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Variation of Some Morphological and Molecular Characteristics of Hungarian *Crivellia* and *Brachycladium* Isolates from Opium Poppy (*Papaver somniferum* L.)

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A destructive seed-borne pathogen, formerly described as *Pleospora papaveracea* affects opium poppy (*Papaver somniferum* L.) plants, grown in Hungary, causing considerable qualitative and quantitative losses. The symptoms of the disease were frequently observed in the field between 1999 and 2006. Seventeen Hungarian isolates were obtained from poppy and cultures were established on malt extract agar from naturally infected seeds, diseased foliage, pods and stem. The pathogens proved to be *Crivellia papaveracea* and a distinct taxon, *Brachycladium papaveris* based on morphological characterization of conidia, conidiophores and cultures, moreover molecular investigation of the ITS region. Significant morphological differences were observed among the isolates originating from distinct plant parts, however, cultural characteristics were similar. Molecular studies revealed that morphological and cultural differences or similarities do not correspond with taxonomic position of the isolates. Morphological variation of the isolates mainly depended on their origin and might be explained with the differences of microclimatic conditions.

Keywords: *Brachycladium papaveris*, *Crivellia papaveracea*, morphological characterization, molecular characterization, *Papaver somniferum*.

The cultivation of opium poppy (*Papaver somniferum* L.), on app. 8 000 ha, plays an important role in food trade and medical sciences in Hungary. The growth and health of this crop is highly influenced by fungal pathogens. A destructive seed-borne pathogen, formerly described as *Pleospora papaveracea* may cause significant quantitative and qualitative losses in the yield. There are numerous international references, which are engaged in clarifying the disease and the pathogen. The anamorph was referred to as *Brachycladium penicillatum* (Corda, 1938), *Dendryphion papaveris* (Sivanesan and Holliday, 1982), *Dendryphion penicillatum* (Fries, 1849), and *Helminthosporium papaveris* (Sivanesan and Holliday, 1982). Because of the uncertain identification through many years, Inderbitzin et al. (2006) carried out an overall investigation and created a new nomenclature for these species. They erected the name *Crivellia papaveracea* as the teleomorph and *B. penicillatum* as the anamorph. A closely related species, *Brachycladium*

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papaveris, of which teleomorph state is unknown, has been recently referred to as the synonym of *D. papaveris* (2010) (www.speciesfungorum.org). However, morphological features are insufficient to distinguish *B. penicillatum* and *B. papaveris* (Meffert, 1950; Farr et al., 2000; O'Neill et al., 2000; Inderbitzin et al., 2006). The presence of microsclerotia on different substrates and macronematous conidiophores on living plant tissue are characteristics for identifying *B. penicillatum* (Inderbitzin et al., 2006). Farr et al. (2000) and O'Neil et al. (2000) found the presence of the sexual fruiting bodies as diagnostic marker as well.

The conservative ribosomal ITS1 and ITS2 regions proved to be eligible to distinguish *P. papaveracea* and *D. penicillatum* (Farr et al., 2000). Inderbiztin et al. (2006) distinguished the two taxa by analyzing the ITS regions, the GDP, EF genes and the mating systems. They confirmed that two, morphologically similar, but distinct taxa infect opium poppy. Nevertheless, correlations between source and molecular or morphological characteristics have not been clarified.

Although numerous authors deal with the disease and the pathogens, the number of experimental results on Hungarian samples is rather insufficient. During previous surveys between 1999 and 2006, significant differences were found in the morphological characteristics of some Hungarian isolates (Nagy, 2006). In this study, we completed this observation by involving several isolates originating from different plant parts and on further production areas in the country. Morphological investigation of the isolates was followed by molecular characterizations. Correlations between molecular and morphological characteristics were looked for by analyzing the variability of the isolates. The present work shall support the suggestion of some former reports that the production and habit of different fungal structures depend on natural or artificial growth substrate (O'Neill et al., 2000).

Materials and Methods

Isolates from 12 cultivars of *P. somniferum* ('A1', 'Alfa', 'Bálint', 'Botond', Unkown-1, Unkown-2, Unkown-3, 'Korona', 'Kozmosz', 'Medea', 'Minoan', 'Tebona') were collected from Hungarian poppy plantations. Symptoms were studied visually on the spot and later on collected plant samples in the laboratory by stereomicroscope. Conidia and mycelia were isolated from the surface of diseased leaves and stems and from dried infected pods on malt extract agar (MEA) (Hawksworth et al., 1995). Fungal structures were isolated from seeds as well. The pathogenicity of the isolates was tested on young, detached opium poppy (*cv.* 'Alfa') leaves – wounded and unwounded – incubated at 19–25 °C under normal photoperiod (12 h daylight, 12 h dark), daily evaluating the disease symptoms.

Morphological characterization was carried out by measuring 100–100 conidia per isolate by compound microscope. Color, shape, size and septation were examined. Differences in cultural characteristics were assessed by measuring mycelial growth, form, color, surface, fructification and microsclerotia production of the cultures.

Sequence analysis was performed to clarify the relationship between the morphologically differing isolates. DNA was extracted from the mycelia and conidia of the fungi grown on MEA according to Sambrook et al. (2001). Fungal DNA was used as template for PCR. Universal primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') were used for the conserved ITS region of the rDNA (White et al., 1990). Amplification was performed in 50 µL reactions containing 2 ng template DNA, 1 µL (20 pmol/µL) of each primer, 3 µL (25 mM) MgCl2, 2 µl (5 mM) dNTPs, 0,5 µL (5 U/µL) Taq polymerase (Fermentas, Lithuania). PCR was performed in a thermocycler (GeneAmp PCR System 9700, Applied Biosystem, USA) with the following parameters: initial denaturation at 94 °C for 6 min followed by 40 cycles at 94 °C for 30 sec, 57 °C for 1 min, 72 °C for 2 min. The final elongation was performed at 72 °C for 10 min. The PCR product was run in 1% TBE agarose gel and visualized by staining with GelRed (Biotium, USA). The PCR product was purified by High Pure Purification Kit (Roche Diagnostics) according to the manufacturer's protocols. The purified PCR product was inserted in to pGEM-T-Easy plasmid vector (Promega, USA). All cloning steps were based upon standard molecular biology protocols (Sambrook et al., 2001). Two independent clones were sequenced with universal primers using an automated DNA sequencer (Applied Biosystems Gene Analyzer 3100). Alignment of sequences was performed with the SEQUED and GAP programs of the Genetic Computer Group (GCG) Wisconsin program package (ver. 10.0) (Devereux et al., 1984). The nucleotide sequences were compared and phylogenetic analysis based on Neighbour Joining (NJ) was performed for single gene dataset (ITS) after a ClustalW alignment on 483 bp by MEGA 4.0.2 software (Tamura et al., 2007). Molecular data from NCBI database were involved in the comparison as well.

Statistical analysis of conidial dimensions and culture growth was carried out using cluster analysis and analysis of variance, respectively in the PASW (Predicted Analysis SoftWare) Statistics 18.0 software program. Pairwise comparison was used to determine whether the isolates from the different parts of the poppy plant can be distinguished and clustered based on the cell size of their conidia. The groups were created by cluster analysis and significant differences inside the groups were examined through pairwise comparison. Cell sizes were estimated based on the conidial length and the number of the septa.

Results

The pathogenecity of the investigated fungal species and their sources on poppy

The target fungal species, *B. papaveris* and *C. papaveracea* could be isolated from seeds, leaves, stem or from the inside of pods of 12 cultivars of opium poppy (Table 1). All isolates proved to be pathogenic to *P. somniferum cv.* 'Alfa'. The most virulent isolate was A1-s_HU. Water-soaked lesions, followed by dark brown necrotic lesions developed on both sides of the leaves – wounded and unwounded as well. Aerial mycelia covered the leaves 11 days after inoculation. Control leaves remained symptomless.

Morphological characteristics of the conidiophores and the conidia

Conidiophores, produced by the isolates on the poppy, could be clustered into two groups on the basis of their morphological features. Conidiophores, developed on the leaves and stems, were dark brown, strongly geniculated, branched mainly from the apex in an acute angle. However conidiophores, developed on seeds, were lighter, pale brown, slightly geniculated and branched in near right angle. Definite macronematous conidiophores developed only in the old cultures of the A1-s_HU seed isolate. Conidia were cylindrical, multiseptate, pale olive brown or yellowish brown, mostly with a thick wall and a visible hylum. The length and width of conidia from leaves were significantly larger than of the conidia developed on seeds. Conidia from leaves measured 46.7 × 8.0 μm (17.9–97.5 × 6.0–12.8 μm) and conidia from seeds 23.5 × 7.2 μm (11.1–40.8 × 4.3–11.1 μm). Conidia from pods were 28.3 μm in length and 7.2 μm in width (16.2–50.2 × 4.3–9.4 m). The smallest conidia (16.4 × 5.4 μm) were produced on the stem. The septation of conidia produced on seeds, stem and in pods reached 3–4, but never exceeded 5 septa. The conidia of the leaf isolates were significantly more septated. The number of septa of the conidia of the Koz-l_Hu isolate even reached twelve (Fig. 1).

Table 1

Sources of investigated isolates in this study

Opium poppy cultivar	Part of the plant	Collection site	Collection year	Name of isolate
A1	seed	Tiszavasvári, HU	2002	A1-s_HU
Alfa	leaf	Budapest, HU	2007	Alf-l_HU
Bálint	seed	Kecskemét, HU	2002	Bal-s_HU
Botond	seed	Budapest, HU	2007	Bot-s_HU
	pod	Budapest, HU	2007	Bot-p_HU
Unknown-1	seed	Budapest, HU	2001	Unk-s_HU
Unknown-2	stem	Budapest, HU	2008	Unk-st_HU
Unknown-3	seed	Budapest, HU	2001	Unk3-s_HU
Korona	leaf	Budapest, HU	2001	Kor-l_HU
	pod	Budapest, HU	2007	Kor-p_HU
Kozmosz	seed	Budapest, HU	2000	Koz-s_HU
Medea	seed	–	2006	Med-s_HU
Minoan	seed	Tordas, HU	2004	Min-s_HU
	leaf	Budapest, HU	2007	Min-l_HU
	pod	Budapest, HU	2007	Min-p_HU
Tebona	seed	Tordas, HU	2004	Teb-s_2_HU
	seed	Budapest, HU	2007	Teb-s_1_HU
	pod	Budapest, HU	2007	Teb-p_HU

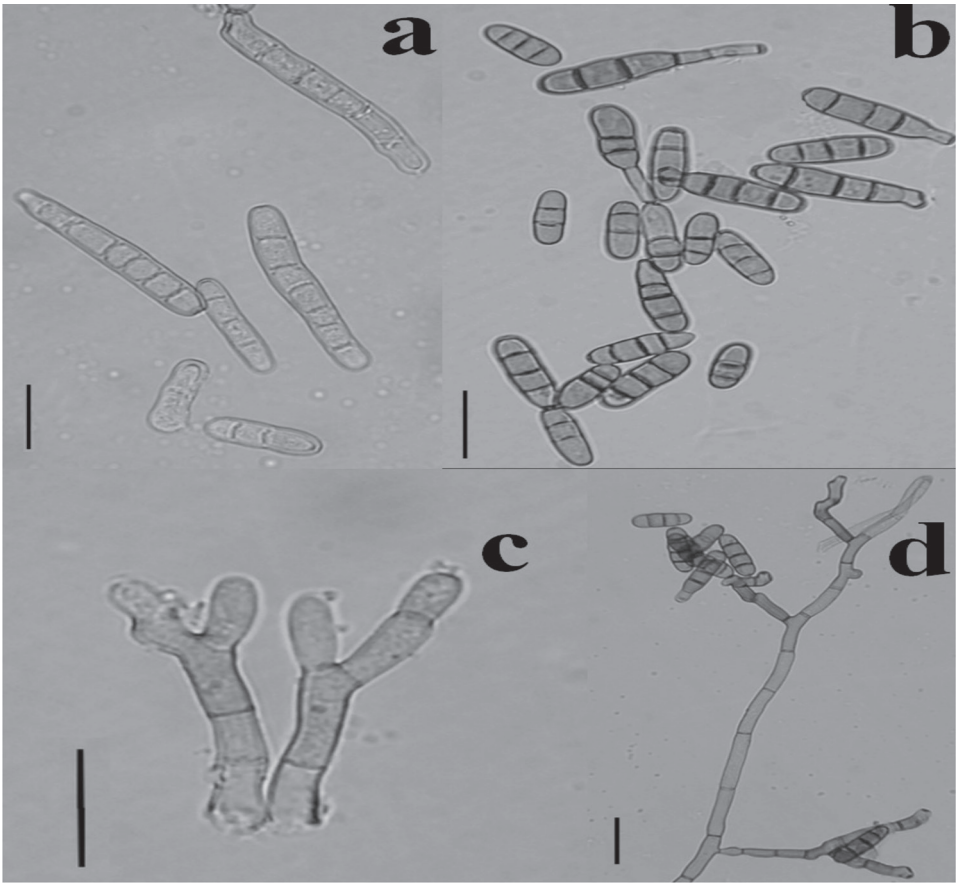

Fig. 1. Typical size and shape of conidiophores and conidia produced by *Brachycladium papaveris* on natural substrates: (a) and (c): collected from leaves, (b) and (d): collected from seeds (scale bar = 20 μm)

Morphological characteristics of the colonies

The 7-day-old colonies of all isolates were circular, olive green, sometimes with a thin off-white margin; with entire, sinuate or slightly crenate edges. Wooly to cottony, white aerial mycelia developed from the middle. Microsclerotia were produced in the cultures of the A1-s_HU, the Koz-l_HU and the Unk-st_HU isolates (Fig. 2). A1-s_HU, Koz-l_HU, Unk-st_HU and Min-l_HU isolates produced chlamydospores. Thin-walled, chlamydospore-like structures were found in the cultures of the other isolates, with the exception of the Teb-p_HU. The isolates A1-s_HU and Unk-s_HU produced globose, dark brown to black, thick-walled chlamydospore-like cells in chains in culture. Somewhat similar structures that could represent an intermediate between chlamydospore and microsclerotia could be found in the cultures of A1-s_HU and Unk-st_HU. Macronematous conidiophores were found only in the cultures of the A1-s_HU isolate (Fig. 2). Mycelia

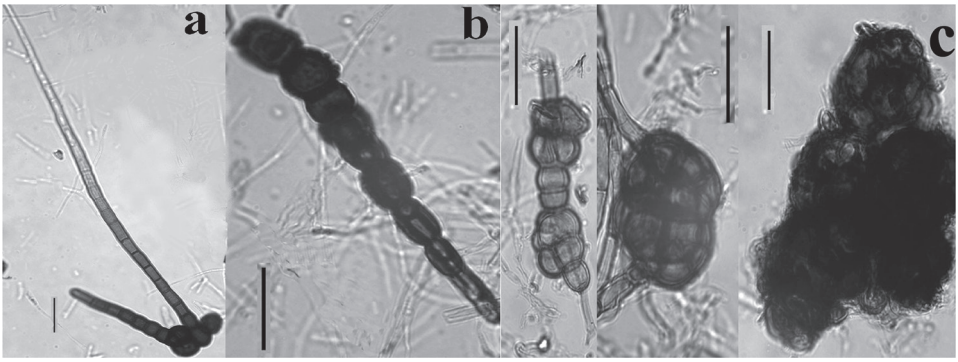

Fig. 2. Macronematous conidiophore (a) and chlamydospore-like globose cells in chain (b) produced by *Crivellia papaveracea* in old culture on MEA medium (sclae bar = 30 μm); different shapes of microsclerotia produced by *Crivellia papaveracea* in old culture on MEA (scale bar = 30 μm) (c)

of the cultures not producing microsclerotia were composed of thinner-walled, lighter hyphae than of the cultures producing microsclerotia. Sexual fruiting bodies could neither be observed in culture of any isolate nor on overwintered poppy stem residues.

The average growth rate of the mycelia was 5.2–5.5 mm/day for the leaf-isolates, 3.5–5.0 mm/day for the seed-isolates and 2.8–7.6 mm/day for the pod-isolates. The average size of the seven-day-old colonies of the isolates of different origin showed high variance. However, according to the univariate ANOVA, the colony size of the microsclerotia producing A1-s_HU seed isolate did not differ significantly from the colony size of the Unk-s_HU, Koz-s_HU and Med-s_HU seed isolates and of the Min-p_HU, Bot-p_HU and Kor-s_HU pod isolates. Significant differences could be observed between the average colony sizes of the seed and of leaf isolates (Fig. 3).

Molecular characterization of the isolates

The molecular analysis of the partial or complete sequence of the ITS1-2 regions and 5.8S gene and the partial sequence of 18S and 28S rRNA gene provided the opportunity to distinguish the isolates. The sequences of the Hungarian isolates were submitted to the NCBI database (www.ncbi.nlm.nih.gov) under the accession numbers GQ995475-GQ995482 and JQ403616-JQ403623. The sequences were aligned by CLUSTALW on 486 bp and compared by neighbor-joining method to all relevant international sequences published previously. Their taxonomic relationship is presented in a phylogenetic tree, shown in (Fig. 4). The sequence of the Teb-s_1_HU isolate, showing 99.9% identity with sequences of *Alternaria* spp. isolates, was selected to represent an out-group.

The isolates could be clustered into two main groups: I: *B. papaveris* and II: *C. papaveracea*, according to the nomenclature of Inderbitzin et al. (2006). The differences between the sequences of group I and II did not exceed 2.7%. The subgroups A, B and C, D, E could be created by sequence homology (Fig. 4). In group I subgroup B, containing Med-s_HU isolate was 99.8% identical with subgroup A. Remarkable that in subgroup A the newly added Hungarian isolates had strong identity with each other (except Alf-l_HU

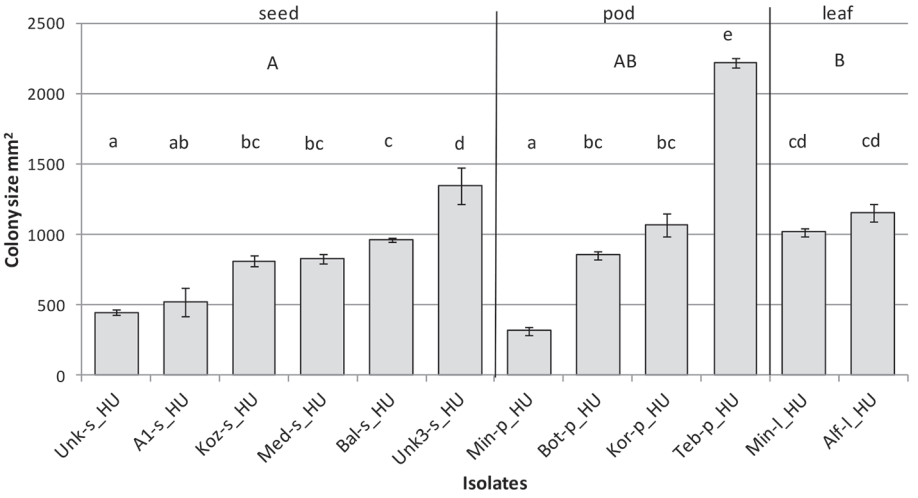

Fig. 3. Average size of the seven-day-old colonies of the isolates of different origin grown on MEA with standard deviations. Homogenous groups (ANOVA, $p \leq 0.05$; Games Howell's test) are indicated by same letters

isolate with 99.2% homology), but they differed significantly from the relevant sequences from the database. In group II subgroup D, containing Unk-st_HU, showed 99.6% homology with subgroup C and only 99.4% with subgroup E. Between subgroups C and E a high level of homology was detected, even up to 99.8%.

The sequence identification revealed that the seed isolates proved to be both *C. papaveracea* (A1-s_HU) and *B. papaveris* (Unk-s_HU, Koz-s_HU, Teb-s_2_HU, Bot-s_HU, Unk3-s_HU, Med-s_HU, Min-s_HU), while on the leaves and in pods only *B. papaveris* (Alf-l_HU, Min-l_HU, Bot-p_HU, Kor-p_HU, Min-p_HU, Teb-p_HU) occurred. *C. papaveracea* was isolated from the stem (Unk-st_HU).

The isolates from leaves, pods, seeds and stem could be clearly divided in 3 groups (A, B, C) based on the pairwise alignment of the average conidial cell sizes (Table 2). The homogeneous group of pod-isolates differed significantly from the other groups, except Unk-s_HU and Unk3-s_HU at $p < 0.05$ level. Two members (Alf-l_HU and Koz-l_HU) of the heterogeneous group of the leaf-isolates showed significant difference. Regarding the most heterogeneous group of the seed isolates significant deviation occurred between Unk-s_HU and Unk3-s_HU isolates. Based on the cell size, the Unk-st_HU isolate might belong to the seed group.

Discussion

Two distinct pathogens, *Crivellia papaveracea* and *Brachycladium papaveris* could be isolated from different parts of *Papaver somniferum*, grown in Hungarian poppy plantations. The molecular identification of the newly collected Hungarian isolates revealed

that *Brachycladium papaveris* occurred in the most cases, while *Crivellia papaveracea* was identified only two times.

According to former results (Ballarin, 1950; O'Neill et al., 2000) significant differences were observed in morphological features of isolates of different origin. As Nagy (2006)

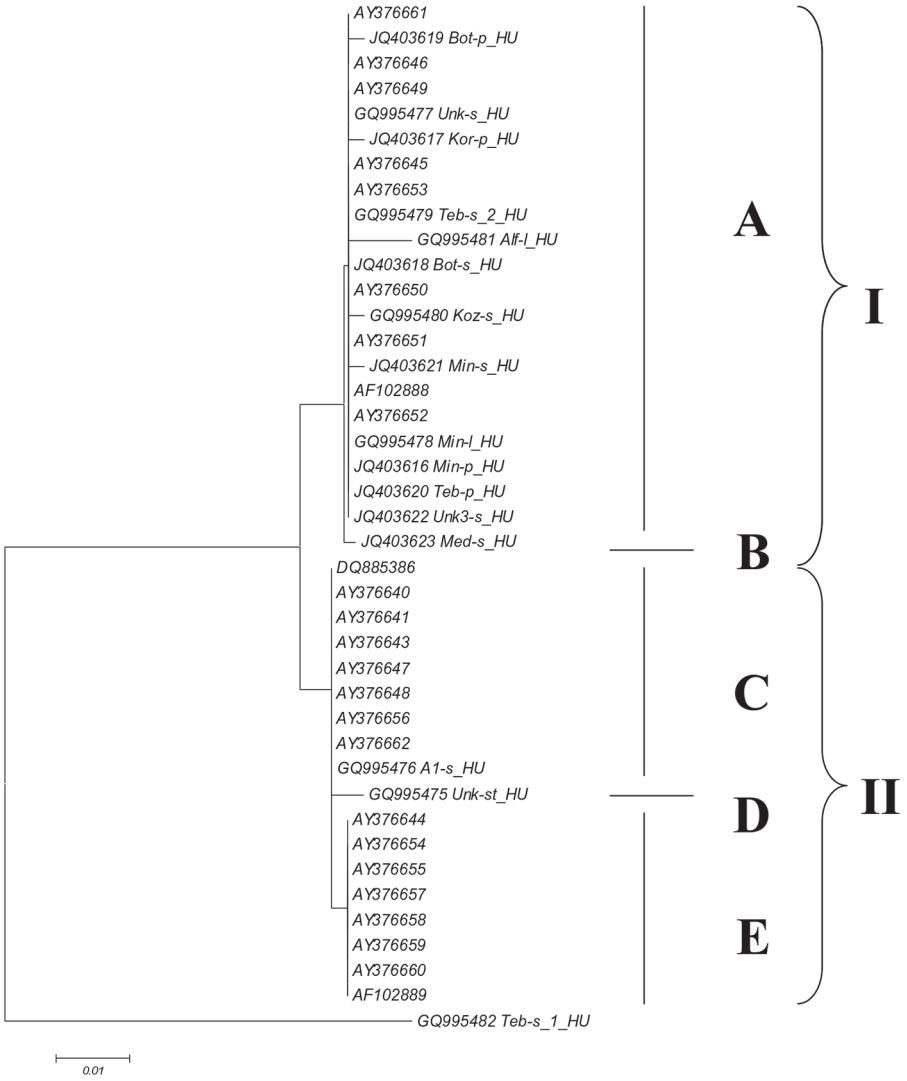

Fig. 4. Phylogenetic tree of the Hungarian isolates and reference isolates from the NCBI database. Sequences of cloned PCR fragments of the ITS region were aligned and used for construction of a rooted tree by the neighbor-joining approach, implemented in the MEGA 4 program. Scale bar represents a measure of the evolutionary distance between sequences analyzed and used for the tree construction

also noticed previously, the differences appeared in the shape of conidiophores and septation, size and cell-wall thickness of the conidia. The clustering analysis of the conidial dimensions of the isolates of different origin confirmed a clear way to divide the samples. At first, four groups were created based on the origin (part of the plant) of the isolates. Inside the groups significant difference could be observed. The isolate from the stem was highly similar to the seed isolates. Leaf isolates produced larger conidia with more septa and stronger cell-wall than seed and pod isolates. Although the samples from pods proved to be homogeneous in the same group, the others showed variability in some degree.

The above mentioned morphological observations could not be supported by the molecular analysis. Molecular analysis of the different isolates showed genetic deviation. Isolates similar in morphological characteristics differed in their nucleotide sequences. On the other hand isolates with different morphological features proved to be closely related taxa on the basis of the sequence of the ITS region. While the clustering analysis of the conidial size data clearly distinguished three groups, the isolates shared about 99% homology in their nucleotide sequences.

Beside molecular-biological results, in agreement with Inderbitzin et al. (2006), reliable distinction between the two fungi can be made on the basis of the microsclerotia production, which characterized only *Crivellia papaveracea* isolates. However, their shapes and sizes are highly variable. The presence of other structures is either occasional (e.g. *Crivellia papaveracea* producing macronematous conidiophores) or could be misleading (e.g. chlamydospores or chlamydospore-like structures, which were found in the cultures of both fungal species). Structures, composed of chlamydospore-like globose cells in chain, were found in the cultures of *Crivellia papaveracea* isolates, whereas Inderbitzin et al. (2006) observed similar structures in the cultures of *Brachycladium papaveris*. Our observations indicate that the mycelia of *Brachycladium papaveris* isolates in culture were composed of lighter and thinner-walled hyphae than that of *Crivellia papaveracea*. According to Farr et al. (2000) and O'Neill et al. (2000) *Crivellia papaveracea* consistently produced sexual fruiting bodies in culture and on plant debris. During our investigation, these structures could neither be found in culture nor on overwintered stems.

Farr et al. (2000) found differences between growth rates of the different species on agar media. According to our observations colony measures of *Crivellia papaveracea* and *Brachycladium papaveris* isolates are varying under standard growth conditions, however, they did not differ significantly in most cases.

The apparent inconsequences between the results of the morphological and molecular assays might be explained with the different microclimatic conditions and exposure of the investigated isolates. This statement is supported by the observations of Misaghi et al. (1978) who found that conidia of *Alternaria alternata* formed in natural habitats were larger and more uniform in size than that of those produced *in vitro* on common agar media. Vakalounakis and Christias (1985) reported that changes in light intensity and in temperature affect conidial morphology in *Alternaria cichori*. The role of other factors such as osmotic potential (Crous et al., 1992) and UV radiation (Braga et al., 2006) in changes of conidial morphology is reported as well. In order to understand the variability of the examined pathogen further studies and observations are suggested.

Table 2

Grouping the isolates with different origin on the basis of their conidial cell size, estimated with pairwise comparison with PASW (Predictive Analytics SoftWare) Statistics program package. A, B and C letters mean three groups, in which each data could be classified after the pairwise alignment.* (asterisk) marks significant difference at $p < 0.05$ level

Origin	Isolates	Groups based on cell size			Species
Leaves	Al-l_HU		B *		*Brachycladium papaveris*
	Min-l_HU		B	C	*Brachycladium papaveris*
	Koz-l_HU			C *	Not identified by molecular tools
Pods	Kor-p_HU	A *			*Brachycladium papaveris*
	Bot-p_HU	A *			*Brachycladium papaveris*
	Min-p_HU	A *			*Brachycladium papaveris*
Seeds	Unk-s_HU	A *			*Brachycladium papaveris*
	Unk3-s_HU	A *			*Brachycladium papaveris*
	Min-s_HU	A	B	C	*Brachycladium papaveris*
	Med-s_HU	A	B	C	*Brachycladium papaveris*
	Koz-s_HU	A	B	C	*Brachycladium papaveris*
	Bot-s_HU	A		C	*Brachycladium papaveris*
	Bal-s_HU	A		C	*Brachycladium papaveris*
	A1-s_HU		B	C	*Crivellia papaveracea*
Stem	Unk-st_HU		B	C	*Crivellia papaveracea*

The exact diagnosis of the target pathogen species is of consequence from practical point of view as well. As Bailey et al. (2000) found, the pathogenicity and aggressiveness of the investigated fungi are differing. Plant protection methods are highly influenced by the pathogenicity of the target pathogens.

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Literature

Bailey, B. A., Apel-Birkhold, P. C., O'Neill, N. R., Plaskowitz, J., Alavi, S., Jennings, J. C. and Anderson, J. D. (2000): Evaluation of infection processes and resulting disease caused by *Dendryphion penicillatum* and *Pleospora papaveracea* on *Papaver somniferum*. Phytopathology 90, 699–709.

Ballarin, C. (1950): Untersuchungen über *Helminthosporium papaveris*. Phytopathol. Z. 16, 399–442.

Braga, G. U., Rangel, D. E., Flint, S. D., Anderson, A. J. and Roberts, D. W. (2006): Conidial pigmentation is important to tolerance against solar-simulated radiation in the entomopathogenic fungus *Metarhizium anisopliae*. Photochemistry and Photobiology 82, 418–422.

Corda, A. C. J. (1938): Abbildungen der Pilze und Schwaemme. Icones Fungorum, Hucusque Cognitorum 2, 1–43.

Crous, P. W., Phillips, A. J. L. and Wingfield, M. J. (1992): Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. Mycologia 84, 497–504.

Dendryphion papaveris (Sawada) Sawada. Species Fungorum. International Mycological Association [online]. (2010): Accessed 14 Apr, 2012. Available from: http://www.speciesfungorum.org/Names/GSDSpecies.asp?RecordID=329803.

Devereux, J., Haeberli, P. and Smithies, O. (1984): A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12, 387–395.

Farr, D. F., O'Neill, N. R. and Berkum, P. B. (2000): Morphological and molecular studies on *Dendryphion penicillatum* and *Pleospora papaveracea*, pathogens of *Papaver somniferum.* Mycologia 92, 145–153.

Fries, E. M. (1849): *Summa Vegetabilium Scandinaviae*. *Sectio posterior.* Typographia Academica, Sweden, Stockholm, Uppsala and Germany, Leipzig, pp. 259–572.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. (eds) (1995): Ainsworth and Bisby's Dictionary of the Fungi, 8th ed. University Press, Cambridge.

Inderbitzin, P., Shoemaker, R. A., O'Neill, N. R., Turgeon, B. G. and Berbee, M. L. (2006): Systematics and mating systems of two fungal pathogens of opium poppy: the heterothallic *Crivellia papaveracea* with *Brachycladium penicillatum* asexual state and a homothallic species with *Brachycladium papaveris* asexual state. Can. J. Botany 84, 1304–1326.

Meffert, M. E. (1950): Ein Beitrag zur Biologie und Morphologie der Erreger der parasitaeren Blattdürre des Mohns. Z Parasitenkunde 14, 442–498.

Misaghi, I. J., Grogan, R. G., Duniway, J. M. and Kimble, K. A. (1978): Influence of environment and culture media on spore morphology of *Alternaria alternata*. Phytopathology 68, 29–34.

Nagy, G. (2006): Mitosporic fungi occurring on medicinal and aromatic plants. [Dissertation]. Corvinus Univ. of Budapest, Budapest

O'Neill, N. R., Jennings, J. C., Bailey, B. A. and Farr, D. F. (2000): *Dendryphion penicillatum* and *Pleospora papaveracea*, destructive seedborne pathogens and potential mycoherbicides for *Papaver somniferum.* Phytopathology 90, 691–698.

Sambrook, J., MacCallum, P. and Russel, D. (2001): Molecular Cloning: A Laboratory Manual (3rd ed.) I–III. Cold Spring Harbor Laboratory Press, New York,

Sivanesan, A. and Holliday, P. (1982): *Pleospora papaveracea.* CMI Descriptions of Pathogenic Fungi and Bacteria, 730 p.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Vakalounakis, D. J. and Christias, C. (1985): Light intensity, temperature and conidial morphology in *Alternaria cichorii*. Transactions of the British Mycological Society 85, 425–430.

White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky and J. J. White (eds): PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.