PREMIO SCARAMUZZI

ATOXIGENIC *ASPERGILLUS FLAVUS* ISOLATES AS CANDIDATE BIOCONTROL AGENTS IN MAIZE

A. Mauro

Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122, Piacenza, Italy

In Italy, nearly 90% of the maize is produced in five regions in the north of the country (Emilia Romagna, Friuli Venezia Giulia, Lombardy, Piedmont and Veneto). Although contaminations by fumonisins are very frequent in these areas, high levels of aflatoxins (AFs) were detected for the first time in 2003, a situation that turned worse in 2012. Aflatoxins are secondary metabolites produced by several members of the genus *Aspergillus* section *Flavi*. Among these, aflatoxin B_1 (AFB_1) is the most dangerous and has been classified by the International Agency for Research on Cancer as class 1 toxin, due to its confirmed carcinogenic and teratogenic activity in humans. The main responsible of maize AFB_1 contamination is *Aspergillus flavus*, a filamentous fungus with a vegetative incompatibility system that limits hyphal fusion and gene flow between individuals belonging to different vegetative compatibility groups (VCGs). Isolates are assigned to VCGs with functional vegetative compatibility analyses (VCAs) typically using nitrate nonutilizing auxotrophs (*nit*$^-$ mutants). The ability to synthesize AFs varies very much within *A. flavus* population, with fungal isolates that produce anywhere from over 1000 ppm AF to no toxin at all (atoxigenic isolates). The genes involved in AFs biosynthesis (confirmed 25) are contained within a 65 to 70 kb cluster, and several distinct lesions within this cluster have been described that may each be responsible for atoxigenicity in various isolates. The main objectives of this study were: (i) VCAs of *A. flavus* isolates from northern Italy, (ii) selection of atoxigenic isolates potentially useful as biocontrol agents to reduce AFB_1 contamination in maize. A high number (45%) of the isolates tested on autoclaved maize were unable to produce detectable levels of aflatoxins. *Nit*$^-$ mutants were obtained from all isolates within 10 days on chlorate-amended medium. More than 1500 mutant sectors were obtained and phenotyped revealing 72% to be *niaD*, 21% *nirA* and 7% *cnx*. Forty-six VCGs were identified; 24 contained only atoxigenic isolates, and the remaining 22 only aflatoxin producers. Members of the largest atoxigenic VCG were found in four of the five areas sampled and in different years of sampling. Molecular analysis of the genes in the aflatoxin biosynthesis gene cluster showed six deletion patterns. In particular, no deletions were detected in 10 atoxigenic isolates, whereas eight isolated had the entire cluster deleted. The capability of 18 atoxigenic isolates to reduce AFB_1 production by a toxigenic strain was evaluated on maize. Each atoxigenic strain was co-inoculated with a toxigenic, in the same proportion (1:1) or in a reduced amount (1 atoxigenic : 4 toxigenic). The extent of reduction of AFB_1 ranged from 60% to 89%, and the best five isolates, belonging to five different VCGs, showed a percentage of reduction similar to or greater than that achieved by a *A. flavus* strain registered in the USA as a biocontrol agent. Although through the proportion 1:1 (atoxigenic *versus* toxigenic) the highest percentage of reduction was achieved, the efficiency, intended as the ability of the inoculum unit of an atoxigenic strain to reduce AFB_1 produced by a toxigenic one, was greater in the 1:4 proportion. This study analysed for the first time the VCG structure of a *A. flavus* population isolated in the major maize-producing districts of Italy. Several atoxigenic fungal strains were identified as potential biocontrol agents for mitigating aflatoxin contamination, thus laying the basis for the development of a reportedly very effective biocontrol strategy in Italy.

Research work recipient of the "Professor Giovanni Scaramuzzi" award for 2012 for the best Ph.D. (Dottorato di Ricerca) thesis, sponsored by the Italian Society for Plant Pathology.

Corresponding author: A. Mauro
Fax: +39.0524.599259
E-mail: antonio.mauro@unicatt.it