Brown rot strains of *Ralstonia solanacearum* caused devastating potato bacterial wilt in the highlands of Madagascar

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Background

Suspicious outbreak of bacterial Wilt (BW), caused by *Ralstonia solanacearum* (Rs), occurs in the Vakinankaratra highland since 2009 and severely limits potato production in Madagascar. Losses can reach 70 to 100 %. Endemic strains belong to biovar 1 [1] and 3 (Prior, *comm.pers*), which assigned to phylotype III or I [2], respectively. While BW was succesfully managed from years in this major potato area through resistant/tolerant potato cultivars (*CIP /Fifamanor accessions*), these behave now as susceptible to BW.

Knowledge of genetic and phenotypic diversity of *Rs* populations prevalent in Madagascar is the corner stone in genetic and epidemiological studies and hence in use of durable wilt-resistant cultivars to control potato BW.

Survey (2013)

A total of 763 strains were isolated from wilted potato plants, diseased tubers, rhizosphere, irrigation water within main representative potato growing areas (37 sites) in the Vakinankaratra highland. All strains are maintained in -80°C at CIRAD Réunion (*RUN 2276 to RUN 3006*).

2. Brown rot diagnosis

Phylogenetic assignation of 32 strains belonging to phylotype II from highly diseased plots. All were **phylotype IIB sequevar 1 lineage**.

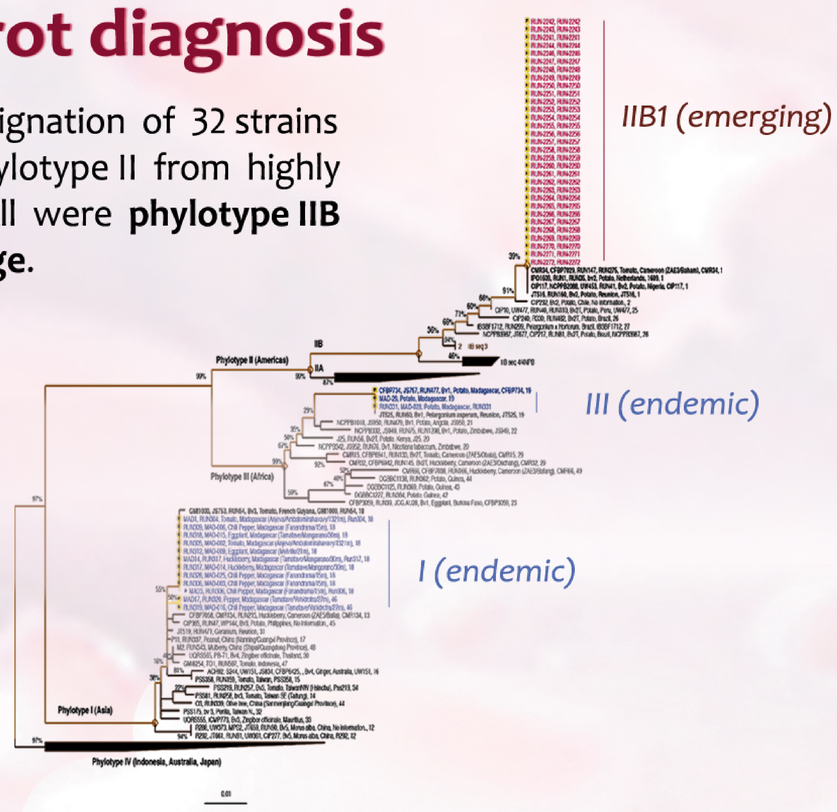

Figure 2 - Neighbor-joining tree based on the partial (750 bp) egl-gene sequences sequences corrected by Jukes and Cantor with 2000 bootstraps resamplings. Strains in blue : malagasy Rs strains already in database (phylotypes I, III); black : worlwide collection; red : strains tested.

1. Phylotype profiling

Phylotype-specific multiplex-PCR showed two lineages of strains : **phylotype III** *(132 strains)* and **surprisingly, phylotype II** *(631 strains)*.

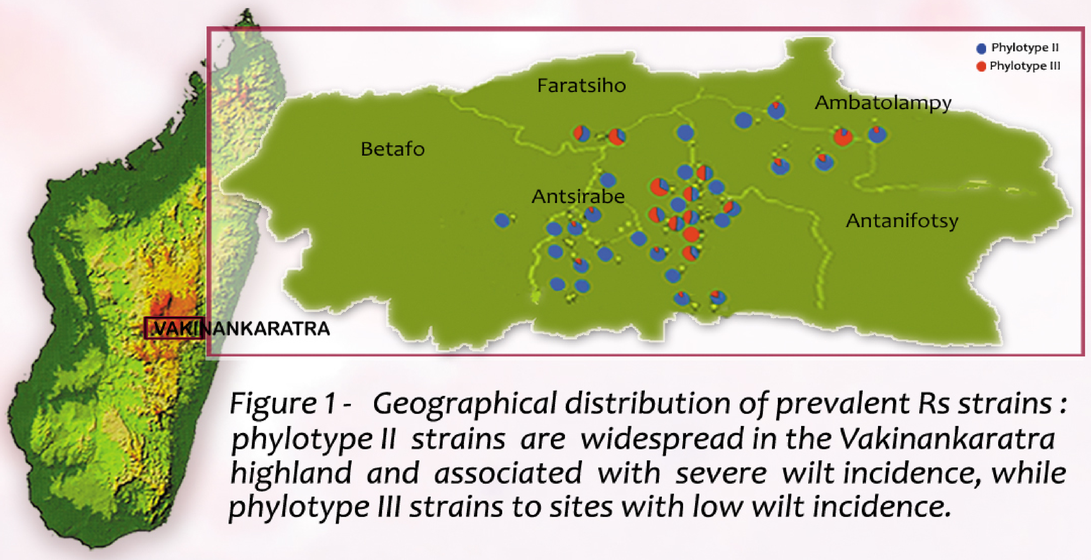

Figure 1 - Geographical distribution of prevalent Rs strains : phylotype II strains are widespread in the Vakinankaratra highland and associated with severe wilt incidence, while phylotype III strains to sites with low wilt incidence.

3. Population structure (VNTR markers, *microsatellites*)

Phylotype III (endemic) : **Malagasy Rs strains are anticipated genetically diverse. 8 VNTR** were **newly developed** in this study. They were completed with 6 VNTR available from published phylotype III MLVA-11 scheme [3]. A total of 14 polymorphic VNTR were selected for the MLVA analysis to explore phylotype III population.

Phylotype II (emerging) : A population with a narrow genetic basis is anticipated from the literature. Origin of introduction(s) will be investigated. We will use 17 VNTR markers : the MLVA-12 s cheme by N'Guessan *et al.* (2013) complemented with the MLVA-5 scheme by Parkinson *et al.* (2013).

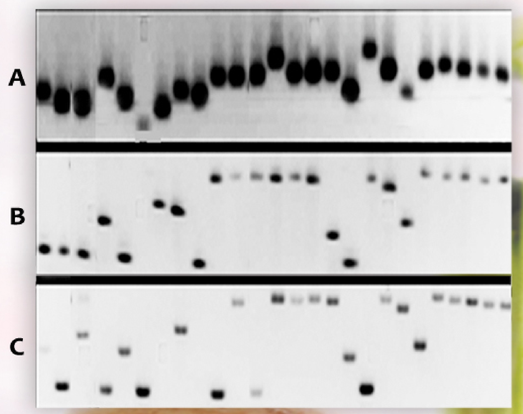

Figure 3 - Example of allelic polymorphism at three VNTR loci selected for phylotype III MLVA. (A) locus CMR026 ; (B) locus CMR1071 ; (C) locus ch0754C.

Conclusion

- **This is the first report of brown rot causing strains in Madagascar.**
- The brown rot select agent well established itself in Madagascar.
- Preliminary VNTR screening reveal a **broad genetic diversity** within members of malagasy *Rs* strains population that belongs to African phylotype III.

Future prospects

- Application of VNTR to discriminate the whole malagasy *Rs* strains and investigating genetic diversity.
- Description of genetic structure in *Rs* population.
- Investigation of the molecular epidemiology and evolutionary history of prevalent *Rs* strain in Madagascar.

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