Microscopic observations of early events during infection of rice leaves by Pyricularia oryzae Cav.: a possible way of recognizing cultivars with quantitative resistance

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Microscopic observation of early events during infection of rice leaves by *Pyricularia oryzae* Cav.

A possible way of recognizing cultivars with quantitative resistance

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Abstract. In Burundian high elevations swamps rice was introduced in 1980 with the release of Yunnan 3, a cultivar from China. Six years after this cultivar was seriously attacked by blast, caused by *Magnaporthe grisea* Hebert-Barr (anamorph *Pyricularia oryzae* Cavara). Since then, the breeding programme is aimed at controlling the disease by the use of resistant cultivars especially by selecting quantitative resistance which would ensure durable protection and stable yield.

The main obstacle is the recognition of cultivars with a high level of quantitative resistance. Our study has dealt with the observation of interactions, at cellular level, between the blast fungus and rice cultivars with differing degrees and types of resistance: Yunnan 3, a susceptible one, hybride 57 with effective vertical resistance to Burundian isolates, IRAT13, IR36, IR64 et Tetep with a good level of quantitative resistance.

The reactions occurring after penetration were classified in five types according to their microscopical appearence: A: no visible host reaction; B: light brown and aniline blue stained cell walls; C: fine cytoplasmic granulation; D: cell necrosis; E: coarse cytoplasmic granulation. Microscopic slides were prepared after treatment of the leaves by the whole-leaf staining method described by Peng et al. (1986).

The resulting data enabled us to distinguish the three groups of cultivars by the chronology of appearance of different cellular reactions, especially of the B reaction. In hybride 57, with effective vertical resistance towards Burundian isolates, staining of cell walls with aniline blue occurred at more than 90% of the infection sites 24 hours after inoculation; while in Yunnan 3 and the others the same reaction was observed 24 h later (48 after inoculation) and at a lower rate of infection sites.

In addition, the B reaction evolved differently according to the resistance type: in hybride 57, the B type reaction evolved to C after the third day, whereas it evolved to D for cultivars with quantitative resistance IRAT 13, IR36, IR64 and TETEP. In the susceptible Yunnan 3, sites without reaction outnumbered those with. This would be probably linked to differences in regulation mechanisms of the cellular reaction.

The results suggested that the time course of the reaction occurring at infection sites may be used to recognize cultivars with a high level of quantitative resistance. This could be helpful in selection for quantitative resistance to blast.

I – Introduction

Rice growing is being practised in Burundi for centuries in upland and flooded conditions. Its introduction in high elevations swamps is very recent. The first cultivar to be released was Yunnan 3, a cultivar from China, in 1980.

In 1982, the bacterial sheath rot caused by *Pseudomonas fuscovaginae* was identified in that new rice cultivation area (Autrique and Maraite, 1983).

In 1986, rice blast broke out and caused serious yield losses to Yunnan 3. It was urgent to replace this cultivar by blast resistant ones.

As a preliminary to breeding for blast resistance, the pathogen variability had to be well known. A high pathogenic diversity in the *M. grisea* population from high elevation swamps in Burundi was encountered despite the limited number of rice genotypes grown in this environment. All the known resistance genes

were broken down by Burundian isolates except Pi-ta2. The latter was, however, matched by isolates from other geographic areas (Bahama and Notteghem, 1990).

Some of the cultivars tested were completely resistant towards Burundian isolates but susceptible when inoculated with isolates from other geographic areas such as CM28 from Cameroons and PH11 from Philippines. The resistance they carried was thus specific and should break down sooner or later.

Facing such variability and in a subsistence system, more emphasis should be put on selecting quantitative resistance which ensures durable protection and more stable yield.

Our study was therefore aimed at finding out methods that could allow to distinguish cultivars with quantitative resistance from those without. To achieve the goal, we observed, under light microscope, the interactions between rice cultivars with known types of resistance and two *M. grisea* isolates from the first day after inoculation with two isolates till the seventh.

The results presented below show the time course of cellular reactions at infection sites in susceptible, completely resistant and cultivars with quantitative resistance.

II – Materials and methods

The cultivars used in this study have been chosen on the basis of previous results on blast resistance in artificial conditions as well as in field tests:

- ❏ Yunnan 3, widely spread in Burundian high elevation swamps and very susceptible to blast;
- ❏ Telorirana, a Malagash cultivar introduced for its tolerance to low temperatures, also susceptible;
- ❏ IRAT 13, an upland cultivar with a good level of quantitative resistance; presently used as parent in breeding for blast resistance in Burundi;
- ❏ Tetep, also with a good level of quantitative resistance; widely used in crosses for resistance by several international institutes;
- ❏ Hybrid 57, a cultivar selected in Burundi; completely resistant towards Burundian isolates but susceptible to isolates from other countries.

These cultivars were sown in pots on a substrate made of 1/3 of field soil, 1/3 of sand and 1/3 of peat. They were watered every day with the Hoagland solution. The temperature of the glasshouse varied between 22°C (night) and 28°C (day) and the relative humidity was kept at about 70%. Plant at five-leaf stage were inoculated with a spore suspension containing 50 000 conidia per milliliter. The inoculated seedlings were kept at 100% of relative humidity during 48 hours and then transferred in greenhouse at almost 80%. Two isolates were used as inocula, BD60 and CM28 from Burundi and Cameroons respectively.

Cellular reactions were observed by the whole-leaf staining and clearing method described by Peng et *al*. (1986). The fifth leaves sampled from the first to the seventh days after inoculation were fixed and decolorized in lactophenol. Decolorized pieces of leaf were treated by a solution of NaCl 0.75% acidified with 15% acetic acid and incubated at 95°C for 2 hours. They are then dehydrated in 50, 70 and 95% ethanol series for 5 minutes respectively and finally in absolute ethanol for 10 min.

Staining was accomplished by autoclaving the leaf pieces in a mixture consisting of absolute ethanol, lactic acid and phenol in equal volumes (at 121°C, 1 atm. for 15 min). They are finally cleared in a solution of phenol-lactic acid (4/1 v/v).

The above stained and cleared pieces of leaves were mounted on glass slides and observed under light microscope with emphasis on the temporal changes of cellular reactions referred to as type A, B, C, D and E:

- ❏ A: infected cells without any change detectable by light microscopy;
- ❏ B: infected cells with light brown and stained cell wall;

- C: fine cytoplasmic granulation;
- D: cell necrosis;
- E: coarse cytoplasmic granulation.

One hundred and sixty infection sites were observed for each cultivar at each date. The results are presented on figures displaying the evolution of the relative frequency of each reaction type during time.

III – Results

The results of our experiments are shown on figures 1 and 2. The cellular reactions at infection sites made it possible classifying rice cultivars according to their resistance type:

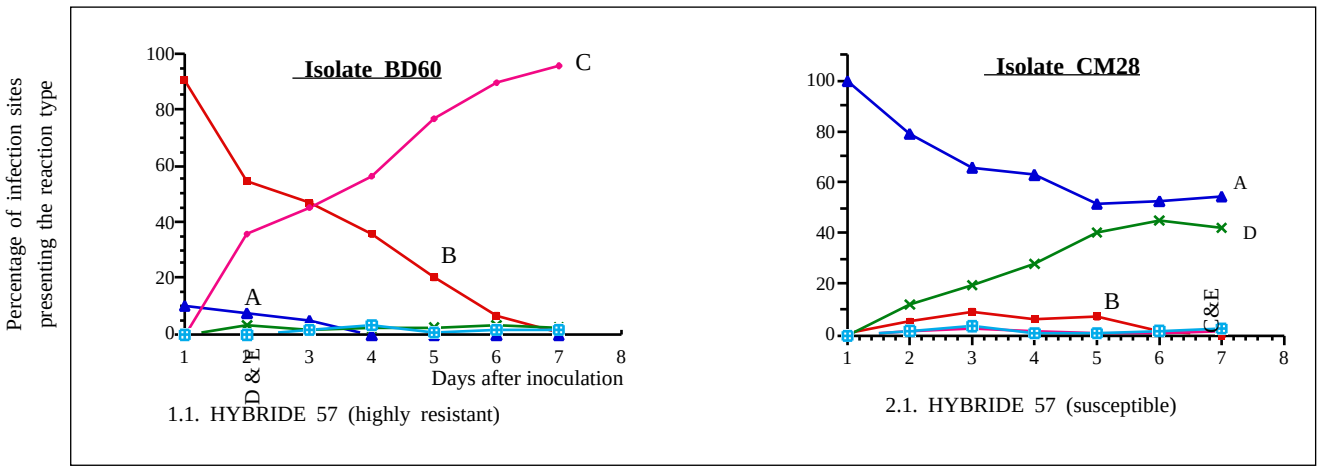
- One day after inoculation, in hybrid 57 with a complete resistance towards the Burundian isolate BD60, the cell walls expressed the brown light and staining with aniline blue (type B) at 90% of infection sites.
- Two days after inoculation a rapid decrease of sites with light browning and high affinity to aniline blue (54% of B) was observed while the fine cytoplasmic granulation increased (from 0 to 35.6%).
- Seven days after inoculation this type of reaction reached 95% and there was no more sites without reaction since the fifth day.

At the opposite, with inoculation by CM28, no visible reaction was observed when the cultivar was inoculated with CM28, virulent to it. The sites without reaction remained predominant till the seventh day (54% of A type). An important number of sites presented the cell necrosis (41% after 7 days) and almost never was fine cytoplamic granulation observed in this case.

Another pattern of evolution was observed in cultivar Yunnan 3 susceptible to both isolates. As a matter of fact, sites without reaction outnumbered those with till 7 days after inoculation (more than 54% in both cases). An increase of cell necrosis was also observed, reaching about 40% of infection sites 7 days after inoculation. The cell wall staining occurred one day later than in hybrid 57 and at only 10% of infection sites at most.

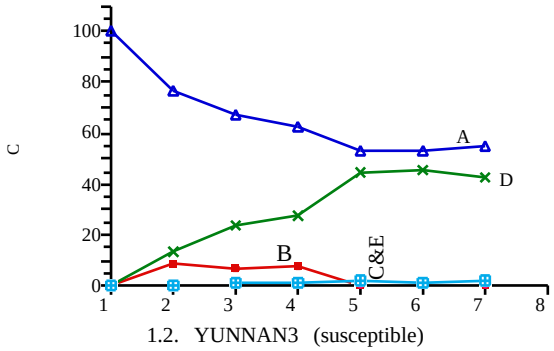
Cultivars with a high level of quantitative resistance behaved like susceptible ones in the first 24 hours after inoculation. Indeed, no reaction is observed 1 day after inoculation. However, after 2 days differences in reactions were conspicous, the B reaction occurred at 60% of infection sites at least and cell necrosis about 20%. Subsequently, most of infection sites presented cell necrosis (D reactions) whereas B merely disappeared after 4 days.

Figures: Evolution of cellular reactions of rice seedlings infected with two isolates of *Magnaporthe grisea* Hebert-Barr. A: infected cells without any detectable change; B: infected cells with light brown and stained cell wall; C: fine cytoplasmic granulation; D: cell necrosis; E: coarse cytoplasmic granulation.

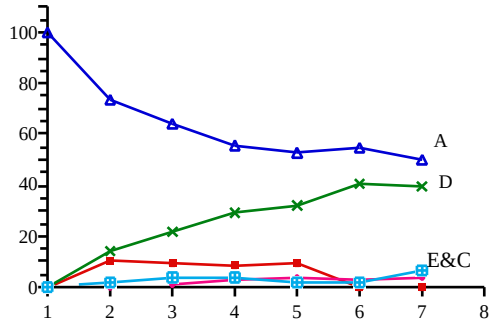


1.1. HYBRIDE 57 (highly resistant)

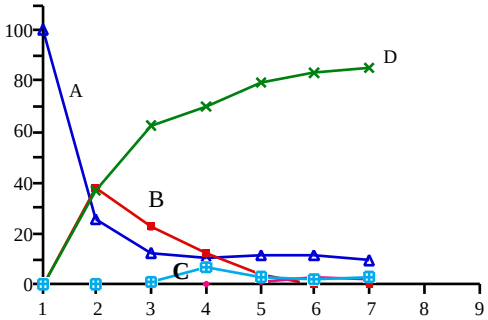
2.1. HYBRIDE 57 (susceptible)



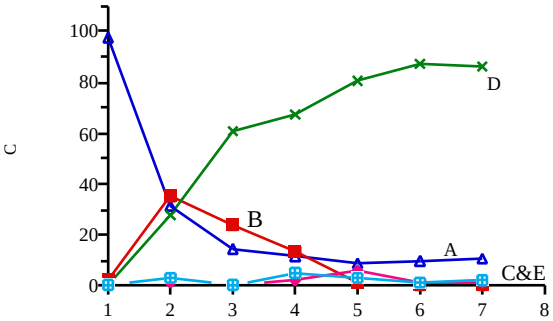
1.2. YUNNAN3 (susceptible)



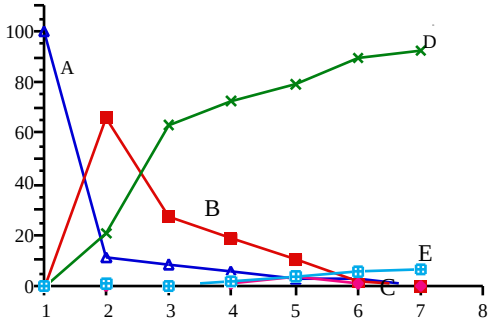
2.2. YUNNAN 3 (susceptible)



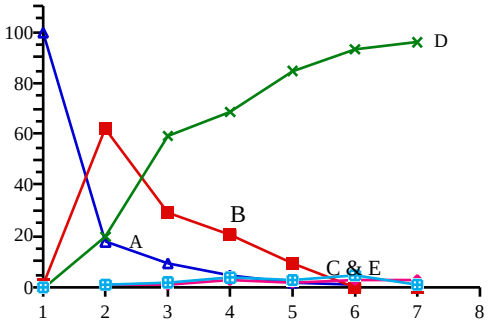
1.3. TELORIRANA (intermediate)



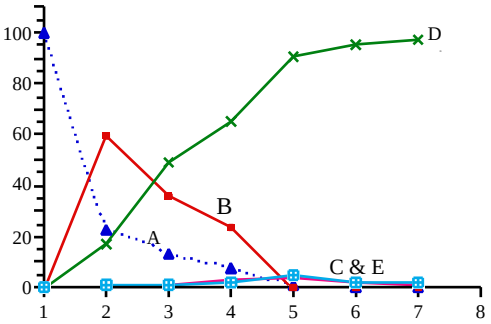
2.3. TELORIRANA



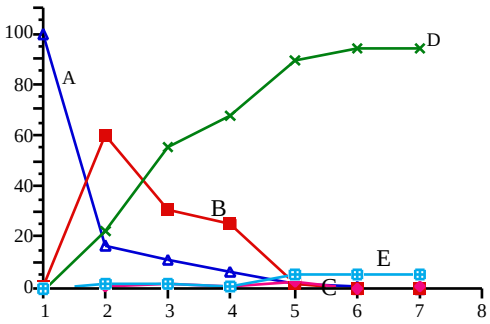
1.4. IRAT 13 (quantitative resistance)



2.4. IRAT 13



2.5. TETEP



1.5. TETEP (quantitative resistance)

IV – Discussion

Our results show it is possible to distinguish different resistance types in cultivars on the basis of reactions occurring at infection sites. Indeed, the complete resistance is characterized by a rapid occurrence of the light browning and cell wall staining with aniline blue, its subsequent decrease parallel to an increase of the cytoplasmic granulation reactions as from two days after inoculation.

The susceptible cultivars show no reaction at more than 50% of infection sites till the seventh day after inoculation whereas quantitative resistance cultivars reacted by producing B reaction and D at most infection sites on and after the third day.

In cultivars with effective complete resistance, the rapid appearance of the B reaction could be a hypersensitive response. It appeared that cultivars with quantitative resistance also presented a similar reaction even later and at a lower number of infection sites. This suggest that the B reaction is likely the reflect of hypersensitivity and is the primary defence mechanism triggered by the penetration of the pathogen in epidermic cells; the other reaction, such as cell necrosis and cytoplasmic granulation, would have resulted from secondary mechanisms.

Wood (1967) suggested that hypersensitive response is apparently a general phenomenon in infected plants. When the process is slower and involves larger areas of host tissue, the phenomenon borders on susceptibility; in this case other mechanisms are responsible of the ultimate restriction of the pathogen. This seems to be the case in our experiment where the B reactions were delayed in cultivars with quantitative resistance and the susceptible ones.

Hahlbrock & Scheel (1987) on their part stated that biochemical responses of plant to pathogens in host or non host are similar; variations might occur most at the level of relative amounts, location and timing of induction than in terms of an all-or-none response. As for Crute (1985), the expression of single resistance genes can vary with the genetic background it occurs.

The above statements agree with our results: the B reaction was the first to appear in both cultivars with quantitative resistance and cultivar completely resistant. However, it occurred more rapidly and at a higher rate of infection sites for the former. The gene responsible of this reaction is likely the same but the intensity and the timing of the induction depends on the resistance type the cultivar is carrying. The regulation mechanisms are obviously different.

Moreover there seem to be secondary mechanisms involved in the subsequent evolution of the B type reactions: it completely disappeared in IRAT 13 and Tetep with quantitative resistance concomitantly with the increase of cell necrosis (the D reaction) 4 days after inoculation while it evolved in cytoplasmic granulation (C type) in cultivars with complete resistance. In the former, the D reactions play an important role. As suggested by Keen (1990), they may act either as reservoirs for accumulation of metabolites toxic to the pathogen and as secondary elicitors that may stimulate defence in surrounding plant cells.

Up to date, quantitative resistance was established after a durable use of a cultivar as resistant. These results make it evident that expression of quantitative resistance can be observed in the early stages of infection. Therefore, the microscopic observations of cellular reactions should be used in selecting cultivars with a high level of quantitative resistance to rice blast.

References

• **Autrique A. & Maraite H.** (1983). La pourriture brune de la gaine causée par *Pseudomonas fuscovaginae*. FAO *Plant Protection Bulletin*, 31: 94.

• **Bahama J.B.& Notteghem J.L.** (1990). "Analyse de la virulence des souches de *Pyricularia oryzae* du Burundi et de la résistance des variétés de riz expérimentées dans ce pays". Rapport de stage 13.

• **Crute I.R.** (1985). The genetic basis of relationships between microbial parasites and their hosts. In: *Mechanisms of resistance to plant diseases*, Frasser R.S.S. (ed) Nijhoff/Junk, Dordrecht, pp.80-142.

• **Hahlbrock & Scheel** (1987). Biochemical responses of plants to pathogens. In: *Innovative approaches to plant disease control*. Ed. Ilan Chet.

• **Keen N.** (1990). Gene-for-gene complementarity in plant pathogen interactions. *Annu. Rev. Genet.* 24: 447-463.

• **Peng Y.L., Shishiyama J. & Yamamoto M.** (1986). A whole-leaf and clearing procedure for analysing cytological aspects of interaction between rice and rice blast pathogen. *Ann. Phytopath. Soc.* Japan 52: 801-808.

• **Wood R.K.S.** (1967). *Physiological plant pathology*. Blackwell Publication, Oxford.