

## O.65 - Evolution of *Magnaporthe grisea* populations and adaptation to upland rice in the Vakinankaratra region of Madagascar

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### Abstract

In Vakinankaratra, where irrigated rice has been cultivated for several hundred years, blast damages increased heavily in the newly developed upland rice. Using epidemiological records, pathogen population genetic data, and pathogenicity test, we studied blast pathogen colonization of the new upland agrosystem. Genotyping with microsatellite markers, showed a significant differentiation between upland and irrigated isolates. Comparison of populations from 3 irrigated valleys (Antsirabe, Manandona, Mangalaza) showed the first two having no differentiation while Mangalaza showed slight differentiation with the two others. That is probably due to selection by the host since Mangalaza had more diversified varieties than the 2 first locations. There is no differentiation between the early isolates causing leaf blast and those causing neck blast in upland agrosystem, differentiation was observed in Mangalaza between 2004 and 2005, and in Andranomanelatra between 2005 and 2006 isolates. Differentiation from one season to the next could be observed during the study for upland isolates. However for each location, ie in Mangalaza, in Manandona and in Antsirabe, differentiation could not be observed between the 2004 and 2005 irrigated isolates. The 99 isolates selected from different branches of the genetic dendrogram formed two distinct groups according to their pathogenicity. The first group brings together irrigated isolates. They have narrow virulence spectrum, ie they could not attack most of the upland cultivars. Even irrigated isolates collected from geographically distant areas like Antananarivo, Lac Alaotra or Vakinankaratra exhibited narrow virulence spectrum. The second gathers upland isolates of Vakinankaratra region. They have a broader virulence spectrum than the irrigated ones. Among the upland isolates, those collected in 2001 showed narrower virulence spectrum than isolates collected in 2005 or 2006, suggesting that the virulence spectrum enlarged with time. Such increase of virulence was observed during surveys. The genotypic and pathotypic structure of populations for the blast fungus in the Vakinankaratra region of Madagascar suggests local adaptation of isolates from irrigated agrosystem to upland agrosystem. In the absence of recombination, and because of short distance migrations, selection for an increased virulence spectrum to attack upland cultivars led to differentiation between populations from the two rice agrosystems and also led to a specialized pathogen population highly virulent to the widely cultivated varieties in this new upland rice agrosystem.

Blast is the most destructive fungal disease of rice. It causes heavy losses particularly in upland rice agrosystems. Emergence of new pathotypes has been reported in different regions, causing the breakdown of cultivars developed with single major resistant genes. (Ou, 1980) It is now well established that interactions between *Magnaporthe grisea* (Hebert) Barr and rice are governed by gene-for-gene relationship (Silué 1992). So, evolution of the pathogen for increased pathogenicity spectrum is expected to occur by simple mutations. However, population evolution in the field was understudied.

On the contrary, extensive research work has been done in different countries to understand blast pathogen population structure and diversity. Studies using DNA fingerprinting analysis revealed that blast populations are organized in discrete clonal lineages, (Levy, 1993; Zeigler, 1994; Zeigler, 1995; Roumen, 1997) a structure in agreement with the clonal reproduction observed in the field. However, in some Asian countries, diversity is higher and the clonal structure is not so obvious. (Chen, 1995; Don, 1999; Kumar, 1999; Sook-Young, 1999). Pathotyping revealed that, depending on the geographic region, each lineage could consist of one to many pathotypes.

The rice agrosystem of Vakinankaratra is an interesting case for studying the structure and evolution of blast pathogen populations. Having an altitude between 1500 and 1750m, and consequently low temperatures, it is less suitable for upland rice. In this region irrigated rice was the only practice in the region for hundreds of years. Upland rice has been developed over the past 20 years and now accounts for several thousands of hectares. Blast attacks were first observed in experimental sites 15 years ago and are now a severe threat to the development of upland rice culture.

We hypothesised that populations on upland rice arose from local populations on irrigated rice by selection of new pathotypes. To test this hypothesis, we characterised the genetic diversity and pathogenicity of populations collected on irrigated and upland rice in nearby fields. Geographic structure was also assessed at different scales to evaluate the importance of gene flow. In addition, the population evolution over time was monitored during three consecutive years to evaluate if genetic changes could be detected over a relatively short period of time.

Disease surveys were done from 2003 to 2006 in the region and isolates collected. In addition to these contemporary populations, isolates collected in 1995 and in 2001 were also genotyped. We used 13 newly developed microsatellite markers. Such markers are adapted to monitor diversity on a relatively short time scale and allow many isolates to be rapidly characterised. Disease incidence and severity were recorded and more regular isolate collection was done in the experimental field at Andranomanelatra for a more detailed studies. Pathogenicity of 99 isolates was assessed by inoculation on 14 differential rice varieties and 12 cultivars from the Vakinankaratra region.

In irrigated rice, the highest disease pressure was observed in 2003 with a frequency of attacked fields varying from 10 to 25% in different localities for the Vakinankaratra plains. This frequency decreased considerably in 2004 (1 to 15%) and remained at a similar level (0.1 to 10%) in 2005. In 2003, disease frequency in the upland was high (10 to 90 %) and this was the first time that the variety Fofifa154 was noticed to be widely susceptible. In the experimental site at Andranomanelatra, with Fofifa154 grown in a ploughed plot, disease was severe in 2005 and 2006. Usually, the blast prevalence recorded was at least 10 times higher in upland rice compared to irrigated rice in the Vakinankaratra region between 2003 and 2005.

At the field level, there was no change between the population sampled at the beginning of the epidemic (leaf blast) and the population sampled at the end (panicle blast). In the large experimental field at Andranomanelatra, there was no differentiation between the early population causing leaf blast and the later one causing neck blast. This was observed during two consecutive years on two different varieties.

No significant differentiation between 2004 and 2005 populations was observed for upland rice isolates in Andranomanelatra. A limited ( $F_{st} = 0.01$ ) but significant differentiation was observed between 2005 and 2006 populations. Differentiation was highest between 2004 and 2006 populations ( $F_{st} = 0.05$ ). These results suggest that population changes can be observed over a three-year period.

Genotyping using microsatellite markers showed a relatively small ( $0.05 < F_{st} < 0.12$ ) but significant differentiation between populations from upland and irrigated rice from nearby fields. This differentiation was observed during the two years when these populations were compared (2004 and 2005). Populations collected in distant sites (20km) on irrigated rice were not significantly differentiated. Even populations on irrigated rice from Antananarivo (170km) and Alaotra lake (400km) were not differentiated from populations from Vakinankaratra. On the contrary, populations collected on upland rice in the Vakinankaratra region were significantly and highly differentiated ( $F_{st}$  up to 0.36). Altogether, these results are in agreement with the existence of an ancestral *Magnaporthe* population on irrigated rice and with the adaptation to upland rice. High differentiation between upland rice populations suggests several adaptation events (founder effect).

The 99 isolates selected to represent the genetic diversity sampled and to allow comparison between populations from upland and irrigated rice, formed two distinct groups according to their pathogenicity. The first group gathers isolates collected on irrigated isolates. They show narrow virulence spectrum and cannot attack most of the upland cultivars. Even irrigated isolates collected from distant areas like Antananarivo, Alaotra lake or Fianarantsoa where different varieties are grown, show this narrow virulence spectrum. The second group gathers isolates from upland rice. They show a broader virulence spectrum and can attack upland cultivars and irrigated rice cultivars. Among them, isolates collected in 2001 showed narrower virulence spectrum than isolates collected in 2005 or 2006, suggesting that virulence spectrum enlarged with time. Such a gain of virulence was observed during disease surveys. These results strengthen the hypothesis of an adaptation of local population of *Magnaporthe* to newly developed upland rice cultivars.

The genotypic and pathotypic structure of populations for blast fungus in the Vakinankaratra region of Madagascar suggest local adaptation of isolates from irrigated agrosystems to upland agrosystems. In the absence of recombination, and because of short distance migrations, selection for increased virulence spectrum to attack upland cultivars led to differentiation between populations from the two

rice agrosystems and a specialised pathogen population highly virulent to the widely cultivated varieties in this new upland rice agrosystem.

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