

Genetic and mycotoxigenic diversity of isolates belonging to the *Fusarium incarnatum-equiseti* species complex, and recovered from maize and banana in China



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Introduction

In the frame of a research project aiming at detection and control of *Fusarium* and related mycotoxins in banana fruits and maize in China, plant samples were collected in four provinces (Fig. 1). In the southern provinces of Hainan and Yunnan, cultivated and wild banana plants coexist, together with maize. In Hubei and Henan, the *Fusarium* diversity was investigated on maize at the end of the growing season.

Beside 14 *Fusarium* species, an important number of isolates belonging to the *Fusarium incarnatum-equiseti* complex species (FIESC) was detected. In order to understand the impact of this finding at both the genetic and mycotoxigenic levels, their phylogeny was studied on basis of the Elongation factor-1 α (EF-1 α) gene, and the AFLP technique was used to provide a preliminary picture of their genetic diversity on maize.

Results

Besides numerous other fungal species, a total of 204 *Fusarium* isolates belonging to 15 species were obtained from the 4 visited provinces. Interestingly, the taxonomic status of 83 isolates remains uncertain. Indeed, their EF-1 α were highly similar (>99%) or even identical to isolates of the *Fusarium incarnatum-equiseti* species complex from human beings, investigated recently by O'Donnell et al. (2009), or from sorghum (Funnel-Harris et al., 2010).

An important phylogenetic diversity was observed (Fig. 2) : 33 EF-haplotypes were distinguished, grouped into 8 clusters (I to VIII), from which 7 were already described as phylogenetic species (MLST) by either O'Donnell et al. (2009) or Funnel-Harris et al. (2010). One new phylogenetic species (VI), represented by 5 isolates was detected on banana. Most isolates belong to the the *incarnatum* clade while only 1 isolate belongs to the *equiseti* clade.

No host specificity was observed : several strains from banana and maize belong to the same phylogenetic species, since some others belong to different ones. There was no correlation with geographical origin.

For maize, the 4 phylogenetic species found were perfectly supported by the AFLP clustering (Fig. 3). A similarity of less than 60% confirms a true species rank of these isolates. Furthermore, an important diversity was observed within groups, without any correlation with the geographical origin of the isolate origin.

Using PCR, it was demonstrated that the strains isolates s of the FIESC groups have NO FUM gene.

Fig. 1 Collect mission locations

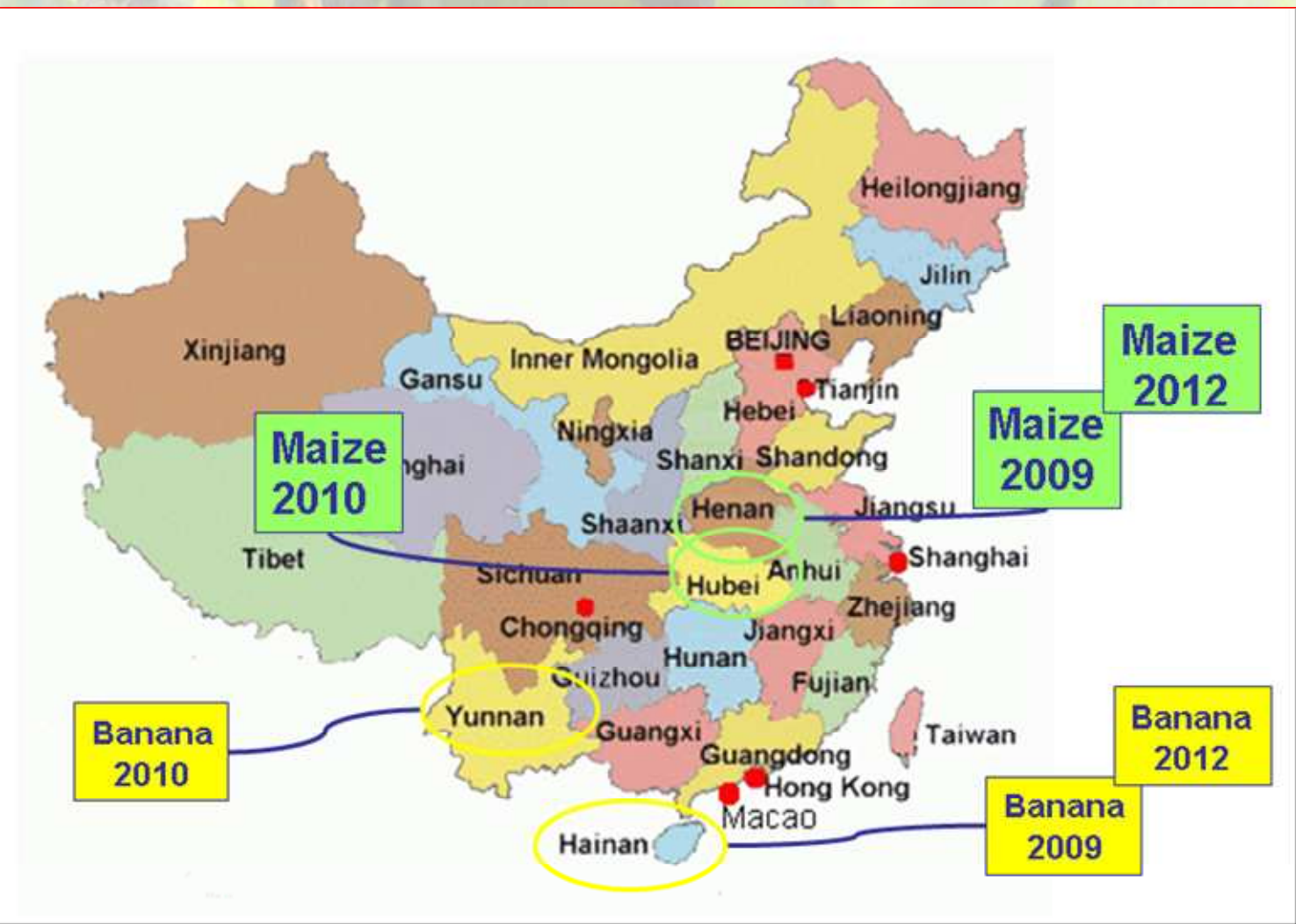
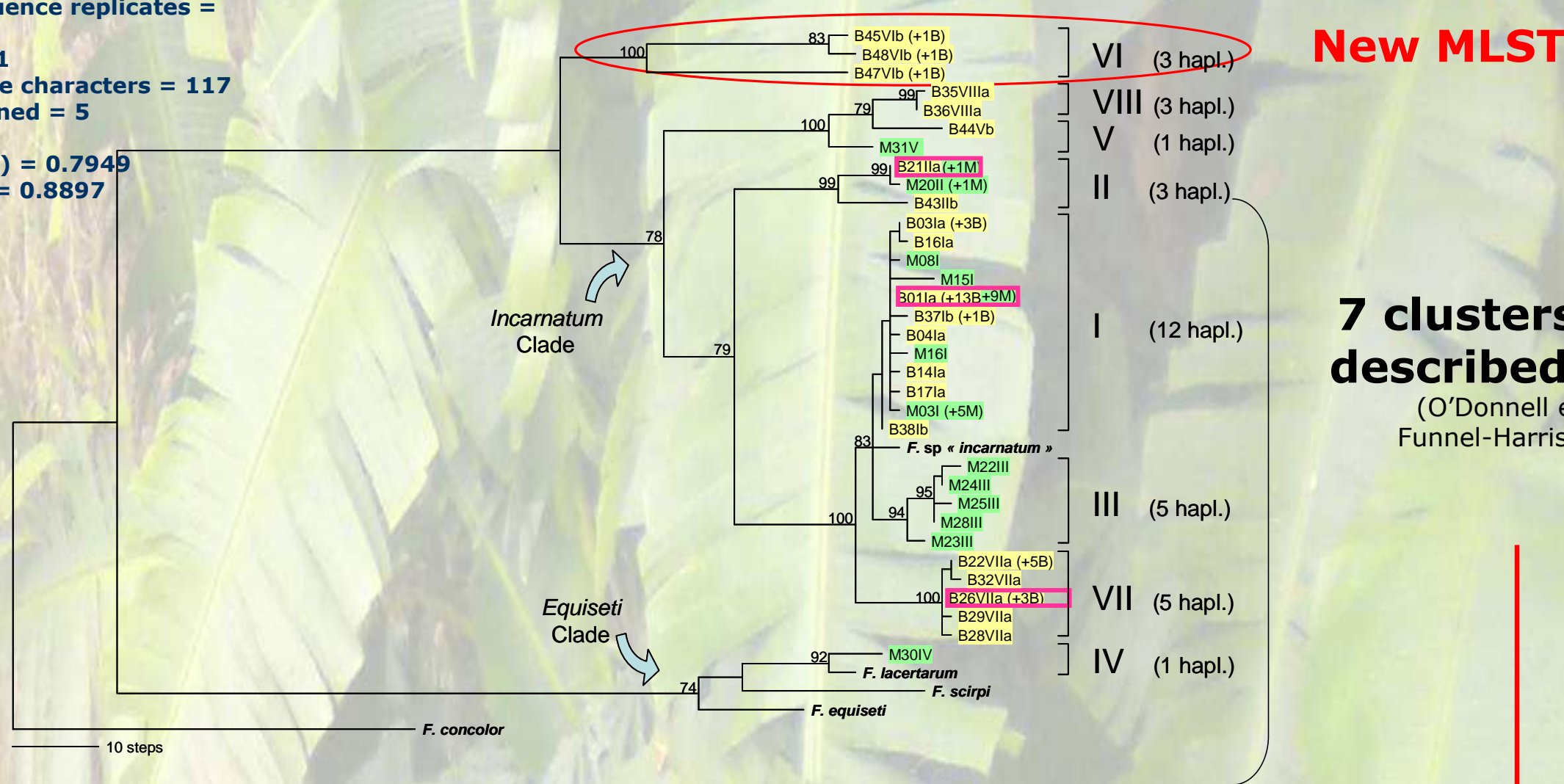


Fig. 2 Phylogenetic analyses

Bootstrap replicates = 1000
Random addition sequence replicates = 1000
Total characters = 601
Parsimony-informative characters = 117
Number of trees retained = 5
Tree length = 351
Consistency index (CI) = 0.7949
Retention index (RI) = 0.8897

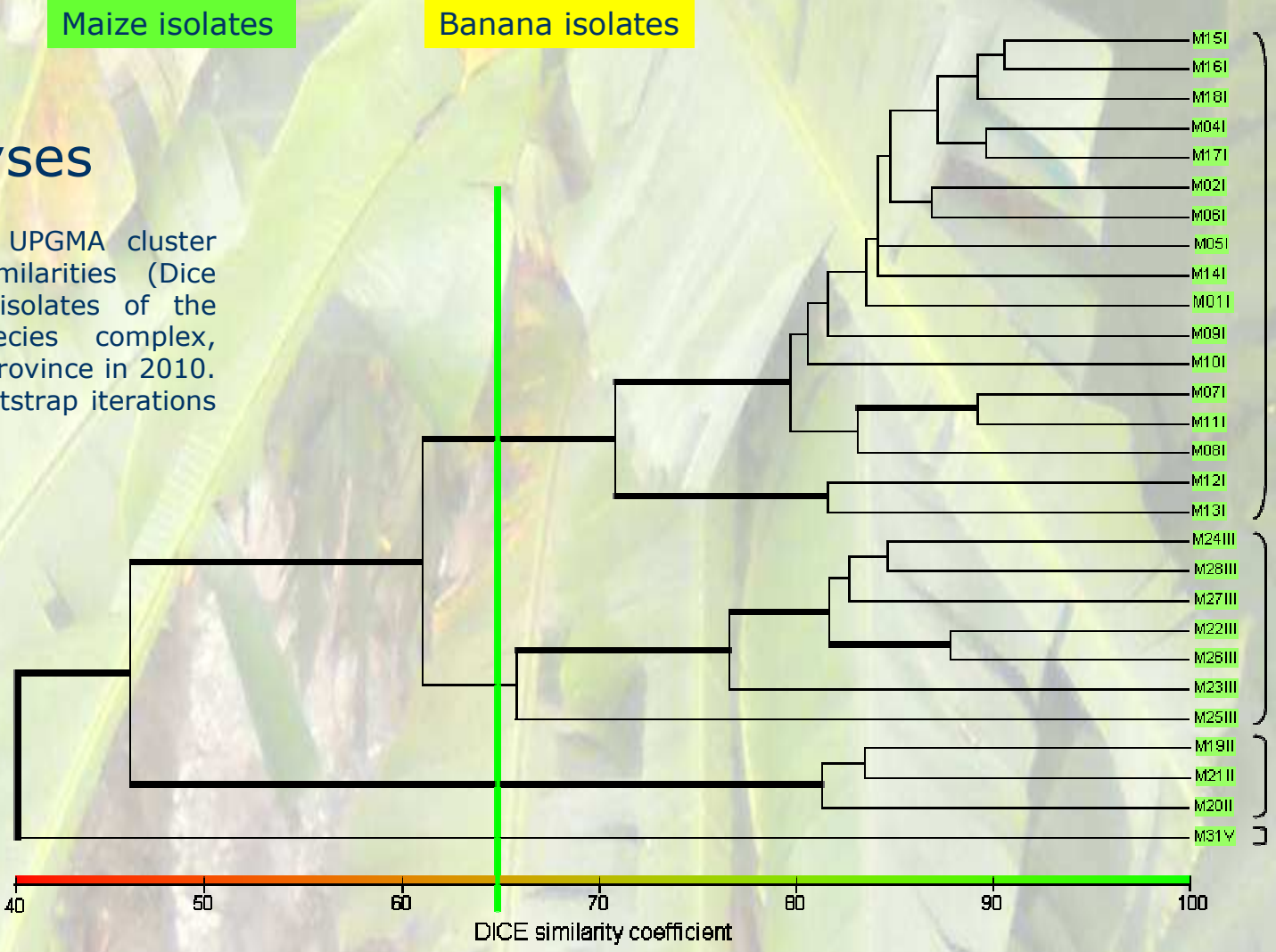


New MLST

7 clusters already described as MLST
(O'Donnell et al, 2009; Funnel-Harris et al, 2010)

Fig. 3 AFLP analyses

AFLP dendrogram generated from UPGMA cluster analysis showing the genetic similarities (Dice similarity coefficient) among the isolates of the *Fusarium incarnatum-equiseti* species complex, obtained from MAIZE in the Hubei province in 2010. Bold traits = support from 1000 bootstrap iterations with values above 70%.



... fully supported by AFLP on a larger number of maize isolates
-> species rank

Discussion and perspectives

The molecular results obtained on a large number of isolates allow to conclude that the MLST previously described could be reconsidered as species. AFLP data analyses are in progress for the banana isolates, and will be compared to those on maize. The similarity of isolates from human beings and plants should require more investigations!

Members of the FIESC have been reported in literature to produce type A and B trichothecene mycotoxins (O'Donnell et al., 2009) what led us to question about a possible multiple origin of the trichothecenes detected (or not!) on maize in China, besides those produced by *F. graminearum*. The occurrence of trichothecenes on banana is also in question.

The occurrence of FIESC isolates on both banana and maize, often cultivated near to each other, raises the question of cross contamination banana<->maize, and of the epidemiology of the disease.

Finally, the occurrence of *Fusarium* on wild banana, in an area of origin of the host, questions the occurrence of a possible coevolution phenomenon between both organisms.

Material & Method

- Identification** - the monoconidial *Fusarium* spp. isolates were molecularly identified using the Elongation factor-1 α (EF-1 α) gene sequence.
- Phylogeny** - parsimonious analyses (PAUP 4.0b10) were performed separately for isolates from the 2 plant hosts. *F. equiseti*, *F. scipri*, and *F. lacertarum* were included into the analyses; *F. concolor* was used as outgroup (O'Donnell et al. 2009). See legend Fig.2 for details of the analysis.
- AFLP** - after restriction with Eco R1 and Mse, ligation, pre-selective amplification, selective amplification (EAC-MCC ; EAC-MCG ; EGG-MCC ; EGG-MCG) and capillary gel electrophoresis, the polymorphic bands, visualized by Genographer 1.6.0, were scored 1/0. See legend Fig.3 for details of the UPGMA phenetic analysis.
- Mycotoxin potential** - PCR detection of the *FUM21* gene was performed using two home-made primer pairs targetting the *FUM21* gene in the fumonisin gene cluster:
>-fvh87, 5'-GTAATGGCWCAAACCCCTTGCAATCA-3' / fvh88, 5'-GTCTGGRCGCAAMGGCKGCATC-3'
>-fvh110, 5'-TTGATGGCTCGGATSCACAG-3' / fvh111, 5'-GAAWGGTCTCTGRGAGCGAAGCTT-3'

References

- Funnel-Harris D., Pedersen J.F. and Sattler S.E. 2010. Alteration in lignin biosynthesis restricts growth of *Fusarium* spp. in brown midrib sorghum. *Phytopathology* 100 (7), 671-68124
- Munaut F., Zhang D., Wu A., Van Peteghem C., De Saeger S. and Van Hove F. 2010. Biodiversity of *Fusarium* species on wild banana plants in the Chinese Province of Hainan. 11th European *Fusarium* Seminar, 19-23/09/10, Radosikow, Poland.
- O'Donnell K., Sutton D.A., Rinaldi M.G., Gueidan C., Crous P.W. and Geiser D.M. 2009 Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum*-*F. equiseti* and *F. chlamydosporum* species complexes within the United States. *J. Clin. Microbiol.* 47, 3851-3861.