records about rice blast were made in China and date from 1637 (Ou, 1972). Since then, this disease has been reported in almost all world areas with Rice production. It is considered the most important and destructive rice disease.

Rice blast can occur on all above-ground parts of the plant and is observed since the earlier growing stages until the final grain production. Symptoms in leaves start as small brown necrotic lesions that evolve to larger elliptical or spindle-shaped and whitish to gray with darker borders (Figure 1A). On leaf margins, mainly on the flag leaf ligule area, the presence of encircling lesions can cause the leaf to fall. In the presence of favorable conditions, the lesions may enlarge and coalesce to kill the entire leaf and sometimes under severe conditions, the plant can be killed. Symptoms are also observed on culm, culm nodes and panicle neck node. Internodal infected areas are darker blocking the distribution of xylem sap and causing plant lodging. Nodal infection causes the culm to break at the infected node. Infection of panicle neck node is known as blast neck and is a critical factor for final productivity (Figure 1B). If infection occurs near the panicle exertion, grains are not formed and the panicle remains upward. When the panicle is infected at later stages, grains are partially formed, and sometimes the grain weight can cause panicle break at node region.
2.1.1 Etiology

The Rice blast disease is caused by the fungus *Pyricularia oryzae*, firstly described by Cavara in 1891 (cited by Ou, 1985). *P. oryzae* teleomorph is the filamentous ascomycete *Magnaporthe oryzae* originally described as *M. grisea* (Herbert, 1971). During some time, it was treated that isolates that infected Rice would be formally designated as *oryzae* and isolates affecting other species as *grisea*. Because there were not morphological differences that could be used to discriminate the rice isolates from isolates of other species, it was established that all members of the group would be designated as *M. grisea* or *P. grisea* (Rossman et al., 1990). However, Couch & Kohn (2002) results using multilocus gene genealogies demonstrated that isolates infecting *Digitaria* spp. were distinct and it was established that these isolates would be designated as *M. grisea*, whereas isolates affecting rice and the other species would be identified as *M. oryzae*.

The anamorph *P. oryzae* produces piriform shaped conidia with one to two transversal septa, slightly darked or hyaline, linked to conidiophore by its larger bottom. Conidiophores are septated, simple, rarely branched, showing sympodial growth and slightly browned. The teleomorph *M. oryzae* has not been found in nature, but it has been produced after crossing appropriate compatible isolates in laboratory. The teleomorph stage produces hyaline ascospores, typically fusiform shaped with three-septate and enveloped by a unitunicate asci.

2.1.2 The disease cycle

During maturation, the conidia, who remains tied to conidiophore, produces a mucilage that in presence of water helps its fixation to the host (Hamer et al., 1988). This mucilage is mannose rich and presents affinity with lectin concanavalin A found in plants (Hamer et al., 1988).

The conidia germination and appressoria formation are controlled by environmental stimulus and germ tube is not formed if conidia were maintained in water, avoiding anticipated germination (Lee & Dean, 1993). The principal stimulus is related to hydrophobic conditions on plant surface. According Hamer et al. (1988), as more wax is disposable on leaf surface where conidia are deposited more effective is the formation of appressoria. The appressoria capacity on penetrate the plant surface is dependent of turgor pressure. The lack of capacity to form this high pressure is related to chemical components or genetic alterations that could interfere on melanin biosynthesis. Melanin is a compound of appressoria cell wall acting as barrier against permeability, allowing increase on some cytoplasmatic solute concentrations (Howard & Ferrari, 1989). In presence of high turgor pressure, the penetration peg formed by appressorium press the epidermic cells until its rupture, allowing fungi penetration. On cell lumen, *M. oryzae* colonizes tissue adopting hemibiotrophic lifestyle. Invasive hyphae are involved by a membrane that separates it from cell host cytoplasm, a characteristic of biotrophic fungi (Kankanala et al., 2007). The fungi progresses to the next cell without cause damages to the cell wall, probably because this happens by plasmodesmata (Kankanala et al., 2007). Invasion of the neighbor cell coincides with the loss of viability of the previously infected cell, initiating the necrotrophic phase and appearance of lesions, around 74 to 96h after conidia deposition on plant surface. On
presence of high relative humidity (≥93%), conidia are released less than 24h after lesions appearing, with the highest conidia production occurring among 3 to 8 days after sporulation beginning. Each lesion from a susceptible host can give rise to more than 20.000 conidia over several days (Barksdale & Asai, 1961).

The infection and colonization described above occurs in aerial plant tissues such as culms and leaves and is different from the process that takes place on root system. On roots, appressoria are not formed and penetration pegs are produced directly from hyphopodium initiating penetration on rhizodermal cells (Sesma & Osburn, 2004; Marcel et al., 2010). On next step, a differentiation of invasive hyphae occurs and new cells are invaded, but the previously infected cells are not damaged. So, a biotrophic interaction occurs without producing lesions on infected tissues. However, around four weeks after root infection, symptom disease appears on plant stems and leaves, showing the systemic pathogen distribution (Marcel et al., 2010).

2.1.3 Epidemiology

The primary inoculum is originated from infected seeds, crop residues and spores dispersed by wind from neighbor farming areas. Kingsolver et al. (1984) reported that the mycelium present on field remainder rice straw could survive for around six years. Infected seeds can cause blast on initial steps of vegetative development, but rarely causes disease epidemies. More than 50 other *M. oryzae* hosts are known, including cultivated species (Metha & Baier, 1998) and weed species (Mackill & Bonman, 1986), however cases of crossed infections are rare. The main inoculum sources for secondary infections are the sporulating lesions on infected leaves, characterizing a polycyclic disease.

The rice plants presents its highest vulnerability period when young plants hold 3 to 4 leaves and during flowering. Blast disease is also favored by temperatures among 20 to 30 ºC and relative humidity over 90%, with production of conidia on lesions beginning when humidity surpasses 93%. Frequent occurrence of dew, mist and drizzle are suggestive of ideal conditions to the pathogen. Disease severity is also affected by soil fertility. High levels of organic matter or excessive use of nitrogen can lead to the disease increase when the cultivar resistance is not specific. However, nitrogen deficiency can predispose plants to disease (Prabhu et al., 1996).

![Fig. 1. Rice blast symptoms on leaves (A) and on panicle neck node (B).](image-url)
2.2 *Magnaporthe oryzae* genetic diversity

2.2.1 Genetic diversity

In order to know the *M. oryzae* population structure around the world, many studies were done using molecular markers mainly based on MGR 586 repetitive DNA sequence (Hamer et al., 1989), and pathogenicity tests on differential set of rice varieties. Levy et al. (1991) analyzed 42 isolates obtained from an isolates bank collected during 30 years representing the most common lineages infecting USA commercial rice cultivars and verified the occurrence of eight lineages. On the Philippines, 1156 isolates were obtained on 38 cultivars sowed in two growing seasons and after analysis were grouped in only ten lineages (Chen et al., 1995). Simple population structure was also observed in Europe (Roumen et al., 1997) and Africa, when isolates from four different countries were separated in nine lineages (Takan et al., 2011). Results found in the literature reports a limited number of the pathogen lineages, showing a clonally typical population structure (Levy et al., 1993; Xia et al., 1993; Xia et al., 2000). Despite the low genetic diversity presented by *M. oryzae*, a high pathogenic variability was observed in pathogenic populations. In Colombia, 39 *M. oryzae* pathogenic races were identified (Levy et al., 1993). In Brasil, a similar study identified 45 races (Anjos et al., 2009), with a frequent change on *M. oryzae* races composition (Ribeiro & Terres, 1987).

Among the factors affecting the low *M. oryzae* population diversity are the uniformity of rice culture management and adverse environmental conditions to the pathogen. In this way, Kumar et al. (1999) realized a study in a Himalaya area (India northern) belonging to rice diversity Center. At this region, there is a high diversity of rice cultivars that remained isolated in different valleys during thousands of years and cultivated under different growing systems, in an heterogeneous environmental that favored the pathogen. Using 458 isolates collected in 29 valleys, it was possible group them in 56 lineages, showing a considerable higher genetic diversity than that obtained out of rice origin center.

The low genetic diversity with a substantial pathogenic diversity found in some places and a higher genetic and pathogenic diversity showed in other areas suggests that different mechanisms are acting in order to create genetic variability in *M. oryzae*.

2.2.2 Variability mechanisms

2.2.2.1 Sexual recombination

*M. oryzae* is a heterotallc fungus with sexual recombination controlled by a single locus (MAT1), that presents two mating types, MAT1-1 e MAT1-2. Both of them are essential for sexual recombination (Kang et al., 1994). *M. oryzae* isolates can be hermaphrodite, male fertile or sterile, but crossing only occurs between fertile isolates from different mating types, and when at least one isolate is hermaphrodite (Zeigler, 1998). Many studies were conducted in order to evaluate the frequency of the different mating types around the world. In Argentina, analysis of 125 isolates collected between 2000 and 2003 showed that all of them belonged to mating type MAT1-1 (Consolo et al., 2005). Similar results were obtained in Korea, when the analysis of 254 isolates demonstrated that all of them also belonged to MAT1-1 (Park, et al., 2008). In Africa studies revealed that 29% of isolates belongs to MAT1-1 and 71% to MAT2-2, but none of the isolates was hermaphrodite, avoiding crossing among them (Takan et al. 2011). When two *M. oryzae*
populations from Himalayas were analyzed, it was found that 22% of the isolates belonged to MAT1-1 and 43% to MAT1-2, and isolates male fertile and hermaphrodite were detected, suggesting a possible occurrence of sexual recombination in this region (Kumar et al., 1999).

There is a consensus that the majority of the *M. oryzae* populations lost its capacity of sexual reproduction. One reason could be the founder effect, because when rice culture was introduced in a new area, probably only one mating type was established (Zeigler, 1998). It is also possible that in some cases the MAT locus could be linked to an avirulence gene, with no compatibility with the prevailing Rice cultivar, and this could represents a drawback for fitness (Notteghem & Silue, 1992), being eliminated along time. The frequent chromosomic rearrangement derived from DNA mobile genetic elements (Chuma et al., 2011) can cause the degeneration of sexual behavior without producing a drawback of fitness.

One role of the sexual reproduction is to form resistance structures for safeguarding the fungi on adverse situations. When rice is cultivated on areas where the weather conditions enable the fungi to survive on a mycelia form during many fallow seasons, mutations that remove sexual reproduction could be fixed because of high energy costs of this character. It is also possible that rice was introduced in worldwide areas where weather conditions would be adverse for sexual reproduction occurrence (Zeigler, 1998).

### 2.2.2.2 Parasexual reproduction

Parasexual recombination in *M. oryzae* can be an alternative to the sexual cycle, because these cycle rarely occurs. Parasexual cycle involves the formation of anastomosis (haploid hiphal fusion) resulting in heterokaryotic cells that can undergo karyogamy and become a diploid cell and then be able to realize mitotic recombination. The return to the haploid stage occurs by successive lost of one chromosome from each pair of homologous chromosomes (aneuploidy) (Crawford et al., 1986, Noguchi, 2011). Parasexual recombination possibility was first suggested by Yamasaki & Niizeki (1965) that observed anastomosis formation and obtained variants matching auxotrophy isolates. Concomitant culture of genetically distinct *M. oryzae* isolates originated higher new haplotype frequency spontaneously than pure cultures (Xia et al., 1993). Zeigler et al. (1997) carried out matching of genetically different isolates and observed growing of mycelium tufts between cocultured isolates. From formed anastomosys new haplotypes were found, indicating the presence of recombination. The fact that isolates from the same or from different populations can easily recombine corroborates the theory that vegetative compatibility is weakly controlled in *M. oryzae* (Crawford et al., 1986). When virulence spectrum and adaptability from parasexual recombinant isolates were evaluated, it was verified that they could infect Rice cultivars that were not infected by its parents (avirulent) (Noguchi et al., 2007). This study also found that the pathogenicity of recombinant isolates is stable and did not change after seven generations, and that the adaptability is intermediate when compared to the parents. But it was also observed that aggressiveness of new *M. oryzae* recombinants can increase after some years of rice cultivation (Fujita & Suzuki, 1982). Parasexual recombination is a process that generates genetic variability, but could also be a mechanism to restore the genome, preventing that the mutation accumulation endangers pathogen fitness, but it remains unproven at this date.
2.2.2.3 Mutation

A common trait observed among *M. oryzae* is the high genetic instability of characters related to morphology, fertility and pathogenicity when isolates are maintained in laboratory (Valent & Chumley, 1991). The *Bufl* gene, which is involved in melanin synthesis, an essential role for penetration process, presents a mutation rate that can reach 20% (Chumley & Valent, 1990).

The *M. oryzae* x *O. sativa* interaction belongs to gene-gene system (Jia et al., 2000) and mutations in avirulence genes (*Avr*) can be decisive for plant-pathogen relationship. Some of *M. oryzae* avirulence genes are located in telomeric and sub-telomeric regions, as is the case of *Avr-Pita* (Orbach et al., 2000), *AvrMedNoi-1; AvrKu86-1* (Dioh et al., 2000), *Avr-Piz* (Luo et al., 2002), *Avr-Pii* (Yasuda et al., 2006), *Avr-P15* (Ma et al., 2006), and *Avr-Pit* and *Avr-Pia* (Chen et al., 2007). Deletions in these regions could result in *Avr* genes lost, resulting in virulence gain, as demonstrated by Orbach et al. (2000). These authors identified spontaneous mutants that suffered deletions on *Avr-Pita* gene and so they were able to infect cultivars with resistance codified by the correspondent *Pi-ta* gene. With *M. oryzae* complete genome sequencing it was verified that 9.7% of the genome was constituted by repetitive DNA sequences (Dean et al., 2005). Substantial part of these sequences are transposons, belonging to eight families, five of them of retrotransposons and three of DNA transposons (Dean et al., 2005). Some of these transposons presented more than hundred copies dispersed over the genome (Kachroo et al., 1994). The highest transposon concentration was observed on telomeric regions, hosting around 24% of these elements (Rehmeyer et al., 2006). The presence of multiple transposon copies in regions next to *Avr* genes could result in recombination among copies of the same element, resulting in deletion of one copy, which could take away one *Avr* gene (Rehmeyer et al., 2006). Farmanet et al. (2002) studied the *Avr1-CO39* gene structure on isolates from different origin and verified that these structure present variation and is not functional in most of the cases. Analysis of regions flanking *Avr1-CO39* allows the identification of RETRO5 and REP1 transposons suggesting that recombination among copies of these elements is related to the frequent alterations observed on *Avr1-CO39* gene structure.

Transposons are recognized as hotspots for recombinations, but can also operate actively introducing themselves in codifying genome regions, resulting in inhibition or alteration of gene expression. Kanget et al. (2001) showed that avirulent *M. oryzae* isolates to cultivars whose resistance was mediated by *Pi-ta* gene could infect the cultivar when transposon Pot3 was inserted on *Avr-Pita* regulatory region. Transposon insertions on avirulence genes were observed also for the gene *Avr1-CO39* (Farman et al., 2002), for genes controlling morphologic traits as *Acr1*, which are involved on conidia morphological control (Nishimura et al., 2000). Recently, it was demonstrated that transposons can move through the genome and can carry *Avr* genes located next to them. Chuma et al. (2011) verified that the *Avr-Pita* gene could be located in different places of the same chromosome, in different chromosomes and also in supernumerary chromosomes, and that it was flanked by other transposons possibly involved with its translocation. This work also suggests that the *Avr-Pia, Avr-Pik* and *Avr-Pii* genes also suffered multiple translocation events.

Presence of *Avr* genes on *M. oryzae* supernumerary chromosomes indicates its involvement on avirulence genes dynamics. These chromosomes are known to be easily lost from the
genome, probably because they are unnecessary (Johnson et al., 2001). This property could be adaptively advantageous because pathogen population can adapt quickly to cultivars carrying correspondent \( R \) genes by losing supernumerary chromosomes. As these chromosomes containing \( Avr \) genes could be horizontally transferred (Akagie et al., 2009) by recombination to isolates that had lost briefly its \( Avr \) genes because of cultivar resistance, these restored \( Avr \) genes could confer some fitness to the pathogen population.

### 2.2.3 Resistance to blast

Two basic resistance types commonly recognized can be used to control rice blast disease, partial and complete resistance. The complete resistance is race specific and governed by one or few \( R \) genes, dominant or recessive, that recognizes the product of the pathogen \( Avr \) gene (Jia et al., 2000). In this cases, when resistance is effective, absence of disease symptoms are observed, whereas when resistance is not effective, disease symptoms occurs in large scale. On the other hand, partial resistance is not race specific and is controlled by QTL’s (Quantitative Trait Loci) characterized by reducing the number and size of lesions (Vergne et al., 2010), acting in a similar manner for different pathogen races. But specifically for rice, some exceptions can be considered. Zenbayashi-Sawata et al. (2005) identified an avirulence gene (\( Avr-Pi34 \)) correspondent to the resistance gene \( Pi34 \), that confers partial resistance. Analyzing the resistance spectrum of a set of rice QTL’s, it was verified that only three of them conferred broad spectrum resistance (Ballini et al. 2008). These results suggest that partial resistance is sometimes specific and does not necessarily have a broader resistance spectrum when compared to complete resistance (Ballini et al., 2008).

A careful analysis of a set of previously described rice blast resistance genes allowed identification of 85 resistance genes and around 350 QTLs (Ballini et al., 2008). Until now, 15 \( R \) genes have been cloned (Table 1, adapted from Liu et al., 2010) and others are mapped (Ballini et al., 2008), being available for use in breeding programs. If we consider the \( M. oryzae \) genetic variability and its capacity to adapt to new rice cultivars, the use of blast resistance genes in isolate, alternate or simultaneous manner in different cultivars could cause the overcome of each of them individually. This could allow the pathogen population to form a ´pathogenic memory´, or in other words, to select races able to infect the cultivars carrying each of the resistance genes. To overcome the resistance, pathogen populations just need change races frequency according the cultivars.

The short living effectiveness of cultivars with single gene resistance because of race changes in pathogen populations had lead resistance breeding strategies usually take strategies as the gene stacking or the multiline or cultivar mixture approach. The gene stacking approach incorporates different resistance genes into a plant selection that is then grown as a homogeneous population, whereas the multiline and mixtures strategies disperses resistance genes among different plants that are grown as a heterogeneous population (Wilson et al., 2001).

In front of this, we propose that the highest possible number of genes assembled in the same cultivar, in a process known as pyramiding of genes (Servin et al., 2004) could be the more effective way to face the rice blast disease in our rice culture conditions. But pyramiding genes could enable the emerging of a pathogen ´super-race´ able to overcome resistance in a single event. As it was previously discussed, the overcome of a \( R \) gene by the pathogen is
possible because of the lost of the \textit{Avr} function. Some works had demonstrated that \textit{Avr} genes are involved with pathogen adaptability acting on processes of host infection and colonization (Leach et al., 2001). So, the higher the number of \textit{Avr} genes that the pathogen need to lost, the lower would be its capacity to cause the disease, until a level that it would fail on cause symptoms. Assuming that this is true, why rice genotypes with stacking genes were not naturally be selected during evolution? Possibly because pyramiding genes represents an additional physiological cost for the plant fitness. Tian et al. (2003) measured the introgression cost of the resistance gene \textit{Pseudomonas syringae RPM1} in \textit{Arabidopsis thaliana}, verifying that isogenic lines that received the gene produced around 9\% less seeds when compared to normal plants. This resistance cost is also observed on plants that were submitted to chemical resistance induction. Wheat plants treated with acibenzolar-S-methyl to induce resistance, showed reduction on number of tillers and number of spikelets in each ear (Heil et al., 2000). So, if a rice cultivar with pyramiding genes against blast disease would be developed, possibly it would present the similar results, increasing resistance and decreasing productivity. There are also cases where resistance genes are linked to agronomic undesirable traits, as occurs with \textit{Pi-21} gene, that is linked to a gene codifying for chalky on rice grain (Fukuoka et al., 2009).

\begin{table}[h]
\centering
\begin{tabular}{lll}
\hline
\textbf{R gene} & \textbf{Codified protein} & \textbf{Reference} \\
\hline
\textit{Pib} & NBS-LRR & Wang et al., 1999 \\
\textit{Pi-ta} & NBS-LRR & Bryan et al., 2000 \\
\textit{Pi9} & NBS-LRR & Qu et al., 2006 \\
\textit{Pi2} & NBS-LRR & Zhou et al., 2006 \\
\textit{Piz-t} & NBS-LRR & Zhou et al., 2006 \\
\textit{Pi-d2} & Kinase receptor & Chen et al., 2006 \\
\textit{Pi36} & NBS-LRR & Liu et al., 2007 \\
\textit{Pi37} & NBS-LRR & Lin et al., 2007 \\
\textit{Pikm} & NBS-LRR & Ashikawa et al., 2008 \\
\textit{Pit} & NBS-LRR & Hayashi and Yoshida, 2009 \\
\textit{Pi5} & NBS-LRR & Lee et al., 2009 \\
\textit{Pid3} & NBS-LRR & Shang et al., 2009 \\
\textit{Pikh} & NBS-LRR & Rai et al., 2009 \\
\textit{Pikp-1} & NBS-LRR & Yuan et al., 2011 \\
\textit{Pikp-2} & NBS-LRR & Yuan et al., 2011 \\
\hline
\end{tabular}
\caption{Cloned rice blast resistance genes and the proteins codified by them.}
\end{table}

So, the selection of genes for pyramiding must be done carefully, in order to introgress genes with broader resistance spectrum and whose \textit{Avr} genes are not easily mutated, as occurs with \textit{Avr-Pita} gene.

3. Breeding rice for resistance to blast

The \textit{Oryza} L. genera belongs to the Poaceae (Gramineae) family and encompasses around 25 species, of which 23 are considered wild and only two are cultivated, \textit{O. sativa} L. e \textit{O. glaberrima} Steud (Khushi, 1997). \textit{O. sativa} species is cultivated worldwide and \textit{O. glaberrima} only in Africa.
The commercial exploitation of a reduced genetic base and the prevalence of a small set of landraces in the breeding process has been the general approach for several crops species. Compared with other crop species, the genetic diversity in the world rice germplasm is quite large. Despite the richness of genetic resources, only a small proportion of the world rice germplasm collections have been used in breeding programs. As a consequence a high genetic similarity is found within several commercial rice germplasms around the world. The limited use of the rice genetic diversity available worldwide has been a concern in Latin America since the late 1980s. Although differences in genetic diversity and relatedness are observed within rice germplasms of different countries, the general feature is a very close relationship among cultivars (Cuevas-Perez et al., 1992; Guimarães et al., 1996; Fuentes et al., 1999). For example, a study demonstrated that irrigated rice varieties currently under cultivation in Venezuela were closely related among them and with cultivars from Colombia, Brazil and Ecuador (Cuevas-Perez et al., 1992). In Brazil, studies demonstrated that Brazilian rice cultivars presents a narrow genetic basis, with only 10 parents responsible for 68% of the genetic pools (Rangel et al., 1996). The same study also showed that only Tetep and Tadukan cultivars were used as gene sources for blast resistance to all Brazilian commercial rice cultivars at that time. But there was little progress in this way, and this still a troubling condition if we consider the very large areas that are cultivated with rice in Brazil.

The Brazilian experience has demonstrated that rice cultivars with complete resistance were short lived. As an example, rice cultivars Metica 1 and Aliança were overcome by pathogen only one year after released. Javaé cultivar presented high resistance levels, but was overcome two years after. In 1997, Javaé cultivar was substituted by Formoso cultivar, and the first results were promising. However, in the 1999/2000 growing season, the resistance was overcome, causing great losses for farmers who did not used fungicides.

So, efforts are being done in order to better understand the rice-Magnaporthe pathosystem, on aspects like search for resistance genes and establishment of pathogen population structure for development of cultivars with long live resistance. Efforts are also being done about cultural practices that can help farmers to produce rice without damaging environmental and his own health, and so, supply the final consumer with highest quality rice.

3.1 The experience of Epagri’s rice breeding programme

Rice is one of the major crops for Brazil and for the state of Santa Catarina, and the annual production value for this state represents around US$ 300 millions. The state of Santa Catarina has an area of 1.12% of the Brazilian territory, although the area that could be farmed is only 12% of its territory, nevertheless, this state is ranked the fifth food supplier in Brazil. The total state rice area of 149,000 ha in 2010 growing season is cultivated by 8,500 farmers, in 11,000 small farms belonging to 83 counties and the seeding system is water-seeded (Figure 2).

The Santa Catarina State rice yield in 2009/10 was estimated in 7.0 t/ha, whereas in some regions, like High Itajaí Valley, the yield can reach up to 12 t/ha (Figure 3). In fact, yields of 10 t/ha are very common among farmers that use the recommended technologies, including modern varieties. The establishment of paddy rice as a major crop and its economic
feasibility in the small family owned farms in Santa Catarina, is the result of the technologies developed by public research and rural extension efforts.

The consequences, in this sense, are the attainment of very high rice yields, which can, in great part, be attributed to the use of the modern rice varieties developed by Epagri (Santa Catarina State Agricultural Research and Rural Extension Agency). In the late 70’s yields were around 2.3 t/ha. Epagri’s Breeding Program started in 1976 at Itajaí Experiment Station. Since 1980 Epagri has released 17 varieties (Table 2). This was decisive to improve rice yield in Santa Catarina. Epagri’s varieties are spread all over the rice irrigated areas in Brazil, reaching also other countries like Paraguai, Bolivia, Argentina and Venezuela.

The general goals of Epagri’s Rice Breeding Program are developing improved genotypes of long grains, to improve blast resistance and grain quality. Recently, the program is directed to promote genetic variability and also to develop technologies that have lower environmental impact.

In the beginning of the research program, local grown varieties brought by immigrant farmers from Italy, were replaced by new lines from national and international institutes.

The methods currently used in the Epagri’s breeding program are pedigree, recurrent selection and induced mutations. Annually, around 230-300 different crosses and backcrosses are done. These originate at least 100,000 individual F₂ and F₃ plants which are submitted to field selection to compose the preliminary studies which include 300 F₄ families, and in the next year, 200 F₅ families. The F₁ to F₅ populations are hand transplanted to the field (Figure 4). In the sequence about 30 to 50 F₆ lines form the advanced experiment in broadcast sowed plots. In the final stages, normally not early than in F₇, the advanced lines (around 20) are tested in 5 different locations (on-farm

Fig. 2. Aspect of rice culture in Santa Catarina State, with rice growing in water-seeded system.
trials) for three years through the three major rice areas of Santa Catarina. The management of this rice fields follows Epagri’s recommendations. In these experiments, each variety is broadcast sowed in 4 x 15m plots.

Fig. 3. Overview of a rice crop field on High Itajaí Valley.

Fig. 4. Overview of Epagri’s Rice Breeding Program experimental field.

It is unavoidable to conclude that Epagri’s Rice Breeding Program is successful and has contributed, recommending varieties from abroad and, developing new varieties, to increase rice yield in Santa Catarina (only paddy rice considered). The highly quality and yielding varieties released by the Breeding Program of Epagri contributed for the State to hold the highest rice yield in Brazil.

But in recent years, blast disease emerged as a potential destructive disease for rice culture in Santa Catarina State. And new strategies were planned in order to face the problem and improve genetic resistance in Epagri’s rice released cultivars. These strategies include cross elite rice lines with known resistance sources, increase of the genetic base by crossing stabilized lines with other Oryza species and developing of cultivars with pyramiding genes.
3.1.2 Crosses with resistance sources

Epagri’s Rice Breeding Program have been using rice cultivars and lineages known and tested for resistance to blast, as Tetep, Oryzica Llanos 5, NP-125, Raminad Str. 3, WC 299 and WC 277. This blast resistance sources were all used in crosses with elite rice cultivars and the progeny was conducted by Pedigree method. Despite all efforts made to improve the efficiency for selecting and developing rice cultivars, blast resistance is continuously being lost in breeding lines after the F3 – F4 or later generations. In our experience, Raminad and Oryzica Llanos 5 produced the higher number of plants with some good agronomic traits and resistance to blast, but they failed in final comparing assays related to productivity and grain cooking quality. So, until now, Epagri’s released cultivars were not directly related to these blast resistance sources.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Releasing year</th>
<th>Yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empasc 101</td>
<td>1980</td>
<td>6.79</td>
</tr>
<tr>
<td>Empasc 102</td>
<td>1980</td>
<td>6.66</td>
</tr>
<tr>
<td>Empasc 103</td>
<td>1981</td>
<td>6.38</td>
</tr>
<tr>
<td>Empasc 104</td>
<td>1985</td>
<td>7.41</td>
</tr>
<tr>
<td>Empasc 105</td>
<td>1987</td>
<td>7.79</td>
</tr>
<tr>
<td>Epagri 106</td>
<td>1992</td>
<td>6.86</td>
</tr>
<tr>
<td>Epagri 107</td>
<td>1994</td>
<td>7.27</td>
</tr>
<tr>
<td>Epagri 108</td>
<td>1995</td>
<td>7.83</td>
</tr>
<tr>
<td>Epagri 109</td>
<td>1996</td>
<td>8.90</td>
</tr>
<tr>
<td>SCS BRS 111</td>
<td>2000</td>
<td>7.00</td>
</tr>
<tr>
<td>SCS 112</td>
<td>2000</td>
<td>7.29</td>
</tr>
<tr>
<td>SCSBRS Tio Taka</td>
<td>2002</td>
<td>8.84</td>
</tr>
<tr>
<td>SCS 114 Andosan</td>
<td>2005</td>
<td>9.07</td>
</tr>
<tr>
<td>SCS 115 CL</td>
<td>2007</td>
<td>7.90</td>
</tr>
<tr>
<td>SCS 116 Satoru</td>
<td>2010</td>
<td>9.40</td>
</tr>
<tr>
<td>SCS 117 CL</td>
<td>2011</td>
<td>8.90</td>
</tr>
</tbody>
</table>

Table 2. Epagri’s Rice Breeding Program released cultivars, with respective year or release and yield.

In 2010/2011 growing season, twelve lines originated from crosses with blast resistance sources are being evaluated for agronomic and resistance traits in final assays. Six of these lines were originated from crosses with Oryzica Llanos 5, three from WC 299, two from WC 277 and one from NP 125. On next growing season, this lines would be tested again in field assays order to observe their agronomic and resistance traits. These assays are done for three years in five different counties covering the several rice growing areas in Santa Catarina State.

3.1.3 Crosses with other Oryza species

The wild species of Oryza contains numerous genes of economic importance and could be used as alternate sources of resistance or tolerance to biotic and abiotic stresses to enrich the cultivated rice gene pool (Jena, 2010).
Efforts were also done related to crosses with other rice species, as *O. rufipogon*, *O. nivara*, *O. latifolia* and *O. glumaepatula*. These four species were introduced in Epagri’s Rice Program in 2006 and since then are used in crosses with elite cultivars (SCS 116 Satoru, SCS 114 Andosan, SCSBRS Tio Taka, Epagri 109, SCS 112, SC 339 and SC 213, among others). The main objective for use this species is the increase of genetic variability of the Epagri’s rice elite lines and the introduction of new genes related to resistance to diseases and pests. This are being done by the backcrossing method, because these species also present undesirable traits for a modern rice cultivar.

These four species are known for its traits related to tolerance/resistance to biotic and abiotic stress (Thanh et al., 2011; Nonomura et al., 2010; Vaughan et al., 2005, Rangel et al., 2006). In the 2010/2011 growing season, when evaluating the F3 generation, segregant populations originated from crosses between *O. glaberrima* and SCS 112 presented good agronomic traits and a productivity of more that 10 t/ha. Despite this promising results, these progenies will be carefully observed in the next segregant generations for its traits related to disease resistance, mainly for blast disease. Also in this growing season, F4 segregating populations originated from the cross *O. glumaepatula* / SC 355 // SC 213 presented productivities ranging from 8,5 to 10 t/ha, and interesting agronomic traits related to grain quality. The species *O. nivara* and *O. rufipogon* were recently crossed with Epagri’s rice lines, and in the 2010/2011 season were initial segregating generations F2.

Crosses with these species are being done in the last four years, and the resulting progenies are being observed and selected in the four first segregating generations. If the promising initial results becomes true, we hope that in the next years we could release a rice cultivar with desired agronomic traits, quality and productivity allied to traits related to blast resistance.

### 3.1.4 Breeding for durable resistance

Considering the significance and the potential losses that blast causes, rice breeding programs since early dispended efforts on developing cultivars with resistance to the disease. Therefore, crosses among elite cultivars and lines with resistance genes were extensively done. However, during breeding process, evaluations were done based on resistance phenotypic traits, leading to selection of lines/cultivars with complete resistance. This happen because it is difficult to identify and evaluate the partial-resistance on this segregating lines, and this leads to erosion of this resistance form. As a final result, cultivars with resistance based on one or few *R* genes were obtained and released, causing an overcome of this resistance after two or three years.

With the conclusion of the rice genome sequencing (Goff et al., 2002; Yu et al., 2002) that allowed cloning and mapping several *R* genes and QTLs, a high number of molecular markers were available for MAS (marker assisted selection), mainly to follow traits as resistance gene during segregant generations (Jena & Mackill, 2008). So, it is possible to obtain lines that carry genes codifying for partial and complete resistance. These lines are interesting because if the pathogen overcomes the *R* genes, plants are not completely vulnerable, remaining with partial resistance.

Incorporation of durable disease resistance into susceptible rice backgrounds has often been difficult using conventional breeding methods. Conventional breeding programs have been
limited to monogenic, race-specific resistance genes since they are easily introgressed into susceptible rice elite lines through simple backcrossing techniques. However, single gene resistance has often proven ephemeral and highly vulnerable to dynamic and diverse plant pathogen populations. Therefore, breeders are endeavoring to shift to breeding durable forms of resistance by pyramiding race-specific genes into a single cultivar. Conventional gene pyramiding requires extensive disease screenings with several races of the pathogen due to the race specificity of many of these genes after each cycle of crossing. Further complicating a pyramiding effort is the frequent absence of an effective selection method due to a lack of differentiating races. Testcrossing to susceptible genotypes is required in each cycle to detect the presence (or absence) of the masked genes due to epistatic interactions between many resistance genes. In addition, as highly effective resistance genes that are effective to many races of the pathogen are incorporated into breeding populations, valuable hypostatic genes are easily lost in each backcross cycle. MAS of hypostatic genes would facilitate their maintenance in pyramiding populations. Molecular markers tightly linked to resistance genes have facilitated their pyramiding into single elite cultivars, with the possibility of creating more durable or broad spectrum resistance (Saghai-Maroof et al., 2008).

Breeding for durable resistance against blast disease would be more effective with a previous knowledge of the pathogen populations from the country or place where the future cultivar will be cultivated. This characterization can be done by pathogenicity tests on international set of rice varieties (Atkins et al., 1967) or by molecular markers (George et al., 1998). When pathogen population structure is known, effective resistance genes could be easily identified. At the present time, isolines (Mackill & Bonman, 1992) and lineages holding only one known resistance gene are available, in order to help plant breeder in this first steps to develop a resistant cultivar.

We have done a previous study on *M. oryzae* pathogen population covering Santa Catarina State (unpublished data) using Pot-2 marker. We were able to identify 13 haplotypes that were inoculated on international set of rice varieties (Atkins et al., 1967), allowing us to classify the isolates in six distinct races. The inoculation of isolates representing these six races on isolines developed by Mackill & Bonman (1992) suggests that *Pi-1, Pi-11 e Pi-4b* genes ensure resistance against all tested races, despite the fact that pathogenic variations among races are known and documented. Based on these informations crosses are being done in order to incorporate these three genes on elite lines and cultivars. The next step is to follow up these genes by molecular markers. On each generation the lines are being submitted to anther cultivation in order to stabilize segregation. The regenerated lines would be analyzed by MAS and submitted to pathogenicity tests to allow selection only of lines with homozygous resistance genes only.

A study covering all the country is also being done to evaluate *M. oryzae* population present on main Brazilian Rice production areas. This is being done by Embrapa (Empresa Brasileira de Pesquisa Agropecuária) and has the objective to suggest to plant breeders the more effective resistance genes to be incorporated on new rice cultivars.

4. Future perspectives

During the 20th and 21st centuries, widespread, intensive, and systematic efforts have been made and continue to be made by plant breeders throughout the world to breed plants that
The most useful genes for higher yields, better quality, uniform size of plants and fruits, uniform ripening, cold hardiness, and disease resistance. In searching for new useful genes, plant breeders cross existing, local, cultivated varieties with one another, with those of other localities, both here and abroad, and with wild species of crop plants from wherever they can be obtained (Agrios, 2005).

Because of the genetic variability of *M. oryzae* and the possibility of parasexual recombination added to favorable environmental conditions to the disease in the tropics (high temperature and relative humidity), the practice had demonstrated that the resistant cultivars lost its effectiveness in less than five years, causing great yield losses. This also happened to Epagri’s rice released cultivars, which were selected for exhibit resistance. As Santa Catarina State is considered a new production area (culture ‘boom’ begins in late 1970), the first Epagri’s cultivars showed resistance for many years. But with intensive use of same culture field year after year, some years ago several problems related to pests and disease began to emerge, among that the rice blast. And today almost all 17 Epagri’s released cultivars are affected by blast in some level. This prompts us to search for strategies to manage the problem. The main of them is to develop cultivars with durable resistance.

Developing durable resistance requires strategies that limit the ability of pathogen populations to adapt to host resistance. The ephemeral effectiveness of resistance to some diseases with great potential has resulted in considerable research into the nature of durable disease resistance, but despite the efforts devoted to understand it, very few successful examples have been confirmed (Wilson et al., 2001). To date, the acknowledged forms to face plant diseases are the use of resistant cultivars, the use of multilines or cultivar mixtures and the use of fungicides. Culture management techniques as crop rotation, control of weed plants and incorporation to soil of crops residues are also recommended for rice culture.

The development of blast resistant rice cultivars by pyramiding genes is a process that is hard-working and time-consuming (at least 8 to 9 years by traditional breeding methods) and the expected result is that the resulting cultivar would show effectiveness and longevity. Theoretical models have been used to estimate the longevity of crop protection afforded by three highly effective resistance genes used under different strategies. In absence of stabilizing selection in the pathogen population, the models indicated few differences if the genes were used sequentially (13 to 14 years), stacked into a single variety (13 to 14 years) or used in multilines (12 to 13 years), considering that composition of multigenic and multilines host population was static year to year (Wilson et al., 2001).

The durability of resistance for a large number of fungal and oomycete pathogens was evaluated by McDonald & Linde (2002). These authors proposed that the risk of failure of genetical control depends not on the nature of the resistance genes but on the evolutionary potential of the pathogen. So, the evolutionary potential of a pathogen population is reflected in its population genetic structure. They identified three factors, population size, rate of gene and genome flow (migration) and the reproduction or mating system (i.e. asexual or sexual), from which to produce a risk factor for loss of resistance. The calculated risk for *P. grisea* was considered high because of the large effective population size, mixed reproduction/mating system and medium mutation rate. So, these authors estimated that the average number of years before the pathogen causes detectable damage on previously resistant cultivars was 1-3 years.
According our understanding, the more effective form to control the disease is the use of cultivars with pyramiding genes for blast resistance. As the development of a cultivar often needs a long time to select and test its agronomic traits, there are few rice released cultivars with pyramiding genes actually available, and none evaluated and ready for cultivation in Santa Catarina State. And while there is not a stacking cultivar available, other strategies need to be implemented.

The use of cultivar mixtures or multilines needed to be carefully considered. Different studies had demonstrated that complex races that can attack various resistance genes have been assumed to be unable to increase in a cultivar mixture or multilines because of the costs associated with a lack of avirulence genes (Zhu et al., 2000; Mundt, 2002; Garret & Mundt, 1999; Wilson et al., 2001). But recently, a study suggested that complex races can overcome multiple host genotypes in a multiline system by producing variants that can infect resist lines through parasexual recombination (Noguchi et al., 2007). The variants had a more complex virulence than the parents and exhibited a level of fitness equal to that of the parents. In particular, in a cultivar mixture, leaf blast caused by the variants was more severe than that caused by either parent. These results suggest that parasexual recombination not only alters pathogenicity but also enhances fitness, such as lesion enlargement and spore production, leading to lack of resistance (Noguchi et al., 2011).

So, a specific strategy to Santa Catarina State could be developed. Considering that the majority of rice is cultivated by little farms, owing areas as 1 to 10 ha, the Epagri´s rice cultivars could be grown in a scheme like patchwork, with each farm cultivating a different rice cultivar, maintaining disease levels in a low and manageable scale. But in presence of favorable climatic conditions to the pathogen, the use of fungicides must be considered in order to reduce economic losses.

Considering that we know that M. oryzae presents low genetic diversity in Santa Catarina State, the use of Epagri´s rice cultivars in an organized manner by farmers and allied to recommended cultural management could help to decrease the use of fungicides and to maintain pathogen populations under control until new resistant cultivars will be released.

With the long history of coevolution between pathogens and its hosts, it seems unlikely that we will be able to eliminate plant diseases by engineering cultivars with new genes or receptors or combinations of genes/receptors and putting them into our crops. Pathogens will continue to evolve. However, genetic engineering offers new opportunities to stay few steps ahead of the pathogen, and the technology can be used for helping to create novel pyramids of resistance alleles and transfer them to the cultivars (McDonald & Linde, 2002). The use of new breeding technologies is likely to underpin future gains in crop productivity and to generate new datasets for crop species (Langridge & Fleury, 2011).

Probably the more reasonable and effective strategy to deal with the rice-P. oryzae pathosystem is the use of modern molecular tools in order to access pathogen population structure in spatial and time scale allied to the use of the same techniques to identify potential sources of resistance genes in O. sativa and its related species.

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6. References


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*Magnaporthe oryzae* Genetic Diversity and Its Outcomes on the Search for Durable Resistance