Genetic Differences of Geographically Separated Isolates of the Fungus *Macrophomina phaseolina*

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Introduction

Macrophomina phaseolina (Tassi) Goid. is a soil and seed-borne polyphagous fungal pathogen that causes charcoal rot of more than 500 crop species, including sorgum, sunflower, soybean, cowpea and cotton (Fig. 1). The ability of *M. phaseolina* to infect roots and its aggressiveness is associated with hot and dry weather early in the growing season (Manici et al., 1995 and 1997).

According to the Intergovernmental Panel on Climate Change (IPCC, 2007) the warming of the climate system is expected to increase. Since chemical control is generally not effective and the selection of tolerant genotypes to reduce yield losses has limited success, strategies for controlling *M. phaseolina* under changing climatic conditions are needed.

The main objective of the present study was to investigate the genetic variability of several Macrophomina populations isolated from different climatic areas collected during two periods: 1981-1992 and 2005-2007. More specifically, we investigated the dynamics of Macrophomina spread to climatic areas other than those of origin of the pathogen (subtropical) and the potential impact of charcoal rot in agricultural land of mid-latitudes in view of the forecasted climate changes.

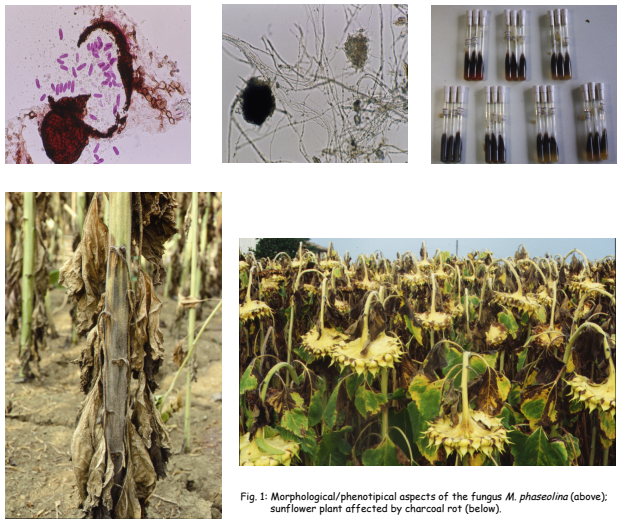

Fig. 1: Morphological/phenotipical aspects of the fungus *M. phaseolina* (above); sunflower plant affected by charcoal rot (below).

Materials and Methods

Fungal Isolates and DNA isolation

A total of 106 isolates of *M. phaseolina* collected from various climatic regions, including agricultural areas of Europe, North America, and Africa and from different hosts, were selected for this study.

Isolates were growth on PDA and genomic DNA was isolates using the DNeasy Plant Mini Kit (Qiagen). DNA was used for sequencing and random amplification of polymorphic DNA (RAPD) analysis.

DNA Sequencing and RAPD

The internal transcript spacer (ITS) region of rDNA of *M. phaseolina* isolates was amplified using the universal primer pair ITS1F/ITS4. PCR products were sequenced using the BigDye sequencing protocol. Phylogenetic trees were constructed from the multiple alignments by the neighbour-joining method.

RAPD analysis was performed using five different random primers (10 mer). Reactions were conducted with single primers and with an imposed low annealing temperature (36 °C). PCR products were separated by gel electrophoresis.

Data Analysis

RAPD profiles were converted into a binary data matrix containing haplotypes. Data matrix was subjected to neighbor-joining cluster analysis and genetic variability was analyzed using ARLEQUIN version 2.0, adopting the three following approaches:

1) Analysis of MOlecular VAriance (AMOVA), using Pairwise difference as distance method;
2) Population comparison, which computed indices of dissimilarity (Fst) between all pairs of populations. Pairwise difference was adopted as distance method.
3) Macrophomina populations were divided according to the main climatic areas in the countries of origin (see Figure 2).

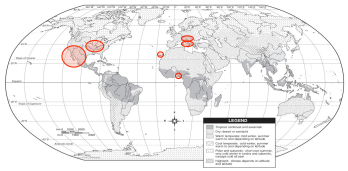

Fig. 2: Red circles indicate the six regions of origin of *M. phaseolina*.

	North Italy	South Italy	South East US,	Mid West US	Africa	CP	2006-07 north IT	2006-07 south IT
north Italy	0.000*							
south Italy	0.023*	0.000*						
SE US,	0.261*	0.170*	0.000*					
	0.238*	0.141*	0.063*	0.000*				
	0.131*	0.045**	0.184*	0.134*	0.000*			
Capo Verde	0.238*	0.142**	0.133**	0.040*	0.309*	0.000*		
2005-07 north IT	0.461*	0.455*	0.532*	0.470*	0.538*	0.654*	0.000*	
2005-07 south IT	0.465*	0.481*	0.522*	0.477*	0.577*	0.735*	0.637*	0.000*

Table 1: Population Pairwise comparison, values represents indices of dissimilarity (Fst).

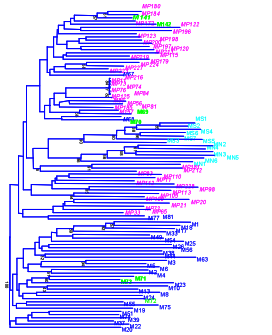

Fig. 3: Phylogenetic trees constructed from the multiple alignments of ITS rDNA sequences, using Neighbour-joining method and 1,000 boots.

Results

- The nucleotide sequences of ITS region of rDNA poorly differentiated the isolates, which were highly homologous. These findings further confirmed the scarce variability of *M. phaseolina* ITS regions .
- RAPD analysis of *M. phaseolina* DNA clearly differentiated populations on the basis of geographical origin (Fig.3), but the highest distance was observed between populations isolated in 1981-1992 and 2005-2007 respectively, with a gap of 15 years during which a global increase of surface temperature has been observed (IPCC, 2007).
- The present study demonstrated the relatively large genetic distance among the populations analyzed on the basis of the temporal factor (Table 1). The temporal factor chosen for this analysis revealed a genetic variability of the fungus much greater than geographical separation and, consequently, strongly reduced the weight of components affecting variability within populations.
- Results of the AMOVA based on temporal factors suggested an increase in spontaneous mutation rates which may be due to changes or an increase in the selective constraints during the 15-year gap.

Conclusions

According to the IPCC (2007) predictions, climate changes are expected to worsen soil conditions (high temperatures and drought) in semi-arid areas such southern Europe, already vulnerable to climate variability (Bullock, 1999) and to reduced water availability.

Those considerations combined with the findings of this study on the genetic variability of Macrophomina populations in mid-latitude areas, suggest that the potential spread of this endophytic root fungus would increase in coming years.

References

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