GENETIC VARIABILITY OF *FUSARIUM VERTICILLIOIDES* ISOLATES COLLECTED FROM MAIZE IN HUNGARY

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Abstract
Fusarium verticillioides (teleomorph: *Gibberella moniliformis*) is a widely distributed pathogen of maize which is able to cause corn seedling blight, root rot, stalk rot and ear rot, and also can infect maize as an endophyte, without any symptom development. Both symptomatic and asymptomatic kernel infections by *F. verticillioides* can result in decreased quality of corn and economic losses due to contamination by fumonisins, polyketide derived mycotoxins causing various diseases in animals including kidney and liver cancer in rodents, pulmonary edema in pigs, leucoencephalomalacia in horses and a postulated role in human esophageal cancer.
We examined the genetic variability of *F. verticillioides* isolates collected in Hungarian maize fields. The samples were collected during 2010-2011 from various maize-growing areas of Hungary. The isolates were identified at the species level using sequence analysis of part of their translation elongation factor-1 gene. Altogether 39 *F. verticillioides* isolates were subjected to UP-PCR analysis using 8 different primers. 155 bands were taken into account during the phylogenetic and population genetic analyses. On the neighbor-joining tree prepared from the similarity matrix, every isolate formed a haplotype, i.e. all isolates could be separated from each other based on their UP-PCR profiles. Geographic clustering of the isolates could also be identified on the tree: most isolates came from the Transdanubian region of Hungary formed a cluster, while those from the Eastern part formed 3 different clusters separated from the other isolates.
F. verticillioides is a heterothallic fungus. The distribution of the mating type genes was also examined in the population using specific primer pairs. Based on the PCR profiles, 44% of the isolates belonged to the MAT1-1, while 56% to the MAT1-2 mating type. The nearly equal distribution of the mating types in the population indicates that the population reproduces both sexually and clonally. However, population genetic analysis of the data using index of association tests and parsimony tree length permutation tests indicated a predominantly clonal population structure.
Further studies are in progress to examine the fumonisin producing abilities of the isolates, and to correlate these data with their UP-PCR profiles.

Key words: *Fusarium verticillioides*; population structure; UP-PCR analysis; maize

Introduction

Fusarium verticillioides is one of the most important pathogens of maize worldwide. This species may cause various diseases of maize including seedling blight, root rot, stalk rot and ear rot, and also can infect maize as an endophyte, without any symptom development. Both symptomatic and asymptomatic kernel infections by *F. verticillioides* can result in decreased quality of corn and economic losses due to contamination by fumonisins. Fumonisins are carcinogenic polyketide-derived mycotoxins which cause various disease symptoms in animals including leucoencephalomalacia in horses, pulmonary edema in pigs, hepatocarcinoma in laboratory animals. Besides, fumonisins have also been implicated as causative agents of esophageal cancer and neural tube defects in humans.
In this study, we examined the genetic variability and population structure of *F. verticillioides* isolates collected from maize in Hungary. The isolates were assigned to species using sequence-based methods, UP-PCR analysis was used to examine the genotypic variability of the isolates, and the distribution of the mating type genes in the population was also examined.

Materials and methods

The *Fusarium* isolates were collected in 2010-2012 from maize kernel in maize fields located in different parts of Hungary. The cultures used for the molecular studies were grown on malt peptone broth using 10 % (v/v) of malt extract and 0.1 % (w/v) bacto peptone, and incubated at 25 °C for 7 d. DNA was extracted from the cells using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. For species identification, part of the translation elongation factor α gene was amplified and sequenced (data not shown). 39 *F. verticillioides* isolates were selected for further studies. The presence of mating type idiomorphs was examined using the primer pairs developed by Kerényi et al. (2004) as described previously (Tóth et al., 2004).

UP-PCR analyses were carried out according to Bulat et al., (2000). The primers used were L45, AS15inv, L15/AS19, AA2M2, L21, 3-2, AS4, AS15 (Bulat et al., 2000, Lübeck et al., 1998). The amplification process consisted of a predenaturation step for 1 min at 94 °C, followed by 35 cycles (30 s at 94 °C, 45 s at 55 °C, and 1 min at 72 °C), plus a final extension of 2 min at 72 °C. The amplification products were separated by the Agilent 2100 Bioanalyzer (Agilent Techn.). All amplifications were repeated at least two times. The faint bands which did not appear in all repeated experiments were not counted during cluster analysis. Altogether 155 fragments were noted and a binomial matrix was created so that presence and absence of DNA fragments were scored as 1 or 0, respectively. Cluster analysis was carried out by using PHYLIP version 3.67 software package (Felsenstein, 2007). A phylogenetic tree was created by using neighbor-joining method (Saitou et al., 1987) with the program NEIGHBOR from the PHYLIP program package. A *F. graminearum* isolate was used as outgroup in these analyses. The final tree was visualized by MEGA4 (Tamura et al., 2007).

Index of association tests (I_A) and parsimony tree length permutation tests (PTLPTs) were performed using the MULTILOCUS 1.2 software with 1000 randomizations (Agapow and Burt, 2001). For the I_A tests, the observed data were used to simulate recombination by shuffling (resampling without replacement) the alleles at each locus of the observed data. For PTLPTs, the null hypothesis was recombination, and significance was determined by the fraction of tree lengths based on resampled data that are at least as long as those based on the observed data. The PAUP software package was used for calculating the tree lengths from 1000 randomizations (Swofford, 2000).

Results and discussion

During UP-PCR analysis, 39 *F. verticillioides* isolates were examined using 8 different UP-PCR primers (Figure 1). Based on neighbor-joining analysis of the UP-PCR data, every isolate represented a single haplotype (Figure 2), indicating a high level of genetic diversity of the population. The isolates formed distinct clusters according to their origin: the isolates collected in Transdanubia formed a single cluster, while those collected in Eastern Hungary (mainly the Great Plain) formed two separate clusters. The three isolates collected in Szeged formed a small cluster too.

The distribution of the mating type genes was also examined in the population using specific primer pairs. Based on the PCR profiles, 44% of the isolates belonged to the MAT1-1, while 56% to the MAT1-2 mating type (data not shown). The nearly equal distribution of the mating types in the population indicates that the population reproduces both sexually and clonally.

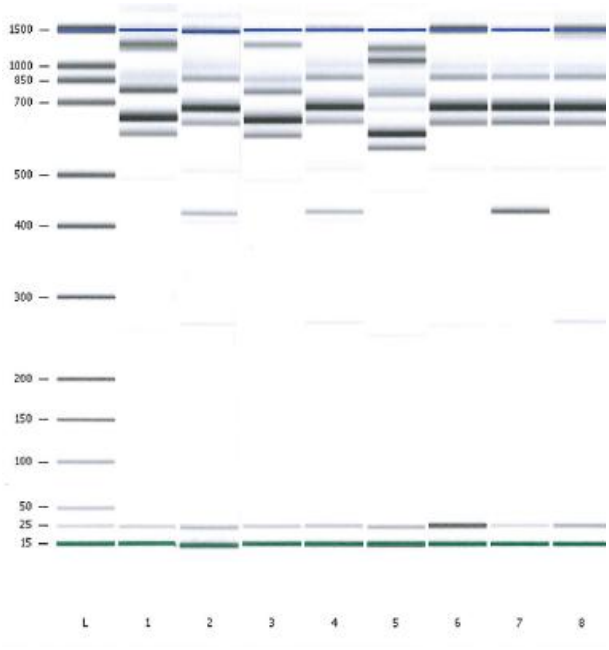

Figure 1. UP-PCR profiles of some *F. verticillioides* isolates using AS4 as primer (L: 1 kb DNA ladder).

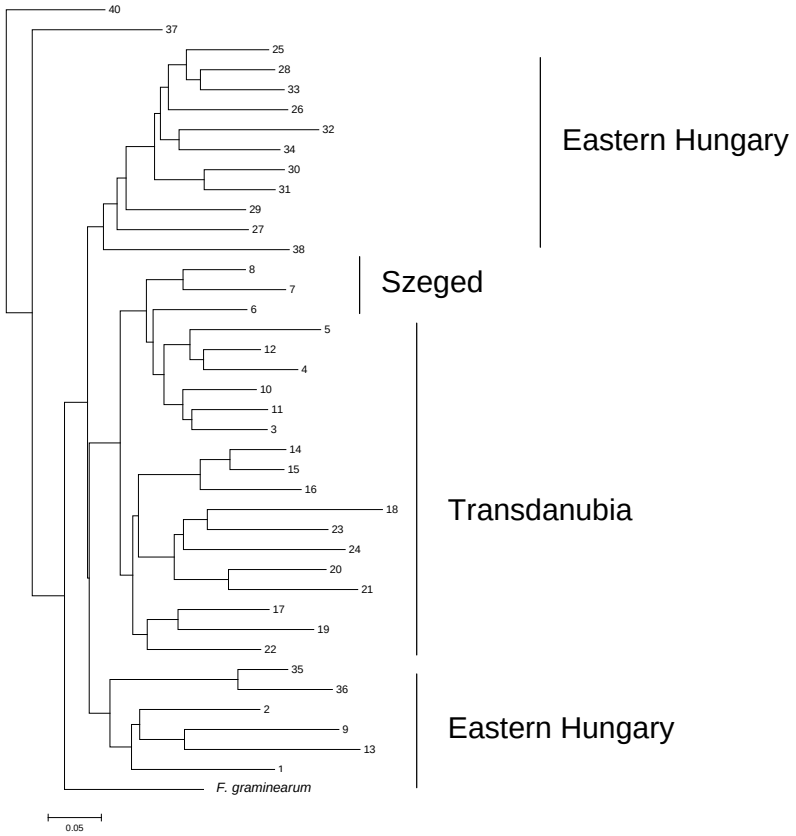

Figure 2. Neighbor joining tree of the *F. verticillioides* isolates based on their UP-PCR profiles.

The population structure of the isolates was examined using index of association tests and parsimony tree length permutation tests. The association index (I_A) test involves the calculation of the variance of genetic distances in a data matrix; in recombining populations the distribution of distances should be normal with low variance, while in clonal populations the variance is high (many distant and close relatives and only a few at the mean; Taylor et al., 1999, Varga and Tóth, 2003). I_A is a rescaled variance, which is zero in strictly recombining populations, and 1 in strictly clonal populations. The observed I_A value is also compared to I_A values obtained for 1000 artificially recombined data sets. The I_A value of a clonal population should be significantly higher than the distribution of I_As for the artificially recombined data sets, while the I_A of a recombining population is within the range of I_A values obtained with the 1000 randomized data sets.

The association index of the *F. verticillioides* population was 3.189, which was significantly higher than that of the artificially recombined data sets (-0.0028; Figure 3), indicating that the population reproduces primarily clonally.

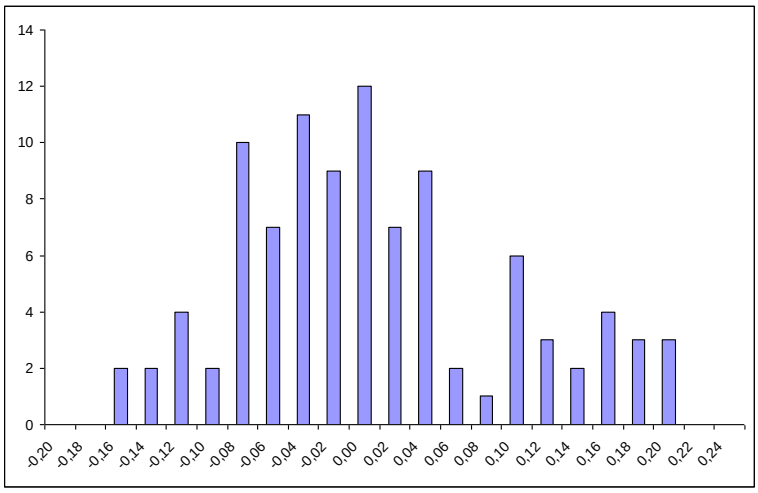

Figure 3. Distribution of I_A in artificially recombined data sets of the UP-PCR data obtained for the *F. verticillioides* population. The actual IA of the original data set was 3.189.

The parsimony tree length permutation test (PTLPT) is derived from methods developed for detecting signals in phylogenetic analyses (Taylor et al., 1999, Varga and Tóth, 2003). Parsimony trees are built for the observed multilocus genotypes and for data sets that have been resampled to simulate recombination as described for the I_A test. A clonal population will support one or a few short, well-resolved trees, whereas a recombining population will support many, longer, and poorly resolved trees. For the *F. verticillioides* population, the actual tree length was 888, while the average of the tree lengths of the artificially recombined datasets was 112, indicating again that the population reproduces primarily clonally (data not shown).

Conclusions

The genetic variability and population structure of *Fusarium verticillioides* was examined in Hungarian maize fields. Although the mating type idiomorphs were distributed in nearly 1:1 ratio, and the genetic variability of the population was high, population genetic tests indicate that the examined population reproduces predominantly clonally. Further studies are in progress to examine the fumonisin producing abilities of the isolates and correlate the toxin producing profiles to the UP-PCR profiles of the isolates.

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