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Evaluation of inoculation techniques for *Fusarium verticillioides* ear rot and fumonisin contamination of maize hybrid genotypes

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In breeding programmes, reliable methods for the screening and evaluation of maize plants for improving tolerance to *Fusarium* attacks are an invaluable tool in increasing crop protection against fungal infection. Some *Fusarium* strains produce mycotoxins which can be formed in infected plants before harvesting, or in grain during post-harvest storage (Bottalico, J. Plant Pathol. 80:85-103, 1998). The occurrence of mycotoxins in cereal grains is a great concern worldwide, because their presence in feed and foods is often associated with chronic or acute mycotoxicoses in livestock and also in humans (Placinta et al., Animal Feed Sci. Technol. 78:21-37, 1999).

Our research is focused on the screening of maize genotypes for tolerance to *Fusarium,* a widely distributed pathogen of maize, causing diseases of roots, seedlings, stalks and grain. In addition to their effects on yield, *Fusarium* species, particularly *F. verticillioides*, can decrease grain quality and produce a number of toxic compounds, including fumonisins. Before starting a breeding program for resistance, efficient techniques for inoculating ears with *Fusarium* ssp. and for detecting mycotoxins are needed to differentiate hybrids as resistant or susceptible under natural infection. Compared with natural infection, inoculation increases disease severity and decreases variability within and among treatments. High levels of resistance identified from inoculated trials will enable breeders to develop more resistant hybrids which are useful to producers during normal growing seasons and growing seasons when disease development is favoured.

Additionally, effective inoculation and subsequent evaluation of corn germplasm may lead to a reduction of fumonisins in grain of commercial corn hybrids and minimize economic disruptions when fumonisin production is favoured (Clements et al., Plant Dis. 87:147-153, 2003). The concentration of mycotoxins and severity of *Fusarium* ear rot are moderately correlated. In fact, fumonisins have been reported at levels of concern in asymptomatically infected grain (Bacon et al., Can. J. Bot. 74:1195-1202, 1996; Robertson et al., Crop Sci. 46:353-361,2006). Therefore, inoculation techniques that are effective for ear rot may not be effective for the assessment of fumonisin content.

The objective of this study was to identify an inoculation technique suitable for the efficient evaluation of a large number of maize hybrids for resistance to *F. verticillioides* ear rot and fumonisins in grain. Furthermore, each entry tested in the artificial inoculation experiments was evaluated in field tests at different locations in North Italy, in order to compare the response of hybrids in different environmental conditions.

For this purpose, 33 commercial maize hybrids (FAO 300-400-500-600-700) were grown in experimental plot designs at 4 different locations. Three inoculation techniques designed to promote the development of *Fusarium* ear rot were applied to self-pollinated and open-pollinated plants. For each inoculation method, 10 replicates for each plot were inoculated with a fresh spore suspension (10^6 spore/ml mixture of 2 *F. verticillioides* isolates from Northern Italy, supplied by Dr. Battilani-University of Piacenza); controls for all the inoculation techniques were non-inoculated and sterile water-inoculated primary ears.

The infection treatments were: i) wounding silk channel inoculation assay (SCIA): syringe injection of 2 ml fungal suspension in the silk channel (region within the husk between the tip of the cob and tip of the husk where the silks emerge) of the primary ear 7 days after pollination (DAP); ii) non-wounding-SCIA: silks of the primary ear were sprayed with 1.5 ml of fungal suspension 7 DAP (Clements et al., 2003); iii) kernel inoculation method: the inoculum was applied 15 DAP by dipping a stainless steel fork in the fungal suspension and stabbing the central-basal of the ear through the husks and into 3 kernels, thus producing a point source of infection from which the fungus may spread (Reid et al., Technical Bull. 1996-5E, Research Branch, Agriculture and Agri-Food Canada, 1996).

Beneficial secondary traits such as husk covering and tightness are factors contributing to fungal pathogen resistance; in general, it was reported that the hybrids with good husk cover show a greater resistance to insect damage and in turn accumulate lower levels of mycotoxins (Betran et al. Crop Sci. 42:1894-1901, 2002). Therefore, in this study, silk channel length at pollination and husk covering at maturity were recorded for each hybrid.

At maturity, ears were manually harvested. For husk cover, visual ratings ranging from 1 (good: tight long husks extending beyond the tip of the ear) to 5 (poor: loose short husks with exposed ear tips) were recorded (Betran et al., 2002). After hand de-husking, the severity of *F. verticilliodes* ear attack was evaluated using rating scales based on the percentage of kernels with visible symptoms of infection, such as rot and mycelium growth. As reported by Reid et al. (1996), for the SCIA (wounding

and non-wounding) method the visual rating scale consists of 7 classes based on percentage of visibly infected kernels (Disease Severity Rating-DSR-: 1=0%-no infection; 2=1-3%; 3=4-10%; 4=11–25%; 5=26-50%, 6=51-75%; 7=76-100%). For the kernel inoculation method, the number of visually infected kernels at the inoculation point were recorded. Individual ear ratings, using a visual scale as described above, allowed a discernible screening of the genotypes tested for *F. verticillioides* resistance.

After visual inspection, ears were dried and shelled and the kernels were bulked within replicates. Ground grain was analyzed for the concentration of fumonisin with an enzyme-linked immunosorbent assay (ELISA). Correlation analyses between visual ear rot ratings, fumonisin content, and ear morpho-physiological parameters (silk channel length at pollination, husk cover ratings) in genotypes derived from the three different inoculation technique experiments are in progress.

*This research was developed in the Program MICOCER