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COMPONENTS OF AGGRESSIVENESS IN *GIBBERELLA FUJIKUROI* POPULATIONS FROM SOUTH ROMANIA

ABSTRACT

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

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that are toxic for consumers (Bush *et al.*, 2004). Among them fumonisins (FUM) are the most dangerous for health. The carcinogenic toxin fumonisin B^1 is usually found in association with moniliformin, beauvericin, and fusaproliferin, in central Europe due to the co-occurrence of *F. subglutinans*; while in southern Europe spread of *F. verticillioides* is reinforced by *F. proliferatum, a* fumonisin B^1, moniliformin, beauvericin, and fusaproliferin producer (Logrieco *et al.*, 2002). In Mexico, even a greater biodiversity of *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 *Fusarium* complexes: *pink ear rot and red ear rot* (*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 *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 *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 *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 *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 sceening 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

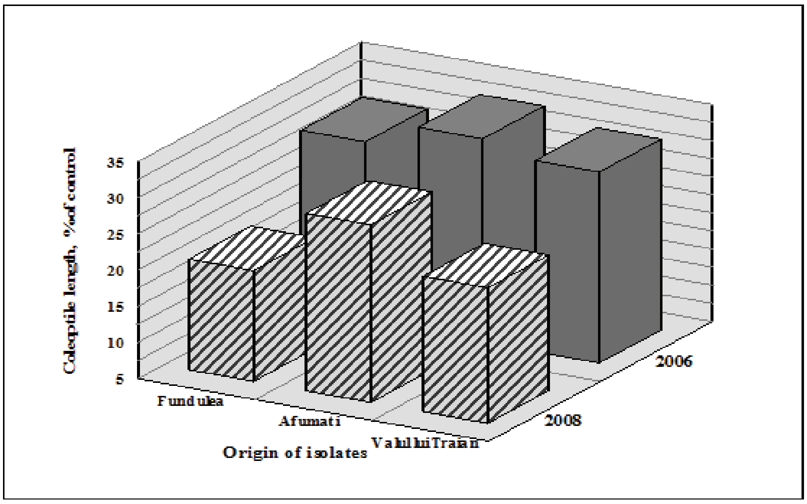

Fig. 1. Variation of aggressiveness under seedling artificial inoculation of three hybrids with 2006 and 2008 *Fusarium* proveniences originated from the southern Romanian locations Fundulea, Afumati and Valul lui Traian (mean values of coleoptyle length, % of control, over three replications)

In field conditions, artificial inoculations performed in 2008 generated visible disease symptoms, allowing a good differentiation of genotypes; a significant, negative correlation (r=-0729) 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 accumulation in grains was evidentiated (*data not shown*), but further confirmation is needed.

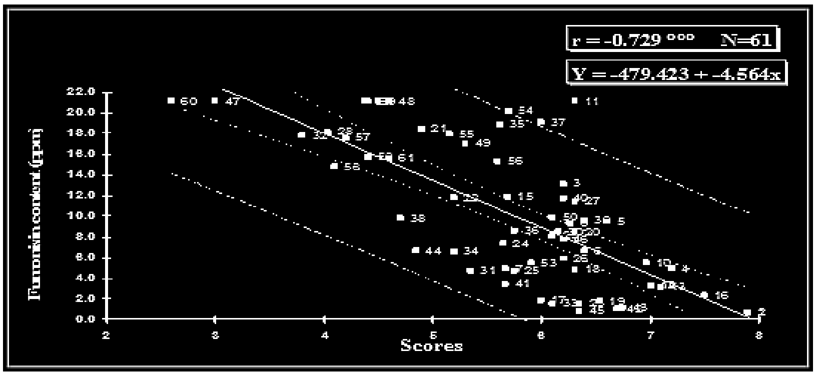

Fig. 2. Relation between pink rot scores and Fumonisin content (artificial inoculation with isolate *FUN 640-1-2/2006,* Afumati, 2008)

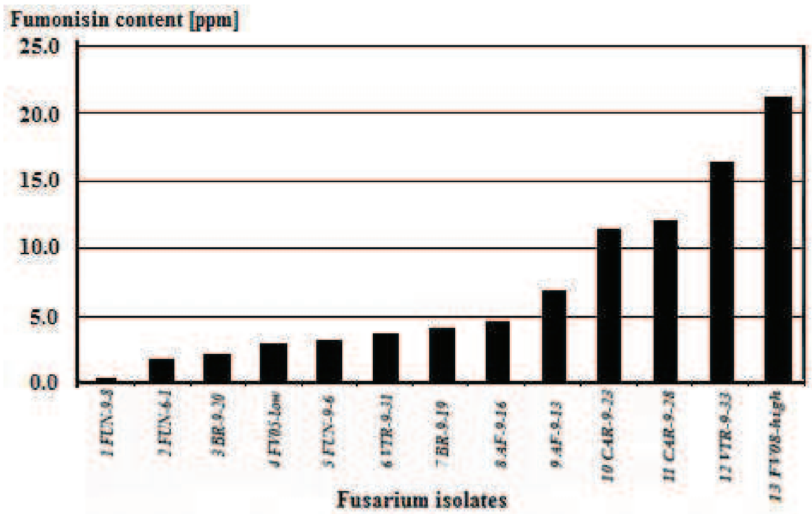

Fig. 3. Variation of fumonisin content, *ppm*, in grains of maize inoculated with 11 Romanian isolates of *Fusarium verticillioides* and two standards (T. Miedaner, *personal communication*) for low *FV-05*) and high (*FV-08*) FUM production (mean values over two genotypes, two culture media and two replications, Afumati 2009)

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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