For breeding purposes, use of reliable Fusarium isolates and inoculation techniques for assessment of host resistance are key components for developing strategies to mitigate the risks caused by this pathogen in maize. Results of evaluating aggressiveness of Fusarium accessions obtained in 2008 from five locations from South Romania, under both seedling and field artificial inoculation in 2009, in terms of coleoptile length (% of control), visual score (1-9) and fumonisin content (FUM, ppm), are reported.

High aggressive potential was found in all populations regardless of their geographic origin. Mean percentage values of coleoptile length across three maize hybrids inoculated with 30 Fusarium verticillioides isolates collected in 2008 (six/per location) and two types of culture media, ranged in seedling stage from 15.8 (Braila) to 19.7 (Valul lui Traian). A large variability in accumulation of FUM in grains corresponding to 26 maize genotype x Fusarium isolate combinations averaged over two culture media, was found in 2009 inoculated experiment. A significant close negative correlation between visual score and FUM content was found when 61 maize genotypes have been inoculated with FUN 640-1-2/2006 isolate in 2008.

Key words: Gibberella fujikuroi, F. verticillioides, maize, corn, fumonisin, aggressiveness, resistance

INTRODUCTION

Pink ear rot, produced by species of Gibberella fujikuroi complex (F. verticillioides, F. proliferatum and/or F. subglutinans), prevalent in drier and warmer climates represents a major constrain for grain quality in maize. Disease leads to accumulation of several secondary compounds (mycotoxins) in grains...
that are toxic for consumers (Bush et al., 2004). Among them fumonisins (FUM) are the most dangerous for health. The carcinogenic toxin fumonisin B₁ is usually found in association with moniliformin, beauvericin, and fusaproliferin, in central Europe due to the co-occurrence of \textit{F. subglutinans}; while in southern Europe spread of \textit{F. verticillioides} is reinforced by \textit{F. proliferatum}, a fumonisin B₁, moniliformin, beauvericin, and fusaproliferin producer (Logrieco et al., 2002). In Mexico, even a greater biodiversity of \textit{Fusarium} species involved in ear rot, than that previously reported was found (Rodriguez-Morales et al., 2007).

Breeding for resistance is the most effective and environmentally friendly component of control strategy aimed to prevent both fungal and mycotoxin contamination in maize caused by \textit{Fusarium} complexes: pink ear rot and red ear rot (\textit{Fusarium graminearum}) (Nagy and Cabulea, 1996, Clements et al., 2002, Miedaner et al., 2004). Up to now, no maize genotype with full resistance was found, while genetic variation for resistance has been reported (Reid et al. 1996, Clements et al. 2004). It is known that resistant materials have substantially lower mycotoxin contents than susceptible ones and selection for reduced ear rot should frequently identify lines with reduced FUM content (Reid et al. 1996, Robertson et al. 2006, Miedaner et al., 2008).

Use of aggressive \textit{Fusarium} isolates for assessment of resistance is crucial for developing appropriate pre-breeding strategies to minimize the risks caused by pink ear rot in maize. In this respect characterization of this trait is a prerequisite step for selection of the most reliable \textit{Fusarium} isolates for phenotyping and molecular approaches of resistance (Ittu and Ciocazanu, 2008, Miedaner et al., 2008).

The objectives of this study were to evaluate components of aggressiveness in \textit{Gibberella fujikuroi} populations sampled in 2008 from naturally inoculated corn ears originating from five locations from southern part of Romania under artificial inoculation, performed in seedling (coleoptile length - % of control) and field conditions (visual score and fumonisin content).

The study was performed at Fundulea (seedling stage in 2009) and Afumati (adult stage in 2008 and 2009).

**MATERIALS AND METHODS**

**Pathogen**

Corn ears displaying clear symptoms of ear rot attack were collected from several targeted locations from South Romania: Fundulea, Afumati Valul lui Traian and Dalga in 2006 and from Fundulea, Afumati Valul lui Traian, Caracal and Braila in 2008. Kernels were plated on water agar in Petri dishes for 48 hours at room temperature (25°C) and emerging \textit{Fusarium} colonies were
transferred on semi selective Fusarium media. A collection of 30 purified F. verticillioides was used for artificial inoculations in seedling stage, while ten selected of these accessions, two standard F. verticillioides isolates for low (Fv-05) and high aggressiveness (Fv-08) (provided by the courtesy of Prof. Thomas Miedaner, State Plant Breeding Institute, University Hohenheim, Stuttgart, Germany), as well as Fundulea 6-1 a proven isolate were used under field conditions.

**Plant host**

Three commercial corn hybrids have been used for seedling assessment (2009) and two for field evaluation of pathogen aggressiveness (2009). Data for 61 hybrids tested in 2008 Afumati screening were also used for FUM x scores correlation.

**Artificial inoculation**

In seedling stage, artificial inoculation was performed by germinating corn grains in a water suspension of conidia (cca 50000 conidia/ml). In adult stage, ears were silk channel inoculated at anthesis with 5 ml of inoculum (cca 1,500,000 conidia/ml).

**Evaluation of aggressiveness**

The length of seedlings coleoptile at eight post inoculation days was measured, aggressiveness being expressed as coleoptile length in inoculated seedlings vs. non-inoculated ones (as % of control). The intensity of attack based on coverage with mycelium was visually scored in harvested inoculated ears (pile of 10 ears per plot), according to a 1-9 scale, where 1=very susceptible and 9=very resistant. FUM content in grains was quantified by the ELISA method. ANOVA was calculated for coleoptile length, visual score and FUM content.

**RESULTS AND DISCUSSION**

High aggressive potential was found in all Fusarium (Gibberella fujikuroi) populations investigated irrespective of their geographic origin, confirming our previous results (Ittu and Ciocazanu, 2008). In 2008, the most aggressive isolates on average were those collected from Braila that drastically reduced the coleoptile length, the mean values registered in inoculated seedlings being only 15.8 % of control. Proveniences from other locations, could be considered less aggressive, with corresponding values ranging from 17.2 (Fundulea) to 19.7% (Valul lui Traian) (Table 1). Evaluation of Gibberella fujikuroi populations sampled from Fundulea, Afumati and Valul lui Traian, suggested that level of aggressiveness was higher on average in 2008 as compared to 2006 (Fig. 1).
Table 1

Range of variation and mean values of aggressiveness, expressed as coleoptyle length, % of control, under artificial inoculation with 2008 *Fusarium* proveniences originated from South of Romania (averaged over three hybrids, two culture media and three replications)

| Origin of isolates | Range of variation | Average |
|--------------------|--------------------|---------|
| Braila             | 9.5-19.8           | 15.8    |
| Fundulea           | 9.8-24.8           | 17.2    |
| Caracal            | 14.8-21.3          | 18.0    |
| Afumati            | 15.7-24.9          | 19.5    |
| Valul lui Traian   | 13.1-24.6          | 19.7    |
| **Average**        | **17.5**           |         |

In field conditions, artificial inoculations performed in 2008 generated visible disease symptoms, allowing a good differentiation of genotypes; a significant, negative correlation ($r=-0.729$) between visual scores (1-9) and FUM content (ppm), in 61 maize genotypes inoculated with isolate *FUN 640-1-2/2006* was observed (Fig. 2). A large variation of FUM content (ppm) in grains of two maize genotypes inoculated with 13 *F. verticillioides* isolates, ranging from 0.3 (*FUN 9-8*) to 16.5 ppm (*VTR-9-33*) was found in 2009 experiment. No values exceeding the accumulation of 22.5 ppm FUM, corresponding to the highly toxigenic standard *FV-08*, in the Romanian collection was registered, but less toxigenic entries as compared to the low standard (*FV-05*) were found (Fig. 3). Generally, an acceptable correspondence between seedling assay and FUM ac-
cumulation in grains was evidentiated (data not shown), but further confirmation is needed.

For breeding purpose the correlation between ear rot scoring and mycotoxin accumulation is important, since mycotoxin analysis are costly and laborious. Although, deviations from this relation could occur in some environments (year/location combination), use of *Fusarium* isolates that combine high pathogenic and toxigenic abilities, could contribute to accelerating of selection gain for better resistance to both components of pathogen aggressiveness in maize.
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