Szent István University

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# IDENTIFICATION OF GROWTH STAGE SPECIFIC GENES IN *GIBBERELLA INTERMEDIA* (*FUSARIUM PROLIFERATUM*)

Abstract of the thesis

Apor Jeney

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School: Biology PhD School

Discipline: Biology

School leader: Dr. Zoltán Tuba
Professor, Head of department
Szent István University,
Faculty of Agricultural and Environmental Sciences,
Department of Botany and Plant Physiology

Supervisors: Dr. László Hornok
CMHAS, Professor, Head of department
Szent István University,
Faculty of Agricultural and Environmental Sciences,
Department of Agricultural Microbiology and Biotechnology

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Dr. Zoltán Tuba
school leader

Dr. László Hornok
supervisor

## Introduction

*Gibberella fujikuroi* (Sawada) Ito & Kimura species complex is composed of at least nine different mating populations (biological species) that are reproductively isolated and are denoted by letters from A to I. These species have a worldwide distribution and cause disease on a wide variety of agricultural plants. Some of these mating populations have been given *Gibberella* species names that parallel the names of their *Fusarium* anamorphs (Leslie et al., 2004). One member of this species complex is *Fusarium proliferatum*. On the basis of fertility, most strains of *F. proliferatum* belong to MP-D (*G. intermedia*). *F. proliferatum* is a well-known, worldwide distributed pathogen of several plants including asparagus, date palm, maize, rice, sorghum, tomato, wheat and onion (Abdalla et al., 2000; Desjardins et al., 1997; Dugan et al., 2003; Elmer, 1990; Leslie, 1995; Moretti et al., 1997). Strains of *F. proliferatum* secrete mycotoxins, such as fumonisins, moniliformin, beauvericin and fusaproliferin that can contaminate animal feed and human food (Nelson et al., 1992; Thiel et al., 1991; Bacon et al., 1996; Marasas et al., 1986; Ritieni et al., 1995; Logrieco et al., 1995). This fungus is an example of a facultative fungal endophyte, its symptomless nature have been observed in several cases (Munkvold and Desjardins, 1997).

Amino acids serve as nitrogen and/or carbon sources or as building blocks for protein synthesis of many eukaryotic cells. Amino acid transporters (permeases) of fungi mediate the selective and strictly controlled uptake of amino acids and the movement of substrate is driven by a proton electrochemical gradient (Young et al., 1999). Some amino acid transporters have specificity and participate in the uptake of only a single compound, whereas others have high affinity for a number of amino acids (Sophianopoulou és Diallinas, 1995). Most amino acid transporters manage only the uptake of amino acids, but some may have other functions, as well. For example, Ssy1 of *Saccharomyces cerevisiae* is a part of Ssy1-Ptr3-Ssy5 sensor complex and acts as a sensor protein with its unusually long N-terminal domain and regulates the expression of a number of other permease genes in this fungus (Didion et al., 1998; Klasson et al., 1999; Forsberg and Ljungdahl, 2001).

Based on their structural and functional characteristics, most fungal amino acid transporters can be assigned either to the AAP (Amino Acid Permease) or to the AAAP (Amino Acid/Auxin Permease) families. These families can further be classified as subfamilies of the APC (amino acid/polyamine/organocation) permease superfamily (Jack et al. 2000). Members of the AAP family share significant sequence similarities and they all contain a typical 12 α-helical transmembrane domain with cytoplasmically located N- and C-terminal hydrophilic regions (Sophianopoulou and Diallinas 1995). Transporters belonging to the AAAP family, which includes auxin and amino acid permeases in plants and animals and vacuolar amino acid transporters of *S. cerevisiae*, share

minimal sequence homology and have 11 α-helical transmembrane domain (Young et al., 1999; Russnak et al., 2001).

The expression of most amino acid transporters is controlled at transcriptional level through substrate induction and/or nitrogen and/or carbon catabolite repression (Sophianopoulou and Diallinas 1995). The nitrogen catabolite repression seems to be a general mechanism to control amino acid uptake in fungi. Certain nitrogenous compounds – ammonia, glutamine and glutamate – are preferentially used by fungi. If these primary nitrogen sources are not available, decreased expression of the genes encoded amino acid permeases could be observed (DeBusk és DeBusk, 1980; Trip et al., 2004b). The transcription factors play important roles in the de novo synthesis of the permeases. GATA-type zinc finger transcription factors activate nitrogen structural genes when preferred nitrogen sources are not available. These factors mediate nitrogen catabolite repression (Marzluf, 1997). Signals of amino acid starvation lead to the activation of the general activator GCN4-like protein which activates the transcription of most genes encoding enzymes involved in amino acid biosynthesis in turn. The GCN4-like protein DNA binding consensus sequence of TGACTC is presented in the promoter region of these genes (Ebbole et al., 1991). However, examples of post-translational regulation are also known. The major mechanisms for post-translation control are feedback inhibition and transinhibition, which occur at the protein level (Sophianopoulou and Diallinas 1995).

Apart from a few exceptions, most transporter-encoding genes are constitutively expressed or activated by nitrogen or sulphur starvation (Gow and Gadd, 1995). The *prnB* gene encoding the major prolin transporter of *Aspergillus nidulans* is upregulated during conidiospore germination and in response to prolin induction and amino acid starvation. Activation of *prnB* expression is a real developmental phenomenon in germinating conidia however transcripts of this gene could be also detected at steady-state level in the fungal mycelia (Tazebay et al., 1997).

No data are available on the amino acid transporters of *Fusarium/Gibberella* species, neither the structure nor the function of these proteins have been investigated. An unusual amino acid transporter gene was identified in the course of the investigation of growth stage specific transcript profiles in *F. proliferatum*. The aim of this work was to characterize this gene and to determine its role in *F. proliferatum*.

## Methods

### Growth conditions

To construct the growth curve of *F. proliferatum* ITEM 2287 and to study germination of Δ*Fpmtr* mutant strains and expression pattern of *Fpmtr* gene one hundred ml complete and minimal medium

was inoculated with microconidia at a final concentration of $10^6$ $ml^{-1}$ and grown as shaken culture (180 rpm) at 26°C, in the dark for 220 hours. Resistance to p-fluoro-DL-phenylalanine (FPA) was assayed by growth in MM, modified by substituting equimolar amounts of $NH_4Cl$ for $NH_4NO_3$. After staying for 3 days at 26°C the dry weight of the mycelia in 20 ml liquid cultures was measured (Margolis-Clark et al., 2001).

**cDNA-AFLP**

Total RNA was isolated from fungal mats and second strand cDNA was synthesized from 2 μg of mRNA. Second strand cDNA was digested by restriction endonucleases, *Eco*RI and *Mse*I and *Eco*RI and *Mse*I adapters were ligated to cDNA fragments (Vos et al., 1995). Pre-amplification was performed with a pre-amplification program in 19 cycles with primers corresponding to the *Eco*RI and *Mse*I adapters, without extension. The PCR products were amplified again with one or two selective base extensions at the 3' end of the *Mse*I and *Eco*RI primers with a cDNA-AFLP touchdown-selective amplification program. The *Eco*RI primers were 5' end labeled with [$^{33}\gamma$P]ATP. Bands, which seemed to be differentially expressed on the basis of visual evaluation were cut from gel and re-amplified. The amplified fragments, separated on a 2% agarose gel, were cloned into pBluescriptIIKS by T4 DNA ligase. *Escherichia coli* strain DH5α was transformed with the resulting plasmid (Sambrook and Russel, 2001). Transformed *E. coli* colonies were checked for the presence of the expected insert by PCR with the same primers used in the selective amplification program. Inserts obtained from these plasmids were labeled with [$^{32}\alpha$P] dCTP and used as probes in Northern-analysis to the RNAs isolated from fungal mats representing different growth stages. The cloned cDNA-AFLP products and the *Fpmtr* gene were sequenced with the Gene Analyzer 3100 by the Sequencing Service of the Agricultural Biotechnology Center (Gödöllö, Hungary).

**Construction of a genomic library of *F. proliferatum* and isolation of the *Fpmtr* gene**

Total DNA isolated from *F. proliferatum* ITEM 2287 was partially digested with *Sau*3*A*I. The excised 10-22 kb fragments were ligated into vector Lambda DASH® II (Gigapack® III Gold Cloning, Stratagene). A 350 bp *Fpmtr* cDNA fragment amplified by cDNA-AFLP from germinating conidia of *F. proliferatum* was used as a probe to screen this genomic library. Phage inserts obtained from positive plaques were restriction mapped and subjected to Southern hybridization with the 350 bp cDNA fragment as probes. Based on these experiments, a 5 kb *Xba*I fragment expected to contain the entire *Fpmtr* gene was ligated into pBluescriptKS and sequenced. The GenBank accession number for the nucleotide sequence of *Fpmtr* is DQ 067573.

**Construction of a replacement vector and transformation of *F. proliferatum* ITEM 2287**

A 3800 bp hygromicin B phosphotransferase (*hph*) cassette was amplified by PCR from pAN7-1 (Punt, 1987), and ligated into pBluescript KS II+ (Stratagene) to give the plasmid pHPH. Another plasmid, pAP was constructed by ligating a 2.8 kb PCR-generated fragment containing the entire *Fpmtr* gene into pSTBlue-1. A 477 bp fragment of pAP, stretching between positions 637 and 1144 of the *Fpmtr* sequence was excised following digestion with *Nco*I and replaced with a 3800 bp hygromycin resistance fragment obtained from pHPH following *Eco*RV digestion, yielding the vector pAPHPH. Preparation of the protoplasts, transformation by the vector pAPHPH, regeneration and selection of transformants were performed according to the method of Proctor et al. (1997). The stable hygromycin resistant transformants were screened for the occurrence of site-specific integration by PCR and Southern analysis.

**Fumonisin analysis**

Fungi were grown in 100 ml modified Myro medium containing 1% (w/v) corn-hull-extract (Dantzer et al. 1996) in 300 ml Erlenmeyer flasks for five days on a rotary shaker (125 r.p.m.) at 28$^{o}$C in the dark. The whole cultures were dried and extracted with acetonitrile. Fumonisins were measured with an ELISA kit (Toxiklon®, Agricultural Biotechnology Center, Gödöllő, Hungary) according to the manufacturer's recommendations (Barna-Vetró et al. 2000).

**Vegetative compatibility test and sexual crossing protocol**

Nitrate non-utilizing mutants were selected on potassium-chlorate containing medium according to Puhalla (1985). Chlorate resistant mutants were assigned to phenotypic classes (*nit1, nit3,* and *nitM*) according to Correll et al. (1987). Complementation of the *nit* mutants was performed on Czapek minimal medium, containing $KNO_3$ as the sole N source.

Strains were crossed according to Klittich and Leslie (1988) using the mating type tester strain, FGSC 7615 as crossing partner. Strains tested for female fertility were grown on carrot agar for one week then sprayed with conidial suspension ($10^6$ cells/ml) of the opposite mating type partner, used as male. Plates were incubated for 47 days at 25$^{o}$C with a photoperiod of 12 h dark/light. Cultures were scored regularly for the presence of perithecia. Mature perithecia were crushed under a stereoscope, and the liberated ascospores transferred aseptically to water agar and spore germination evaluated microscopically.

**Infection of plants**

Sterile glasshouse soil was inoculated with the wild type strain of *F. proliferatum* ITEM 2287 and its Δ*Fpmtr* mutants by using a modified method of Oren et al. (2003). Inoculum was produced by

growing the fungi in normal Petri dishes on MM, covered with 0.5–1 cm pieces of sterilized wheat straw. After 14 days of incubation, 5 g straw, colonized by the fungi was mixed with the upper 5 cm layer of sterile soil in pots of 2 liters. Maize seeds, surface sterilized by soaking for 30 min in 1% (w/v) sodium-hypochlorite were planted into the soil immediately after inoculation. [Sub-samples of the surface sterilized seeds were plated on peptone-PCNB medium (Papavizas, 1967) to check whether the seeds were free of internal infection.] Plants were grown in glasshouse for 3 weeks at 25-28$^{o}$C under natural illumination. Leaf and stem samples, collected at weakly intervals were cut into three pieces, surface sterilized by dipping them in 1% (w/v) sodium hypochlorite for 2 min. Isolated DNA from plants were subjected to PCR assay, to detect fungal DNA. A pair of primers (Waalwijk et al. 2003), specific to *F. proliferatum* was used to detection of the fungus. The same samples were placed on *Fusarium* selective peptone-PCNB medium (Papavizas, 1967) to confirm the presence of viable fungal strains.

## Results

### Identification of growth stage specific transcript profiles in *F. proliferatum* by cDNA-AFLP analysis

To identify growth stage specific genes in *F. proliferatum* a comparative analysis of gene expression was performed by cDNA-AFLP. The 9 (almost all conidia produced germ-tubes) and 202 h-old (mature differentiated mycelium was dominated) fungal material were subjected to comparative cDNA-AFLP. 310 fragments showed strikingly different intensities depending on growth stage. Forty-eight fragments, which showed the most strikingly different intensities, were selected for further examination. Seven of the 48 fragments displayed growth stage dependent expression patterns in Northern analysis. One of these fragments, which was strongly expressed in the early stage of conidial germination and repressed in the late stationary phase, gave significant sequence homology to a neutral aromatic and aliphatic amino acid transporter gene from *Neurospora crassa* (*mtr*) and *Penicillium chrysogenum* (*PcMTR*). The other cDNA fragment shared similarity to the *CLTA1* gene, identified in *Colletotrichum lindemuthianum*. *CLTA1* encodes a putative GAL4-like transcriptional activator and this regulatory gene was found to be involved in the transition between biotrophy and necrotrophy during the life-cycle of this fungus (Dufrense et al., 2000). One of the cDNAs derived from the late growth stage transcripts showed convincing similarity to a myo-inositol transport protein from *N. crassa* and a mannitol transporter from *Apium graveolens* (Noiraud et al., 2001). Another cDNA clone appeared to share significant homology to the SAP155 protein from *Saccharomyces cerevisiae*. SAP proteins are known to be physically associated with the SIT4 phosphatase in the late $G_1$ stage of the cell cycle. The association results in the formation of separate complexes, which remain stable until the middle of mitosis. SIT4 is

involved in a number of late $G_1$ processes, including cytoskeleton organization, cell wall integrity and ribosomal gene transcription. However, SIT4 exerts these activities only in physical association with the SAPs (Luke et al., 1996). Of the remaining 3 clones matched only to hypothetical proteins.

**Characterization of *Fpmtr***

Most amino acid transporter encoding genes are constitutively expressed in fungi, except for a few of them that are activated by nitrogen or sulphur starvation. A special feature of the putative amino acid transporter identified by cDNA-AFLP is its selective expression in germinating conidia, indicating a developmental control of this specific transport system in *F. proliferatum.* We isolated the *Fpmtr* from the genomic library of *F. proliferatum*. The *Fpmtr* sequence contains two exons (157 and 1230 nt long) interrupted by a short intron (53 bp). CAAT elements, putative binding sites of transcription factors were found in the 1097 bp 5'-noncoding region as well as a duplicated TGACTC sequence, known to be recognized by GCN4-like protein, the general regulatory protein of the amino acid biosynthesis in fungi (Ebbole et al., 1991). Three GATA consensus sequences, were identified in promoter region, could serve as recognition sites for major nitrogen regulatory proteins (Marzluf, 1997). *Fpmtr* is a single copy gene and encodes a polypeptide containing 462 amino acids. *Fpmtr* is selectively expressed in early stage of growth. Abundant transcript levels were detected up to 24 h of growth by Northern analysis irrespective of the nutrient status of the cultures. Neither starvation, nor addition of amino acids was needed for transcription induction, suggesting that expression of *Fpmtr* is solely under growth stage specific control. Not much is known about the transcription patterns of other members of the Mtr-class transporters, but the *mtr* gene of *N. crassa* seems to be constitutively expressed (Koo and Stuart, 1991), like most other fungal amino acid transporters (Sophianopoulou and Diallinas, 1995).

Topological analysis of the deduced FpMtr protein (Kyte és Doolittle, 1982) showed that this protein possesses 11 putative transmembrane α-helical segments. This feature is typical of the AAAP (Amino Acid/Auxin Permease) family, which includes auxin and amino acid permeases in plants and animals, vacuolar amino acid transporters in *S. cerevisiae* and Mtr-like transporters (Young et al., 1999). This finding confirmed that FpMtr is an Mtr-class transporter.

To find the specific function of *Fpmtr*, Δ*Fpmtr* knock-out mutants were generated with *hph* casette by gene replacement. PCR and Southern analysis were established integration of the *hph* cassette into *Fpmtr* by double cross-over in two transformants, Δ*Fpmtr*43 and Δ*Fpmtr*47.

There were no significant differences in fumonisin B1 production between the mutants and the wild type. Levels of fumonisin B1 synthesized by these fungi after five days culturing on modified Myro medium ranged from 7 to 8.0 μg $ml^{-1}$. Vegetative growth of the Δ*Fpmtr* mutants was normal

in liquid and solid media, but the germination of conidia was delayed and abnormal germ tube development could be observed.

Δ*Fpmtr* mutants had increased resistance to FPA when the fungi were grown in the presence of ammonium as the sole nitrogen source. Mutations at the *mtr* gene in *N. crassa* ruin the function of the amino acid transport system I, which is responsible for the uptake of neutral and aromatic amino acids. The malfunction of this transport system also results in resistance to toxic amino acid analogs, such as p-fluoro-DL-phenylalanine (FPA) (Stuart et al., 1988). However, this resistance is observed only, if the general amino acid transport system (system II) also is blocked, which can result from the addition of $NH_4^+$ ions (DeBusk and DeBusk, 1980). Growth of the wild type and the two Δ*Fpmtr* mutant strains were compared in Vogel medium containing 25 mM $NH_4Cl$ as a sole nitrogen source and 0–100 μM FPA. Approximately 80% growth inhibition of the wild type was obtained at 65 μM FPA although the mutants grew quite well. We also added FPA to liquid cultures of the three strains at different time intervals. The wild type was more inhibited at 10 h of growth than the Δ*Fpmtr* mutants, but there were no differences in the dry weight of the three strains, if 20-h-old cultures were treated with the toxicant, mutants were sensitive to FPA as the wild type strain. These findings clearly suggest that *Fpmtr* belongs to the Mtr-class of fungal amino acid transporters and its transcription exclusively occurs in early stages of growth.

Endophytic colonization of plant tissues by *G. fujikoroi* has been reported (Munkvold and Desjardins 1997), therefore we compared the endophytic colonization capability of the two Δ*Fpmtr* mutants and the wild type. Maize seeds were sown in artificially infested soil, and one-weak-old, three-leaf-stage plants were tested for the presence of internal fungal infection by species-specific PCR. Fungi from PCR positive plant samples were always recovered by plating on peptone-PCNB medium, but no fungal growth was observed in samples that were free of infection in the PCR assay. The endophytic colonization capability of the gene disruption mutants was significantly retarded, as compared to the wild type.

The two Δ*Fpmtr* mutants and the wild type were equally fertile when used as males in crosses with FGSC 7615. The first perithecia appeared after 8 days incubation on carrot agar. If the mutants were used as female parent the fertility of the cross decreased dramatically. The first perithecium was detected only after four weeks of incubation, while fertility with the wild type strain was similar to when it was used as a male parent. If the two Δ*Fpmtr* mutants were used as the female parent, the number of perithecia also decreased by 98%, as compared to the wild type.

Strain ITEM 2287 is self-incompatible (Correll et al. 1989), i.e. no heterokaryons are formed when complementary *nit* mutants of this fungus are paired on minimal medium containing nitrate as the sole nitrogen source. Pairings of *nit* mutants of any of the Δ*Fpmtr* strains with complementary *nit* mutants derived from the wild type resulted in robust heterokaryotic growth at the contact zone

of the paired fungal colonies, indicating that inactivation of *Fpmtr* abolished vegetative self-incompatibility. The Δ*Fpmtr* mutants were still vegetatively incompatible with the other strains of *F. proliferatum*, suggesting that disruption of *Fpmtr* did not overcome the normal barriers to heterokaryon formation that exist between different strains of the fungus.

## Discussion

A cDNA-AFLP approach was developed to identify growth stage specific genes in *F. proliferatum*. 310 fragments showed strikingly different intensities depending on growth stage. To establish whether the intensity of these fragments correctly reflects the differences in the original mRNA populations of the two growth stages, the fragments were cloned and used as probes in Northern analysis. Seven of these fragments displayed growth stage dependent expression patterns only in Northern analysis. cDNA-AFLP is an RNA fingerprinting technique to display differentially expressed genes. The work of Bachem et al. (1996) established that kinetics of expression revealed by cDNA-AFLP analysis is comparable to those found in Northern analysis. On the basis of our results we could state that final outcomes of the cDNA-AFLP methods should be supported by Northern analysis or Real Time PCR. This cDNA-AFLP investigation resulted in the identification of a putative amino acid transporter gene (*Fpmtr*), strongly expressed during conidial germination and repressed in the late stationary phase. *Fpmtr* showed significant sequence homology to *mtr* and *PcMTR,* neutral aromatic and aliphatic amino acid transporter genes known from *N. crassa* (Stuart et al., 1988; Koo and Stuart, 1991) and *P. chrysogenum* (Trip et al., 2004a).

We isolated the *Fpmtr* from the genomic library of *F. proliferatum*. On the basis of identified sequence motives in promoter region, *F. proliferatum* is supposed to possess GCN4-like and GATA-type transcription factors. Therefore nitrogen catabolite repression plays an important role in the control of amino acid uptake in *F. proliferatum*. To confirm this hypothesis we compared the FPA resistance of the two Δ*Fpmtr* mutants and the wild type in the presence of ammonium as the sole nitrogen source.

Two amino acid transport systems could be responsible for the uptake of FPA in *F. proliferatum* as in *N. crassa* (DeBusk and DeBusk, 1980). In the case of hindrance of a system (perhaps general amino acid permease) in the presence of $NH_4^+$ ions, FpMtr transports FPA into the cells. Our results confirmed that nitrogen catabolite repression plays role indeed in the control of amino acid uptake in *F. proliferatum*. However, the reason why GATA sequence motives are in promoter region of *Fpmtr*, if the amino acid uptake by FpMtr is not blocked in the presence of $NH_4^+$-ions, is not known. Neither starvation, nor addition of amino acids was needed for

transcription induction although promoter region of this gene contained a TGACTC motif, known to serve as recognition sites for GCN4-like regulatory proteins of amino acid biosynthesis.

As the Δ*Fpmtr* mutants grew normally on media amended with various amino acid sources, thus we couldn't establish of which amino acids were transported by FpMtr. What is more we don't know whether the FpMtr uptakes amino acids at all. This dilemma seems to be answered by the different sensitivity of the wild type and the mutants to the presence of FPA. Measuring of some amino acid fluxes with amino acids labeled with radioactive $^{14}C$ isotope would help to characterize the function of the permease.

Topological analysis of the deduced FpMtr protein and FPA assay clearly suggest that FpMtr belongs to the Mtr-class of fungal amino acid transporters. However *Fpmtr* is selectively expressed in the early stage of growth, but the *mtr* gene of *N. crassa* seems to be constitutively expressed in minimal medium (Koo and Stuart, 1991; Wolfinbarger, 1980). Apart from a few exception, most transporter-encoding genes are constitutively expressed or controlled by the nutrient status of the cultures (Gow and Gadd, 1995). The transcription pattern of *Fpmtr* is most similar to that of *prnB,* a proline transporter of *A. nidulans*, which is upregulated during conidial germination. Unlike *Fpmtr*, though, transcripts of *prnB* also could be detected at steady-state levels in the fungal mycelia and this level increased in response to induction by the target amino acid (proline) or to amino acid starvation (Tazebay et al. 1997). To the best of our knowledge *Fpmtr* is unusual among fungal amino acid transporters in its growth stage specific expression, and its insensitivity to amino acid starvation.

Trip et al. (2004a) suggested that the Mtr type transporters may be involved in reception of signaling molecules. This hypothesis was based on the low copy number of Mtr homologues found in fungal genomes, as compared to the relative abundance of the AAP tansporters in these organisms. Phenotypic changes, observed in the Δ*Fpmtr* mutants support the possible sensory function of the Mtr type transporters. Inactivation of this transporter gene delays conidial germination, paralleled with a distorted initial growth of the germ tube that probably results from a transient disturbance in growth.

Endophytic colonization of plant tissues by the members of the *G. fujikuroi* complex has been already reported (Leslie et al., 1990), but it is not known yet why the infection is symptomless and finally what triggers rotting in tissues colonized by fungi (Munkvold and Desjardins, 1997). The presence of *F. proliferatum* was detectable in symptomless maize thus the endophytic colonization capability is typical of this fungus. This capability of the mutants was adversely affected, suggesting that the fungus might have problems adapting to a less than optimal environment if this otherwise dispensable transporter in not present.

It is difficult to provide suitable conditions for perithecial development (Klittich és Leslie, 1988). The genetic background of this physiological process is fairly complicated and mostly unknown. The reduced female fertility of the Δ*Fpmtr* mutants could result from troubles with signal transmission/reception needed for rapid and robust perithecial development. Finally, disruption of *Fpmtr* abolished the heterokaryon self-incompatibility phenotype of the wild type parental strain, and this change in self-recognition also could be attributed to a sensory function that had been lost by inactivation of this transporter.

These data suggest that *Fpmtr* is involved in multiple developmental processes related to both sexual and parasexual recombination events in *F. proliferatum* and FpMtr functions as a sensor/receptor protein rather than a typical amino acid transporter. Furthermore, the fungus might have problems in adapting to a less than optimal environment if this otherwise dispensable transporter has been inactivated. Measuring of some amino acid fluxes would help to characterize the function of the FpMtr protein. Entire amino acid transport system of *F. proliferatum* could be characterized on the basis of available sequences of genes encoded amino acid transporters and transcription factors.

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