Genetic variability of phytopathogenic Fusarium proliferatum associated with crown rot in Asparagus officinalis L.

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Introduction

Fusarium proliferatum (Matsuhima) Nirenberg (teleomorph: *Gibberella intermedia* (Kuhlman) Samuels) [Fig. 2] is an important pathogen involved in the destructive crown and root rot of asparagus (*Asparagus officinalis* L.). Infection of plants with *F. proliferatum* leads to asparagus decline, yielding unprofitable plantations in long-term cultivation of this perennial plant [Fig. 1].

This species as well as *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. verticillioides* are described as producers of the fumonisin mycotoxin. Fungal isolates could not only be isolated from roots and crowns but were also found in asparagus spears obtained within the main harvest period, which does not show symptoms of an infection with *Fusarium* spp. (Goßmann et al. 2005).

Fig. 1: Symptoms of asparagus crown and root rot. 1) infected crown and roots of an 1 year old asparagus plant by *Fusarium* spp.; 2) chlorotic shoots caused by *Fusarium* spp.; 3) Fusarium infested areas in a asparagus plantation. Pictures by courtesy of the Institute of Vegetable and Ornamental Crops (IGZ eV), Großbeeren

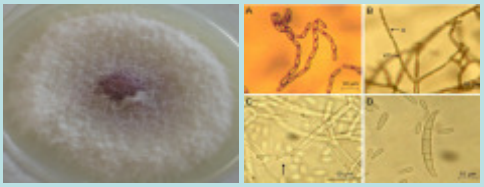

Fig. 2: Morphological characteristics of *F. proliferatum* on SNA (left) and under the microscope (400x), (right) **A and B:** microconidia in chains [K] and on primary conidiophores [KT], **C:** polyphialides, **D:** micro- and macroconidia

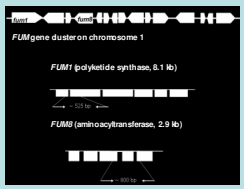

Fig. 3: Fumonisin gene cluster organisation an analyzed gene fragments of *FUM1* and *FUM8*.

Methods and Results

F. proliferatum isolates obtained from perennial asparagus plantations from Austria and Germany were included in a study of genetic variability and detectability of two essential genes of the fumonisin gene cluster and compared with *Fusarium* spp. infecting crops. Genetic fingerprinting of 45 isolates by RAPD- and DAF-PCR revealed genetic heterogeneity of *F. proliferatum* by establishment of 14 different fingerprint groups [Fig. 4]. Most isolates differentiated into 3 main fingerprint clusters, but no association was found between fingerprint group and origin of the isolates. By gene specific PCR it was shown, that in all isolates tested both initial genes of the fumonisin biosynthetic pathway - *FUM1*, encoding a polyketide synthase as well as *FUM8*, an aminoacyltransferase gene - were detectable. In *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. verticillioides* isolates these genes were sporadically detected.

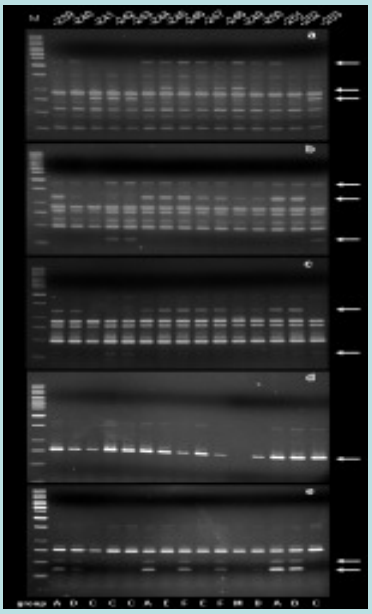

Fig. 4 Genetic fingerprinting of *F. proliferatum* isolates applying RAPD-PCR with Q1 (a) and Q6 (b) primers and DAF-PCR with primer combinations Q2, Q6 (c) Q2, Q20 (d) and Q11, Q20 (e). Fragments of diagnostic value are indicated by an arrow. M = Marker 1 kbp Ladder, Fermentas

The fumonisin biosynthetic gene cluster [Fig. 3] is thought to be exclusively present in isolates of the genus *Fusarium* with fumonisin producing potential (Waalwijk et al. 2004). Verification of *FUM* genes suggests that these fungal strains are able to produce fumonisins and responsible of contamination of asparagus spears with this mycotoxin. Fumonisin B production *in vitro* and *ad planta* has been confirmed for two *F. proliferatum* strains. Amplified *FUM* fragments exhibited nucleotide polymorphisms as shown by sequencing [Table 1, Fig. 7], but were not discriminated by PCR-RFLP [Fig. 5, 6]. Sequence variability of *FUM1* and *FUM8* gene fragments of the *F. proliferatum* isolates was below 1%. *FUM8* fragments exhibited overall higher interspecific sequence divergences than *FUM1* genes. Fragments from *F. verticillioides* shared 84% (*FUM1*) and 77% (*FUM8*) sequence identity with *F. proliferatum* sequences [Table. 1].

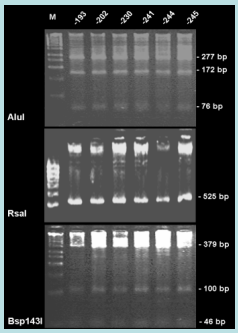

Fig. 5: PCR-RFLP pattern of *FUM1* fragments, amplified from *F. proliferatum* isolates from asparagus, with AluI, RsaI and Bsp143I.

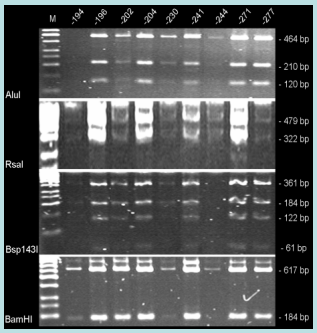

Fig. 6. PCR-RFLP pattern of *FUM8* fragments, amplified from *F. proliferatum* isolates from asparagus, with AluI, RsaI, Bsp143I and BamHI.
M = Marker 50 bp Ladder, Fermentas

Table 1: Nucleotide sequence identity [%] by pairwise comparison of *FUM1* and *FUM8* gene fragments of *Fusarium* spp.

	FUM1				*FUM8*			
	n[1]	[bp][2]	Mean (S.D.)	Min.	Max.	n[1]	[bp][2]	Mean (S.D.)	Min.	Max.
Intraspecific sequence identity										
F. proliferatum	11	484	99.89 (0.253)	99.1	100	10	258	99.84 (0.328)	99.2	100
F. verticillioides	10	482	100.00 (0.37)	100	100	2	257	100	100	100
Interspecific sequence identity of *F. proliferatum* isolates										
Fusarium species complex										
F. fujikuroi	1	484	96.91 (0.17)	96.9	97.1	1	258	98.06 (0.248)	98.4	98.8
F. globosum	1	484	96.26 (0.17)	96.9	96.9	1	258	98.06 (0.248)	98.4	98.8
F. nygamai	1	484	96.94 (0.17)	96.7	97.1	1	258	98.06 (0.248)	98.4	98.8
F. oxysporum	1	484	96.94 (0.17)	96.5	97.0	1	258	98.06 (0.248)	98.4	98.8
F. verticillioides	10	484	96.32 (0.28)	96.5	96.9	2	258	97.67 (0.248)	97.5	97.8
Interspecific sequence identity of *F. proliferatum* isolates to *Fusarium* species complex										
F. oxysporum	1	484	96.26 (0.17)	96.9	96.9	1	257	97.99 (0.248)	97.5	97.8

1 sequences per *Fusarium* species included in the pairwise comparison
2 compared fragment length without primer sequences, ID identical sequences

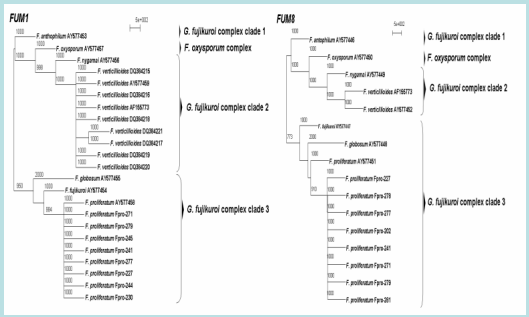

Fig. 7: Maximum-parsimony phylograms of *FUM1* and *FUM8* nucleotide fragments obtained from *F. proliferatum* strains in comparison with corresponding *Fusarium* spp. sequences from the database (indicated by accession numbers behind the *Fusarium* species). Species complexes according to O´Donnell et al. (1998) are indicated in the middle. Bootstrap values (n = 1000 repetitions) over 700 are indicated on branch nodes.

Conclusion

Genome wide variability of *F. proliferatum* occurring in asparagus obtained by RAPD- and DAF markers is mirrored by *FUM1* and *FUM8* gene diversity, but obviously not in the presence or absence of the fumonisin gene cluster within this species.

References

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