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Pathogenicity variation in *Fusarium verticillioides* populations isolated from maize in northern Italy

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ABSTRACT

One hundred and eighty one strains were selected among *Fusarium verticillioides* populations isolated from maize samples collected in three fields located in northern Italy. All the isolates were tested for their pathogenicity on maize seeds by assessing the seed germination percentages and the percentage infection indexes concerning seed colonization, radicle decay and coleoptile rot. *Fusarium verticillioides* strains did not affect seed germination even in presence of high seed colonization, but showed a variable pathogenic behavior according to the maize growth stages. Seedborne *F. verticillioides* population as well as strains isolated at maturity was effective in seed colonization and in inducing coleoptile rot, not causing however serious radicle decay. Only populations isolated at seedling and pre-silking stages showed high radicle decay ability. These results provide baseline information on *F. verticillioides* pathogenicity. They constitute an important input for further investigation of *F. verticillioides* biology in order to define its evolutionary potential.

1. Introduction

Fusarium verticillioides (Sacc.) Nirenberg (synonyms *F. moniliforme* Sheldon, *Gibberella moniliformis* Wineland, *G. fujikuroi* mating population A) commonly infects a wide range of crops and plants throughout the world (Bacon and Nelson 1994). Although *F. verticillioides* shows no host specialization, it mainly occurs on maize and it is in general associated with several diseases including stalk, kernel, and ear rots known as Fusarium rots (White 1999). *Fusarium verticillioides* reduces seedling stands in maize causing seed decay, root rots, damping-off and seedling blight (Soonthornpoct et al. 2000). *Fusarium verticillioides* may occur on maize as a seedborne endophyte or infect the plant at various developmental stages without inducing visible disease symptoms (Munkvold et al. 1997; Venturini et al. 2011a). Moreover, *F. verticillioides* infection of maize may cause an accumulation of mycotoxins such as fumonisins. Contamination of field-grown maize with the mycotoxin fumonisin B_1 is of a greatest concern for food and feed safety because of its causal role in equine leucoencephalomalacia (Marasas et al. 1988), porcine pulmonary edema (Colvin and Harrison 1992), liver and renal carcinogenicity in laboratory rodents, and possibly even human carcinogenicity (IARC 2002) and neural tube birth defects (Marasas et al. 2004).

Many factors, including climate, cultural practices, and host susceptibility contribute to the disease progress and to the fumonisin accumulation in maize (Parsons and Munkvold 2010). Up to now it has not been clearly defined how the

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genetic variability in *F. verticillioides* population may play a role into disease incidence and mycotoxin production. While genetic variability of *F. graminearum* populations, the causal agent of maize Gibberella ear rot (or red ear rot), has been thoroughly investigated (Zeller et al. 2004; Akinsanmi et al. 2006; Fernando et al. 2006), no reliable information is available on *F. verticillioides* population genetics, with the exception of two studies carried out in Argentina (Reynoso et al. 2009) and in the Philippines (Cumagun et al. 2009).

Many phenotypic markers, such as pathogenicity, growth rate, mycelial aspect and conidia production may be used for describing the population biology in *F. verticillioides*; among them, pathogenicity may constitute one of the most suitable characters for investigating fungal population variability (Péros et al. 1997; Zhan et al. 2007; Haque et al. 2008). By considering pathogenicity as population marker, previous studies pointed out relevant differences between mating populations within *G. fujikuroi* clade (Leslie et al. 2005; Wulff et al. 2010). Such differences were aimed to characterize *Fusarium* species associated with plants providing data on *Fusarium* biodiversity and variability. The present work was undertaken to determine the variability in *F. verticillioides* populations associated with maize in northern Italy using pathogenicity on maize seed as population marker. Moreover, by considering the composite *F. verticilliodes* epidemiology, fungal populations were constituted taking into consideration different plant organs and maize growth stages at which strains were isolated.

Pathogenicity can be quantified by several methods, for example, by determining the disease severity (Zhan et al. 2002; Cumagun and Medianer 2003). In the current study pathogenicity was measured by assessing: seed germination inhibition; seed colonization, radicle decay, and coleoptile rot abilities of *F. verticillioides* strains following a modified version of the procedure described by Munkvold and O'Mara (2002). In a previous work, the same *F. verticillioides* populations were phenotypically characterized through determination of mating type and male/hermaphrodite polymorphism (Venturini et al. 2011b). These mating behavior data were further developed by the results of the present work providing a more detailed characterization of Italian *F. verticillioides* populations.

2. Materials and methods

2.1. Fusarium verticillioides strains

One hundred eighty one *F. verticillioides* strains were arbitrarily selected among the *F. verticillioides* strains held in the culture collection at the Department of Agricultural and Environmental Sciences Division of Plant Production – Plant Pathology, University of Milan. All the strains were isolated from maize samples collected during the 2007/2008 maize cropping season in three fields located in Lombardia (northern Italy), Sant'Angelo Lodigiano (SAL), Pontevico (PO), and Pieve d'Olmi (PDO) at the following growth stages (GS): maize residues cultivated in 2007 growing season, seeds (GS00) according to BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale (Lancashire et al. 1991), seedlings with 3 unfolded leaves (GS13), plants with the tip of tassel visible (GS53), fully ripe plants (GS89) (Venturini et al. 2011a). The isolates were maintained on potato dextrose agar (PDA, Difco, Becton & Dickinson Co., Sparks) at 4 °C and as conidial suspensions stored in 20% glycerol at −80 °C.

2.2. Pathogenicity assays

Untreated maize seeds used for experimental inoculations belonged to a non commercial hybrid line 'H6' kindly supplied by Dr. F. Introzzi (FMB-CRA, Sant'Angelo Lodigiano, LO, Italy). In order to reduce the seedborne *Fusarium* contamination in maize, seeds were sterilized following a modified version of the procedure described by Daniels (1983). Seeds were placed in sterile plastic cups, covered with distilled water and stirred for 3 min. Water was removed and 100% commercial bleach (6% sodium hypochlorite) was added to cover all the seeds, which were then stirred in bleach for 20 min on a reciprocal shaker. The bleach was removed, and the seeds were rinsed twice in sterile water. They were then covered with sterile distilled water and allowed to soak for 4 h at 25 °C. They were rinsed twice more, covered with sterile distilled water and placed in a 60 °C water bath for 10 min. After the heat treatment, water was removed and the seeds were immediately transferred to a Petri dish on a sterile filter paper. The kernels were dried under sterile laminar flow hood. The seeds were then placed on the surface of PDA plates previously colonized by *F. verticillioides* after an incubation at 25 °C in the dark for 5 d. The PDA plates were incubated in the dark for 9 d at 20 °C. The assays were carried out on three replicates consisting of 10 seeds for each *F. verticillioides* isolate. A set-up of one hundred sterilized H6 maize seeds plated on uninoculated PDA plates served as negative control. Pathogenicity was evaluated by assessing germination percentage (seeds were considered germinated if the radicle was >5 mm long). In addition, seed colonization (SC), radicle decay (RD) and coleoptile rot (CR) were assessed, for each seed, on a 0–4 scale where 0 = seed surface uncovered with mycelium or well developed root/coleoptile showing negligible decay symptoms; 1 = ≤25% seed surface covered with mycelium or root/coleoptile area showing decay symptoms; 2 = >25 to ≤ 50% seed surface covered with mycelium or root/coleoptile area showing decay symptoms; 3 = >50 to ≤ 75% seed surface covered with mycelium or root/coleoptile area showing decay symptoms and 4 = ≥75% seed surface covered with mycelium or root/coleoptile area showing decay symptoms. The disease severity index in percentage expressed by percentage infection index (I%I) was calculated according the Townsend–Heuberger formula (Townsend and Heuberger 1943).

2.3. Statistical analyses

All statistical analyses were performed using PAST software ver. 1.95 (Hammer et al. 2001). I%Is assessed were grouped by location, maize growth stages, plant organs from which *F. verticillioides* strains were isolated and by mating type and female fertility of the isolates. Box plots were drawn in order to graphically explore the distributions of the data. The agglomerative hierarchical clustering analysis using the Euclidean similarity coefficients under the rule of unweighted