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Characterization of the European population of the blast pathogen *Magnaporthe grisea*

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Abstract. Populations of the blast pathogen *(Magnaporthe grisea)* consist of several or many races. Knowledge of which races are present and in which proportions is helpful for plant breeders in their task of developing blast resistant cultivars.

From 1960 onwards, blast populations have been analyzed by inoculation tests of samples of isolates on rice cultivars carrying different resistance genes. This method is time consuming, and not easy to carry out, and the number of isolates that can be evaluated is limited.

The use of molecular markers allows for a much more detailed analysis of the genetic variability of blast populations because this method can process a much larger number of isolates in a relatively short time. To provide useful information for breeder, the variation for molecular markers should correspond with variation for (a)virulence factors. Recent research carried out in the USA, in Colombia, and in the Philippines has shown that the blast populations consist of several or many clonal lineages that each have a more or less restricted virulence spectrum. The present work investigates the extent of genetic variation and the relation between molecular marker, and virulence type information among isolates obtained from the European countries Italy, Spain, Portugal, France and Hungary.

In Europe, the area grown to rice is limited, and only one crop can be grown per year. In most areas, the climatic conditions are unfavourable for the disease during most part of the growing season. In addition, European cultivars often are extremely susceptible when grown elsewhere in the world. We thus departed on the hypothesis that the blast population in Europe would be extremely simple, having only a few virulence factors. The results indicated the presence of more genetic variation than expected. Within a sample of just 25 isolates, at least 4 or 5 lineages were present, each with a characteristic virulence spectrum. Test using the Japanese differential cultivars showed that virulence was present at least once for the known resistance genes pi-a, pi-i, pi-f, pi-ks, pi-k, pi-t, pi-ta and pi-sh. On the other hand, some cultivars well adapted to European growing conditions revealed a highly effective resistance to all the isolates tested. The symptoms induced on these cultivars did not correspond with that of any of the Japanese differential cultivars, suggesting that the resistance of these cultivars may be controlled by so far undescribed resistance genes.

I – Introduction

The majority of the European rice growing areas have a Mediterranean climate. For most part of the growing season, weather conditions are hot and dry which is unfavourable for blast disease development (see this volume elsewhere). Leaf blast epidemics are therefore rare. Towards the end of the season, conditions become more favourable, with a higher risk of prolonged periods of humid weather. Sometimes epidemics of neck blast occur, causing economic loss. The disease can be controlled by growing resistant cultivars, but, at present, nearly all commercially grown European rice cultivars are highly susceptible. Introducing blast resistance in these cultivars is difficult. Most of the known donors of resistance genes lack the other characteristics that are required for successful growth under European field conditions, especially where it concerns cold tolerance and growth duration. Considering the effort that is needed to incorporate the resistance genes into a cultivar with an acceptable agronomic performance, the choice of the resistance genes used is very important.

Recent studies done elsewhere in the world show that blast pathogen populations are made up of a number of clonal lineages, which each are virulent to a limited range of resistance genes (Levy *et al.*, 1993; Correa-Victoria *et al.*, 1994; Zeigler *et al*., 1994). A better knowledge of the population structure in

Europe may be helpful for breeding for blast resistance. This paper presents some results of an ongoing study on the genetic variability of *Magnaporthe grisea* in Europe.

II – Materials and methods

Isolates were collected in five European countries. The date of collection for each isolate, the cultivar from which it was obtained, and its geographic location are shown in Table 1.

Table 1. Country of origin, year of collection and host cultivar of the isolates studied

Isolate	Country	Year	Host-cultivar
FR1	France	80	Delta
FR3	France	86	Smeraldo
FR5	France	86	Smeraldo
FR9	France	86	Unknown
FR10	France	86	Belgioso
FR13	France	88	Rocca
FR26	France	90	Lido
FR27	France	91	Thaibonnet
FR28	France	91	Lido
FR32	France	92	Koral
FR41	France	94	Sariceltik
HN1	Hungary	93	Öki-2
HN2	Hungary	93	Öki-4
HN3	Hungary	94	Ringola/Sandora
HN4	Hungary	94	Ringola/Sandora
HN5	Hungary	94	Ringola/Sandora
IT2	Italy	86	Vernia
IT3	Italy	86	Thaibonnet
IT6	Italy	89	Ariete
IT10	Italy	89	IAC 164
IT11	Italy	89	Lido
IT14	Italy	89	Ariete
IT16	Italy	90	Unknown
IT20	Italy	90	Thaibonnet
IT21	Italy	90	Icario
IT22	Italy	91	Balilla
PR2	Portugal	90	Pritz
PR3	Portugal	90	Koral
PR6	Portugal	90	Onda
PR12	Portugal	91	Onda
PR13	Portugal	91	Thaibonnet
PR14	Portugal	91	Lido
PR61	Portugal	91	Ringo
PR71	Portugal	92	Koral
PR72	Portugal	92	Koral
PR76	Portugal	92	Thaibonnet
SP1	Spain	86	Unknown
SP2	Spain	94	Thaibonnet
SP3	Spain	94	Bahia
SP4	Spain	94	Bahia
SP5	Spain	94	Lido
SP6	Spain	94	Unknown

To detect pathogenic variants (races), each of the isolates was inoculated at CIRAD to 24 rice cultivars (Table 2). Cultivar 'Maratelli' served as a susceptible check. The rice plants were grown in a greenhouse in plastic trays (45 x 30 x 7 cm) filled with a peat soil. For each isolate, three trays were prepared. Per tray, 8 cultivars were sown in a single row of 15-20 plants each. Ample nitrogen fertilizer was applied. A solution of ammoniumsulfate equivalent to 5 gr N m^{-2} was applied shortly after sowing, when the plants reached leaf stage 4, and again one day before inoculation (Roumen, 1992). Each isolate-rice cultivar combination was assessed in two separate trials, carried out with at least a few weeks interval.

Table 2. Names, known resistance genes, and geographic origin of differential cultivars used to detect pathogenic variants of the blast pathogen in Europe

Cultivar	Resistance gene(s)	Origin
Aichi-asahi (Japan)	A	Japan
Fujisaka-5	I K^s	Japan
Fukunishiki	Z SH	Japan
Kusabue	K SH	Japan
Shin-2	K^s SH	Japan
BL-1	B SH	Japan
K-59	T	Japan
K-1	TA	Japan
Pi-no-4	TA^2 SH	Japan
Tsuyuake	K^m	Japan
Norin-22	SH	Japan
ST-1	F	Japan
Kanto-51	K	Japan
Nipponbare	SH	Japan
K-60	K^p	Japan
Reiho	A TA^2	Japan
Rico-1	K^s	USA
Nato	I	USA
Lido	Unknown	Italy
Aichi-asahi (CIRAD)	Unknown	Japan
Thaibonnet	Unknown	USA
Gigante Vercelli	Unknown	Italy
Estrella	Unknown	Portugal
Maratelli	Susceptible check	Italy

Isolates were cultured from a single conidiospore. Growth and sporulation of the pathogen was induced by placing inoculated Petri dishes filled with a rice polish agar at 27°C under fluorescent light for about eight days. Conidiospores were harvested by adding a little water to Petri dishes with sporulating cultures. The spores were dislodged with a camel brush, the suspension was filtered, and the spore concentration measured using a haemocytometer. Spray inoculation was done spraying 30 ml of a suspension containing 50,000 spores ml^{-1} and 0.5% gelatine to each tray of plants using an artist's paintbrush powered by compressed air. The plants were inoculated when the plants had 5 or 6 leaves on the main culm. Trays with plants were placed on a rotating table and inoculated spraying the suspension in slow systematic movements. Immediately following inoculation, the plants were kept in the dark inside a phytotron at 25°C and 100% humidity for 16 hours. Inoculated plants were returned to the greenhouse the following morning.

The infection type (IT) was scored six days after inoculation using a scale with 7 lesion type categories (Figure 1). The most common lesion type as well as the largest lesion type that developed was noted for each isolate-cultivar combination using the cultivar rows as experimental unit.

DNA fingerprinting of each isolate was done at Purdue University, USA. For the analysis, the probe 'MGR 586' was used, which corresponds to a repeated DNA sequence in the pathogen. This sequence is dispersed randomly through the genome and is not related to fitness. In addition, the use of this probe on gels with digested DNA of pathogen isolates typically produces 50 to 80 bands making it highly sui-

table for fingerprinting purposes (Levy *et al*., 1991). The degree of relatedness among the isolates was calculated according to the presence or absence of bands. Usually, isolates belonging to the same lineage show similarity for 90% of the bands or better.

III – Results and discussion

When we started this work, we assumed the genetic variability of the blast pathogen in Europe to be limited. Compared to other cereal crops, the total surface planted to rice is small. Only one crop is grown per year, with a similar planting season for each of the regions. In addition, most of the rice is grown in regions with a Mediterranean climate under conditions that are unfavourable for disease development during most part of the growing period. The blast pathogen is thus likely to go through large bottlenecks regularly, and such situation favours loss of genetic variability. A bit to our surprise, we found a much larger genetic variability than expected.

Analysis using the MGR 586 probe revealed the presence of six lineages among the isolates tested. Several lineages were restricted to a specific rice growing area. All isolates belonging to lineage 3 were from Hungary. Likewise, those belonging to lineage 6 were from the Delta de Ebro region in Spain. The single isolate from the Spanish Valencia area was found to belong to a distinct lineage 1. The lineages 2, 4 and 5 were present in more than one region. Lineage 4 isolates were obtained from Italy and France. Lineage 5 was present in Italy, France and Portugal. Lineage 2 seemed the most widespread and was present in Italy, France, the Spanish Delta de Ebro region, and in Portugal.

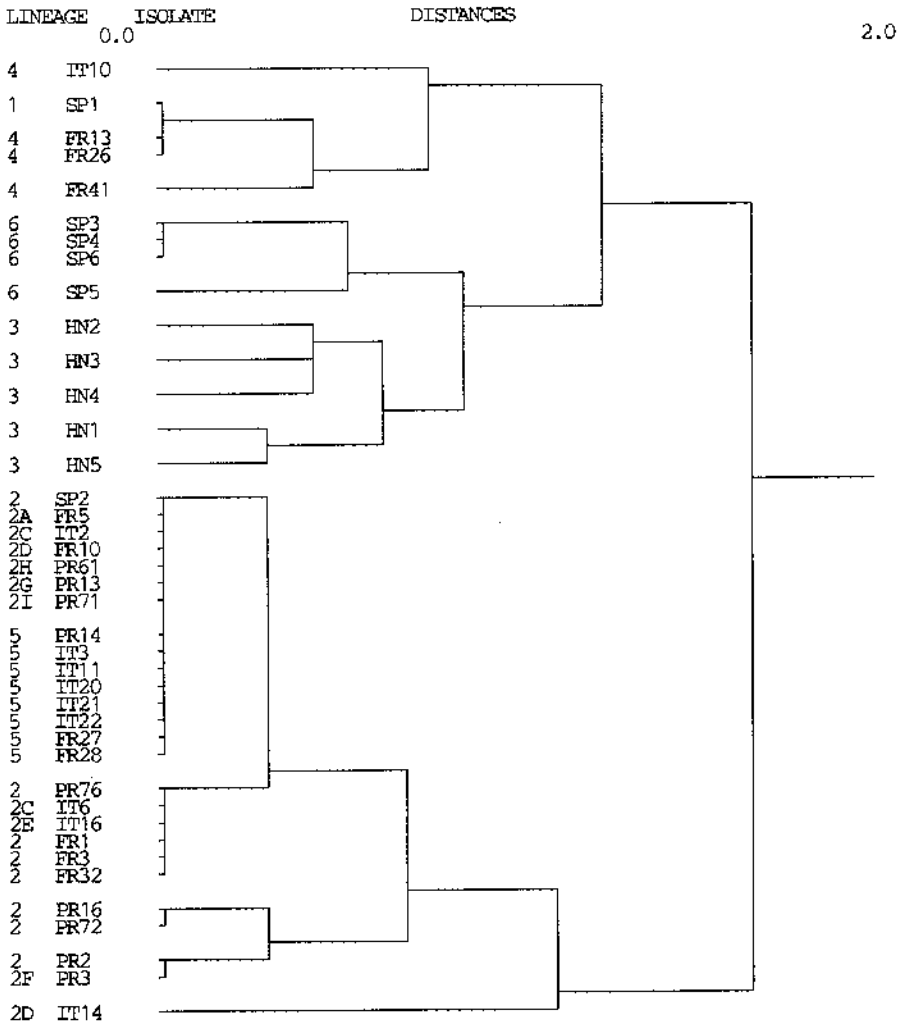
In agreement with results obtained for isolates elsewhere in the world, the data indicate that the European lineages each have a characteristic, restricted virulence pattern. Some cultivars were completely resistant to all isolates (and thus lineages). Examples are ‘Kanto-51’, ‘Kusabue’, ‘Reiho’, ‘Pi-no-4’ and the Italian cultivar ‘Gigante-Vercelli’. Certain cultivars were highly resistant to all isolates of a particular lineage, but not to others. For example, the cultivar ‘Aichi-asahi’ was completely resistant to any isolate of lineage 4. And none of the isolates belonging to lineage 2 or 5 could completely overcome the resistance present in the cultivars ‘Nipponbare’, or ‘Shin-2’ although they could produce sporulating lesions (Table 3). In general, the correspondence between the virulence pattern and the lineage of isolates was remarkable. Cluster analysis of the obtained disease reaction scores produced isolate clusters that closely corresponded with the lineage classification (Figure 2). Within lineage 2, clear genetic variation for avirulence to some of the resistance genes in the rice differentials occurred. Interestingly, all isolates of lineage 5 showed exactly the same virulence pattern, regardless of origin (Italy, France, or Portugal). Genetic variation for avirulence in this lineage might be completely absent. Among the five isolates of lineage 3, isolate HN1 caused a more susceptible infection type on cultivar ‘ST-1’ than the other isolates of this lineage.

Table 3. Observed infection type for some differential rice cultivars after exposure to European isolates of repesentative of their lineage

	Isolate (and lineage)						
Cultivar	PR3 (2)	PR16 (2)	IT16 (2)	FR13 (4)	FR27 (5)	SP4 (6)	HN1 (3)
Aichi asahi	6	6	6	0	6	6	6
K 1	6	6	0	0	0-2	0	0
K 59	3-5	6	6	2	2	2	2
Shin 2	3-5	0-3	3-5	6	3-5	6	6
Nipponbare	3-5	3	3-5	6	3	6	6
St 1	0	0	0	6	0	3-5	6
Maratelli	6	6	6	6	6	6	6

Despite the over-all relation between lineage and virulence pattern, isolates belonging to lineage 5 could not be distinguished from those of lineage 2 by their virulence pattern alone (Figure 2). Likewise, the single lineage 1 isolate had the same pattern as those of lineage 4. It will be worthwhile to try and identify rice cultivars that give a distinct reaction for isolates of these lineages.

Figure 2. Classification of European *Magnaporthe grisea* isolates by cluster analysis of virulence data (Feb. 1995) on differential cultivars. The lineage of isolates is noted in the left margin. For some isolates, data of the second repetition were not yet available



The lineage diversity in Europe seems similar to that found in the USA, but is lower than that found for tropical regions where rice is cultivated since long, such as the Philippines (Chen, 1993). However, it is not unlikely that more lineages are present in Europe. Especially Spain could reveal additional lineages with increased sampling. The sample size of isolates from the Delta Ebro region was very small and the Valencia rice growing area was represented by just one isolate. Isolates from the Southern Spanish rice growing area near Sevilla were not available. Considering the cultivated surface in Italy, a more systematic sampling repeated in time is not unlikely to also reveal the presence of additional lineages for this region.

Regardless of lineage, virulence was detected at least once for the known resistance genes *pi-A*, *pi-I*, *pi-F*, *pi-K*, *pi-Ks*, *pi-SH*, *pi-T*, and *pi-TA*. The use of these single genes for creating resistant cultivars is thus unlikely to be useful. So far, no virulence was detected for the resistance genes *pi-B*, *pi-Kp*, *pi-TA*^{2} and *pi-Z*.

If we accept the theory that lineages have a limited virulence spectrum and are invariably avirulent to certain of the resistance genes, specific combinations of some of the resistance genes can be of interest. For example, assuming that there are no other lineages in France besides lineage 2, 4, and 5, a combination of the resistance genes *pi-A*, and *pi-F* might confer durable resistance. The *pi-A* gene would be effective to any isolate of lineage 4, while the *pi-F* gene would be effective to the isolates of the lineages 2 and 5 (Table 3).

The combination of the information on virulence pattern and lineage of the isolate will permit us to optimize the range of isolates used for screening and evaluating resistance in European rice cultivars. For example, any isolate belonging to lineage 5 will give us results representing the whole lineage and testing just one isolate of this lineage will do. Varietal resistance may vary depending on which isolate of lineage 2 is used. Efficiency of tests is likely to be higher when lineage 2 isolates are used with a different virulence pattern as a lineage 5 isolate, if the latter is also included in the test.

The cultivars 'Thaibonnet', 'Gigante Vercelli', and 'Estrella' showed complete or incomplete hypersensitivity resistance to all isolates tested. The disease reactions obtained with these cultivars suggest they contain major genes that are not yet characterized. 'Gigante Vercelli' and 'Estrella', but not 'Thaibonnet', might be of interest as donors of resistance genes, since they are agronomically adapted to the regional growing conditions. In its country of origin, the USA, 'Thaibonnet' (L-202) is highly susceptible. In a recent test done with Dr. Lima in our greenhouse, a Portuguese isolate collected in 1994 produced a highly susceptible infection type on 'Thaibonnet'. The resistance of this cultivar is thus likely to rapidly succumb to new variants of the pathogen.

Summary

The genetic variability of the blast pathogen present in Europe was larger than expected. Virulence for several of the known resistance genes was detected although these genes are not present in the rice cultivars that are grown in Europe. The virulence pattern of the isolates closely corresponded with their lineage classification.

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