THESIS OF PHD DISSERTATION

DIVERSITY AND ANTAGONISTIC ACTIVITY OF ENDOPHYTIC FUNGI FROM SWEET CHERRY AND PEPPER

NEDA HADDADDERAFSHI

Supervisors:
PROF. DR. NOÉMI LUKÁCS
DR. KRISZTIÁN HALÁSZ

CORVINUS UNIVERSITY of BUDAPEST
Faculty of Horticultural Science

Department of Plant Physiology and Plant Biochemistry

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Name:	Doctoral School of Horticultural Sciences
Field:	Crop Sciences and Horticulture
Head of Ph.d School:	Dr. Magdolna Tóth Academic Professor, DSc Department of Fruit Sciences, Faculty of Horticultural Science, Corvinus University of Budapest
Supervisors:	Dr. Noémi Lukács Academic Professor, DSc Department of Plant Physiology and Plant Biochemistry, Faculty of Horticultural Science, Corvinus University of Budapest Dr. Krisztián Halász Assistant Professor, PhD Department of Plant Physiology and Plant Biochemistry, Faculty of Horticultural Science, Corvinus University of Budapest

The applicant met the requirement of the PhD regulations of Corvinus University of Budapest and the thesis is accepted for the defence process.

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Prof. Dr. Tóth Magdolna Head of the PhD School	Prof. Dr. Noémi Lukács Supervisor	Dr. Krisztián Halász Supervisor

1. Introduction

Endophytic symbionts including bacteria and fungi live within plant tissues without causing any obvious negative effects and have been found in every plant species examined to date (Clay 1992). It became evident that endophytes are rich sources of bioactive natural products, and many different agents have been isolated from these microorganisms with promising applications in development of natural drugs and other industrial products (Berdy 2005). Fungi are among the most important groups of eukaryotic organisms well-known for producing many novel metabolites which are directly used as drugs or function as lead structures for various bioactive products (Kock 2001) (Berdy 2005). Endophytic fungi were first studied in plants in temperate regions, but recently these studies were extended to tropical plants as well. Allegedly, all plants maintain associations with fungal endophytes and epibionts (Ahlholm *et al*., 2002). These associations between fungi and plants are generally a cryptic phenomenon in Nature. Fungal endophytes may inhabit tissues of roots, stems, branches, twigs, bark, leaves, petioles, flowers, fruit, and seeds, including xylem of all available plant organs (Ahlholm *et al*., 2002) (Kogel *et al*., 2006). These fungi affect the ecology of plants, by frequently enhancing the capacity of host plants to survive and resist environmental and biological stresses through mechanisms that are only partially understood (Faeth and Fagan 2002). It is also believed that endophytes have important roles in plant protection, acting against herbivores, insects and pathogens and may also increase plant resistance to biotic and abiotic stimuli (Faeth and fagan 2002) (Arnold *et al*., 2003). The outcome of interaction between host plants and endophytes can vary in a seamless manner from mutualism to parasitism. In most cases, the host plant does not suffer; in fact it often gains an advantage from colonization by a fungus (Kogel *et al*., 2006). Contribution of endophytes to biological functions of the host plant was primarily studied in cool season grasses, particularly in those of agronomic importance, although the interactions between host plants and endophytes in natural populations and woody plants are poorly understood (Faeth and Fagan 2002). It has been documented that higher non-grass plants furnish complex, multilayered, spatially and temporally diverse habitats that support species-rich assemblages of microorganisms. Microfungi are dominant components of those communities and their biodiversities are thought to change specifically in accordance with the harboring tissue, the host species, geographical differences and climate conditions (Faeth and Fagan 2002) (Saikkonen et al., 2004). Considering the presence of endophytes in every known plant species, such characteristics make fungal endophytes as one the most diverse components of the biomass that

are dynamically being modified to adjust to the environmental changes and to host physiology. It is estimated that there are approximately 1 million fungal endophyte species worldwide, however, only a fraction has been described and explored to date (Ganley *et al*., 2004).

Yet, there is a lack of information regarding the features of endophytic fungal communities in different host plants in Europe. Available data are principally originating from studies on characterization of bioactive products of endophytes with industrial or medicinal applications. Nonetheless, understanding the composition and dynamics of endophytic assemblages and impacts of host-specificity and tissue-colonization of these symbionts on physiology, is fundamental to improve the existing knowledge about the bioecology of plant-endophyte mutualism and is required to pave the lane toward finding novel bio-agents with pesticidal, medicinal and industrial applications. Along with other Central and Eastern European countries, Hungary has started to improve the state's capacities for attending the world market of horticultural products. In the last two decades sweet cherry (*Prunus avium*) received an increasing attention (Hrotkó and magyar 2004). To produce high quality products for the fresh market vigorous rootstocks well-adapted to the regional climate and soil condition are needed. That is why different strains of *P. mahaleb* have been introduced as rootstocks in Hungary. To develop the cultivation of sweet cherry in large scales an integrated research program, from basic to applied science, is fundamentally needed to improve productivity of domestic species and their resistance to stresses and natural pathogens. Fungal endophyte assemblages associated with their host plant may have an influence on pathophysiology of the host. Since a lack of information exists in this regard not only in the Hungarian niech but such data are also rare all around the world. Therefore, the present study was carried out to obtain the following objectives:

1. Determining the biodiversity of endophytic fungi in sweet cheryy grafted on different *P. mahaleb* rootstocks
2. Identification of potentialy host-specific or or tissue-specific (leaf, twig, and root) strains and their dynamic changes during the growing season.
3. Evaluation of anti-microbial activities of isolated fungal endophytes.

In additon to sweet cherry we also investigated the endophytic fungi of pepper under different growing conditions and in different cultivars. My task in the latter studies was to help to start the cultures and to monohyphenate / monosporulate the individual strains and establish the strain collection.

2. Materials and Methods

2.1. Biodiversity and antagonistic characterization of fungal endophytes on *Prunus sp.*

2.1.1. Locality and sampling strategy of the study

Samples were selected randomly from 110 trees among 180 sweet cherry trees grown in the orchard of Corvinus University of Budapest, Soroksár, nearby the city of Budapest lies between 19° 07’ 00’’E and 47° 24’ 00’’N geographical coordinates. Trees were planted at a spacing of 4x2 m, resulting in a density of 1,250 trees ha^{-1}. Orientation of the rows was north-south. The same individual plants were subjected to the study in all sampling periods. The study was conducted in autumn 2008, spring 2009 and autumn 2009.

Tissue samples from leaf, twig and root of trees (cultivar Péter grafted on different rootstocks) were collected. Trees were nearly 8 years old when sampled for the first time, and rootstocks originated from four different species: *Prunus mahaleb* L. (Érdi V., Bogdány, SL64, Egervár, Korponay, SM11/4, CEMANY and Magyar rootstocks), a variety of *P. avium* (Vadcseresznye) and *P. fruticosa* (Prob) and from a hybrid inbred rootstock of *P.cerasus* and *P.canescens* (Gisela 6).

2.1.2. Tissue preparation

Samples were obtained from root, twig and leaf of each individual tree and were collected in plastic bags. Samples were transported to the laboratory at the Department of Plant Physiology and Biochemistry, Faculty of Horticultural Sciences, Corvinus University of Budapest (Hungary). In the laboratory, all samples were washed thoroughly by detergent under running tap water. Surface sterilization of plant material was carried out using chlorine bleach (NaOCl) diluted in water to concentrations of 2–10% to treat the specimens. After being sunk in 96% ethanol for 1 min, tissue samples were washed by dipping into hypochlorite 3% solution for 10 min and then were sunk again for 1 min in 96% ethanol. Procedure was followed by washing the samples twice with sterile distilled water for 5 min.

Size of the sampling unit and surface sterilization procedures vary according to the preferences of the investigator, the species of host plant, and host tissue type sampled. A pilot study prior to commencement of main sampling procedure was fulfilled to optimize the techniques for tissue preparation with higher efficiency and obtaining larger numbers of endophytic fungi from each sample. Accordingly, it became clear that the smaller the sampling unit was, the greater the recovery of diverse species/genotypes could be achieved. Also, conversely, the larger the sampling unit was taken, the greater the potential existed to miss rare or slow-growing species and to recover

mixed genotypes of the same species. Thus, two sections from different parts of each tissue compartment, from leaf as 0.5 cm in diameter each and from twig and root two sections as 0.5 cm in length, were randomly cut for isolation of endophytic fungi on the selected media.

2.1.3. Primary isolation of endophytic fungi

Routine mycological media are suitable for primary isolation, sub-culturing and identification of endophytic fungi. Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), 1-2%, were used as pre-culturing media. Each 10 pieces of a single segment were transferred into PDA plates supplemented with 250 mg/l amoxicillin, 250 mg/l cephalexin and 100 mg/l chloramphenicol. Plates were incubated at 22°C and colonies were observed after 1-2 weeks. Fungi with rapid growth were sub-cultured onto media without inhibitors to enhance normal sporulation. Optimal incubation conditions varied according to the provenance of the host tissue and therefore some cultures were incubated for two to three weeks to let the slow-growing fungi emerge. Plates were sealed with Parafilm to prevent desiccation of the media, and were incubated in a growth chamber with a humidity control.

2.1.4. Single spore isolation of endophytic fungi

Single spore isolation was performed to obtain specific fungal subcultures from polyspore isolates. Accordingly, 20 g/l water agar was used as sporulation medium in sterile Petri dishes. After autoclaveing at 121°C for 20 min penicillin (0.5 g/l) was added when the temperature of agar was about 50°C, then the medium was distributed into 90 mm Petri dishes inside a laminar flow cabinet. By employing a fine sterile sampler, spores were picked up from the surface of an individual colony. Different isolation methods were employed based on differences between fungal isolates according to the type of their fruiting bodies. Fungi with closed fruiting bodies such as *Ascomycetes* with cleistothecia or perithecia and *Coelomycetes* with pycnidia were removed from the substrate surface. Fungi with cup shape fruiting bodies as like as *Ascomycetes* with apothecia and *Coelomycetes* with acervuli, were transferred directly by removing the whole fruiting body. Spores from *Basidiomycetes* with gills were obtained by removing a few segments of gills and finally when no sporulation was detected, subcultures were prepared by transferring single thread of hyphae (single hyphae) into PDA plates.

To overcome the problem of bacteria or yeast contamination and prevent the transfer of wrong species, in addition to using antibiotics, spore masses were also diluted in sterile water. A glass container was sterilized using ethanol 70% and wiped with a towel on which ethanol 70% had been

sprayed. A sterilized pipette was then used to transfer about 6 drops of sterilized water into the container and spore masses obtained as explained above were added to make a spore suspension. This homogenous spore suspension was finally transferred onto the water agar plates. A permanent slide was also prepared by using a drop of each spore suspension to check whether the correct fungus had been selected.

The procedure was followed by incubation of the plates at 22°C for 24 hours. Germinated spores were detected by microscope examination and then one single spore was picked up and transferred into another PDA plate. Isolates were incubated at 25°C and were checked frequently till their colony diameter was about 1-2 centimeters.

2.1.5. Morphological study

Cultures on both PDA and MEA media were assessed according to their morphology. Colony appearance, mycelium color and structure, shape of conidiomata, conidia and conidiophore (size, color, ornamentation, etc.) and characters of conidiogenous cells were observed for morphological classification of isolated fungi using a light microscope with 5X, 10X and 40X objective lenses for magnification.

2.1.6. PCR amplification of ribosomal internal transcribed spacer regions

In the present study, amplification of the fungi ITS region was performed using fungal domain specific ITS1 and ITS4 primers. Total DNA was extracted from fungal components by applying a modified CTAB (cetyltrimethylammonium bromide) method. After pre-heating at 65°C in water bath, 15 µl mercapto-ethanol + 2% polivynil-pyrrolidone solution was added to the samples. CTAB buffer containing 2% CTAB, 0.1 M Tris-HCl (pH 7.0-8.0), 20 mM EDTA and 1.4 M NaCl, previously heated at 65°C, was mixed with the content of each tube to the total volume of 600µl. Samples were incubated at 65°C for 30 min and supernatants were collected after centrifugation for 15 min at 12 000 g. Protein content was depleted by adding 600 µl chloroform to each tube followed by centrifugation at 12 000g for 8 . The procedure was repeated one more time, then 900 µl of 96% ethanol was added to each tube and samples were incubated at -20°C for 2 hours. After centrifugation at 12 000xg for 15 min, pellets were washed by 70% ethanol and resolved in 100 µl of sterile Milli-Q water. The PCR reaction mixture consisted of 5 µl fungal DNA, 2.5 µl 10x loading buffer, 0.5 µl 10 mM dNTP mix, 15.6 µl sterile Milli-Q water, 0.4 µl Taq polymerase and 0.5 µl each of the forward and the reverse primers ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' (White *et al.*, 1990) in a total reaction volume of 25µl.

Amplification was performed in a Thermal Cycler (BioRad T100 THERM) and PCR conditions were 15 min at 95°C followed by 40 cycles at 95°C for 1 min, 30 sec at the annealing temperature and 72°C for 1min. Aliquots of each amplified product were electrophoretically separated on a 2% agarose gel in 1x TAE buffer and visualized using ethidium bromide under UV illumination..

PCR amplicons were recovered using the kit. Isolates whose sequences had a similarity greater than 95% were considered to belong to the same species. Sequence-based identifications were made by searching by FASTA algorithms the EMBL/Genbank database of fungal nucleotide sequences. The criterium for species identification was an ≥97% identity to the database sequence, genera were positively identified when the sequence match reached 96.9 – 95.0%. When the similarity was less than 95%, the isolate was considered as unidentified.

2.2. Analysis of endophyte – pathogen antagonism by dual culture method

In the present study, a dual culture method was applied to assess the antagonism of endophytic fungi against pathogens. Two relevant pathogens of sweet cherry were used: *Agrobacterium tumefaciens* strain, kindly provided by Ernő Szegedi from FVM Vine and Wine Research Institute (Kecskemét, Hungary) and *Monilia laxa*, obtained from Géza Nagy at the Department of Plant Pathology, Corvinus University of Budapest, Hungary.

Two culture media were examined, Malt Yeast-Extract Agar (MYEA) and Potato-Dextrose Agar (PDA), of these PDA was found to be more adventageous and was therefore. Fungi which had faster growth on culture plates were selected for antagonism test. With the help of a sampler needle, an inoculum of the selected endophyte and of the pathogen were aseptically planted 40 mm far from each other on a Petri plate (d = 90 mm) containing 30 ml fresh PDA medium. At the same time, inocula of the endophyte and the pathogen were placed separately on a PDA-containing Petri dish as controls. All plates were incubated at 28°C for 7 days. Six replicates were used for each plate. Growth inhibition was tested by measuring the radial growth of each colony in three directions (horizontal, diagonal and vertical) every day during a period of 15 days after inoculation and calculated by applying the following formula:

Growth Inhibition (GI) (%) = $[(DC - DP)/DC]$x100

where DC = diameter of control, and DP = diameter of pathogen colony dual cultured with endophyte. Values were calculated as the averages of all achieved data.

Radial growth measurements of each colony in three directions on an actual day were used also to calculate the growth rate (GR) of colonies. The growth rate value was defined as the slope of measurements logarithmic curve during 15 days and was expressed in mm/day.

2.3 Statistical analysis

Data derived from the present study were assessed with an emphasis on diversity of endophytic fungi isolated from different cherry rootstocks. Leaves, twigs and roots as three different tissue compartments from each rootstock, were also examined to indicate the anatomical distribution pattern of isolated endophytes. Differences in number and diversity of fungal endophytes recovered from cherry rootstocks in three distinctive periods when sampling process had been performed (autumn 2008, spring 2009 and autumn 2009, hereafter: season I, season II and season III, respectively), were also considered for data analysis. Accordingly, relative frequency (RF) of various species was defined as the proportion of recovered colonies belonging to an identified endophytic fungus compared to the total number of isolates during a particular season.

Infection frequency in different organs was expressed as the percentage of endophyte-bearer explants among the cultured specimens from a particular tissue and also by dividing the number of isolated colonies from each organ to the total number of tissue explants, which were defined as colonization rate (CR) and isolation rate (IR), respectively. Relative abundance and species richness were calculated for diversity analysis. Shannon-Weaver index ($e^{H'}$) was used to compare the distribution of endophyte species in all examined tissue compartments of every rootstock in the 3 sampling times. Simpson's diversity index (D) was calculated representing the diversity of endophytic assemblages on different rootstocks.

Endophytic fungal colonies which could not be characterized by morphological or molecular methods, were marked as unidentified and were omitted from analytical calculations.

A two-way variance analysis (ANOVA) was applied to determine the significance of growth inhibitory effect between all groups. Student T test was also used for comparing the growth factors of a group with corresponding control sample. All data were expressed as the mean value (mm) of total measurements for six replicates.

$P \leq 0.001$ was determined as the level of significance.

2.4. Sampling, cultivation and identification of endophytic fungi in *Capsicum annuum* L.

As described in Chapter 2.9, endophytic fungi also occur in pepper (*Capsicum annuum* L.). We investigated two pepper cultivars (Hó F1 and Kárpia F1) and from these cultivars 8 organs such as roots, shoot, leaves, pedicles, pericarp at different developmental stages and seeds. Samples were taken in 3 replicates four times during the vegetation period in 2013 (in April, May, August and October). The plants lived in open field on original sandy soil with drip irrigation or in greenhouse on rockwool. By the sampling in April and May plants were in the seedling stage, such only root, leaf and shoot samples could be collected. Samples were separately put into plastic bags and pre-cultivation started in 6 hours after sample collection.

Surface sterilization was performed by soaking the corresponding organ for 1 min in 70% ethanol, after that for 10 min in 20% hypochlorite, and finally again for 1 min in 70% ethanol. After surface sterilization samples were washed by dipping into sterile distilled water. Tissue samples of 5 mm were cut under sterile conditions. We put 9 pieces from each organ sample on PDA pre-culturing medium supplemented with 1 g/l chloamphenicol. We incubated our samples in 90 mm Petri dishes at room temperature in dark for 2 weeks. After this period we surveyed and evaluated the frequency of the outgrowing colonies. Then representative colonies were selected for further work. Small pieces from these cultures were transferred under sterile conditions to fresh PDA plates and were then monosporulated or monohyphated. my personal contribution to this part of the project was mainly in this last step.

Identification of endophytic fungi was done by PCR and sequencing and selected strains were also investigated for their ITS regions.

3. Results

3.1. Biodiversity and colonization patter of fungal endophytes on *Prunus., Sp.*

A total of 9823 tissue segments (inocula), 3397 inocula from roots, 3233 inocula from twigs and 3193 inocula from leaves of all cherry rootstocks were examined while 1614, 2530 and 1037 inocula showed fungal endophyte infection in cultures from roots, twigs and leaves, respectively. All isolates were primarily identified by morphological characteristics and then were subjected to single spore isolation process where distinguished culture of every individual colony was prepared and phylogenetically examined by molecular experiments. Among isolated genera, two species of *Alternaria* (*Alternaria* sp.1 and *Alternaria* sp.2) with a total of 1931 colonies for *A.* sp.1 and 1473

colonies for *A.* sp.2 had the first and the second largest number of colonies isolated from cherry trees. In contrast, *Ceratobasidium* sp.1 and *Ceratobasidium* sp.2 had the minimum number of colonies (4 and 15 colonies, respectively) among other isolates and were observed only in root samples. In root samples, however, *A.* sp.1 exhibited the highest frequency as 605 colonies of this fungus were isolated from root samples. *A.* sp.2 composed the largest population of endophytes with 1349 isolated colonies (although this isolate was not observed in the root) and the lowest number of isolated colonies belonged to *Glomerella acutata* (21 colonies) which was detected only in twig samples. Similar to root samples, A.sp.1 with 502 isolated colonies was the most frequent fungus in leaves while *Pyronema* sp., with 10 colonies showed the minimum frequency in this tissue. Along with *Pyronema* sp., two other identified isolates, *Rosellinia* sp., and *Xylaria digitata* were only observed in leaf samples. Collection of tissue samples from cherry rootstocks was accomplished in three time periods: autumn 2008 (season I), spring 2009 (season II), and autumn 2009 (season III). According to the results, the total species richness of *Prunus mahaleb* rootstocks (Bogdány, SL64, SM11/4, Egervár, Korponay, Magyar, CEMANY, and Érdi V) was higher than other rootstocks. Average number of distinctive species isolated from *Prunus mahaleb* rootstocks was 12.5 in season I, 10.1 in season II, and 10.1 in season III. During season I, the largest number of species was isolated from SM11/4 (16 species) while Magyar rootstock harbored 9 different species in composition of endophytic fungi associated with this rootstock. Species richness of *Prunus mahaleb* rootstocks in season II ranged from 10 species (SM11/4 rootstock) to 13 species that were detected on Korponay, Bogdány, and Érdi V rootstocks. During season III, the maximum number of species (12 species) was isolated from Korponay, while SL64 had the minimum species richness among other *Prunus mahaleb* rootstocks (8 species) in this sampling period. The difference of species richness between *Prunus mahaleb* rootstocks in three sampling periods was not significant.

Although a number of 12 different species was isolated from Prob rootstock (*Prunus fruticosa*) in season I but the species richness had a significant decrease ($P \leq 0.05$) in season II and season III (7 species, and 8 sepcies, respectively). Gisela6 (*Prunus cerasus, Prunus canescens*) showed a relatively low species richness with no difference in all three sampling periods (6 species, 7 species, and 8 species respectively). During season I, the lowest species richness was observed in Vadcseresznye (*Prunus avium*) (3 isolated species), but this index had an increase ($P \leq 0.05$) in season II (10 species) and in season III (7 species). The average species richness was the highest

in season I (11 species) but had a slightly fall in season II (10.7 species) in compare with season III (9.5 species). However, this difference was not significant. *Prunus mahaleb* rootstocks harbored the most heterogeneous endophyte communities with almost the same species richness in all seasons, but other rootstocks were associated with endophytic fungi community which showed comparatively less species richness. Collectively, root samples had the richest endophytic fungi assemblages regarding the number of identified species in season I (average 7.1 species, maximum= 10 species, isolated from Korponay and SM11/4 rootstocks, minimum= 1 species, isolated from Vadcseresznye rootstock). During the season II, root samples harbored again more distinct species (average 6 species, maximum= 8 species, from Érdi V and SL64 rootstocks, minimum= 3 species, isolated from Gisela6 rootstock), whereas species richness index showed no difference between root and twig as a consequence of increase in number of different species isolated from twig samples in this season (average 4.9 species, maximum= 6 species, isolated from Érdi V, Egervár, and SL64 rootstocks, minimum= 3 species, isolated from Prob rootstock). Distribution of different endophyte species during the season III had a shift toward predominant species richness in twigs (average 6.3, maximum= 8 species, isolated from Korponay and Érdi V rootstocks, minimum= 5 species, isolated from SL64, CEMANY, Vadcseresznye, and Gisela6 rootstocks). Leaf samples had the lowest species richness in all sampling periods ($P<0.05$). Bogdány, Érdi V, SL64, SM11/4, Egervár, Korponay, CEMANY, and Magyar were rootstocks of *Prunus mahaleb* L., examined in the present study.

From Bogdány, a total of 700 colonies were isolated from root, twig and leaf specimens and the highest infection burden was observed in season III (collectively, 336 colonies were isolated at this season). During all three sampling periods, from all identified fungal endophyte species in this study, 22 species were isolated from this rootstock. Maximum species richness in season I was observed in root samples (8 species), in root samples in season II (7 species) but in twig samples in season III (7 species). The evenness of species, indicated by Shannon-Weaver index was comparatively the highest in Season II ($e^{H'}=1.71$), while Simpson index was expectedly the lowest ($D=0.48$) in the same season for Bogdány, demonstrating the highest diversity of the endophytic fungi assemblages on this rootstock during the season II. The highest abundance was detected for *Alternaria* sp.1 and *Alternaria* sp.2 that along with *Epicoccum nigrum* (with comparatively lower abundance) were isolated from all tissue compartments of this rootstock. The largest number of *Alternaria* sp.1 colonies was found on Twig samples in season III and *Alternaria* sp.2; however

was the most abundant on twig samples in season I. *Rosellinia* sp., (RF=70%), *Xylaria digitata* (RF=67%) and *Alternaria* sp.1 (RF=55%) were the most frequent species on leaf samples in season I, season II and season III, respectively. *Rosellinia* sp., was only isolated from leaf samples of this rootstock. In total, 869 fungal endophyte colonies were isolated from Érdi V rootstock. The largest number of isolated colonies was observed in twig samples during season III (199 colonies isolated). Leaf samples during the season I showed the lowest number of isolated colonies (6 colonies isolated), but in season III a number of 159 colonies was obtained from this tissue. The most abundant species in this rootstock were *Alternaria* sp.1 (280 isolated colonies) and *Alternaria* sp.2 (214 isolated colonies) while the first species was obtained from all tissue compartments in season III (120 isolated colonies from twigs, 82 isolated colonies from leaves and 28 isolated colonies from roots) (RF=60%, RF=52%, and RF=36%, respectively) and only from root samples (50 isolated colonies) during season II, but no isolate of *Alternaria* sp.1 was observed in tissue samples from this rootstock in season I. on contrary, *Alternaria* sp.2 was only isolated from all tissue samples in season I and from leaves and twigs, but not roots, in season II. No colony of this species was isolated from this rootstock in season III. Among all identified species, *Rosellinia* sp., was only isolated from leaf samples during the season I (4 isolated colonies). A total number of 898 endophytic fungi colonies were isolated from SL64 rootstock. Maximmum infestion of this rootstock was found on twigs collected in season III (237 isolated colonies). The lowest endophyte burden was indicated in leaves collected during season II (7 isolated colonies), although 165 colonies were collectively isolated from leaves in season III. The most frequent species was *Alternaria* sp.1 identified in all tissue compartments during season III: 139 colonies from twigs (RF=59%), 96 colonies from leaves (RF=58%), and 16 colonies from roots (RF=18%), however this species was not observed in all tissue samples from SL64 during the season I. *Alternaria* sp.2 also showed a reletively high frequency in this rootstock, but was mostly isolated from tissue samples in season II: 5 colonies from leaves (RF=71%), 57 colonies from twigs (RF=77%), and 23 colonies from roots (RF=24%). During season I, this species was isolated from leaves (16 isolated colonies, RF=57%) and twigs (118 isolated colonies, RF=73%), but was not observed in season III. The highest diversity in endophytic fungi communities was calculated in season II ($e^{H'}$=0.96), but the index for season III showed less diverse endophytic assemblages on this rootstock ($e^{H'}$=0.23). Simpson index for season II and season III was D=1.23 and D=1.86, respectively. From SM11/4 rootstock, a number of 634 colonies were isolated showing the highest infection burden

on root samples during season II (141 isolated colonies) but less number of fungi colonies on leaves in that season (5 isolated colonies). During the first season, the largest number of colonies was isolated from twigs (133 isolated colonies) while this rate was almost constant during next seasons (73 isolated colonies in season II and 121 isolated colonies in season III). Regarding other tissue samples, the number of isolated colonies was relatively flactuated. For instance, from leaved 32 colonies in season I, 5 colonies in season II and 54 colonies in season III were isolated. The most frequent species were *Alternaria* sp.1 and *Alternaria* sp.2, predominantly composed the endophyte cummunities associated with SM11/4 rootstock. *Alternaria* sp.1 was isolated from root in season II (60 isolated colonies, RF=43%), and from leaf (20 isolated colonies, RF=37%), twig (54 isolated colonies, RF=45%), and root (11 isolated colonies, RF=31%) samples in season III, but was not observed in season I. Diversity indices demonstrated the most divergent endophytic infection in season II ($e^{H'}$=1.49, D=0.57) and the most homogenic structure of endophyte assemblages during season III ($e^{H'}$=0.58, D=1.6) associated with SM11/4 rootstock. Experiment on Egervár rootstock yielded a total number of 692 endophyte colonies where the largest number of isolates were detected in twig samples. In total, 125 isolated colonies during season I, 74 isolated colonies during season II, and 169 isolated colonies during season III were obtained from twigs. In the first two sampling periods, leaves showed a very low rate of infection, although in season III, 105 colonies were isolated from leaf samples of this rootstock. As a consequence, either twigs or leaves showed the maximum infection burden during the third season, whereas root samples were mostly infested in season II (a total of 95 colonies were isolated from root samples). The most abundant species on this rootstock was *Alternaria* sp.1 (a total of 195 isolated colonies) which was onserved in all tissue samples during season III: 43 isolated colonied on leaf (RF=41%), 83 isolated colonies on twig (RF=49%), and 19 isolated colonies on root (RF=27%), but it was only isolated from root samples during season I and season II (17 isolated colonies, RF=43%, and 33 isolated colonies, RF=35%, respectively). *Alternaria* sp.2 had a slightly lower abundance (a total of 147 isolated colonies), however it was isolated from examined tissue samples of leaves and twigs in season I: 7 isolated colonies from leaf (RF=70%), and 84 isolated colonies from twigs (RF=67%), and in season II: 4 isolated colonies from leaf (RF=80%), and 52 isolated colonies from twig (RF=70%) with relatively high frequency but was not observed in root samples. The recent species was not detected on tissue samples during season III. According to the results, 688 fungal endophyte colonies were isolated from Korponay rootstock. The adundance of isolated colonies was found

the highest in twigs during season I (107 isolated colonies) and season III (154 isolated colonies), but the largest number of colonies was isolated from roots (102 isolated colonies) in the second sampling period. The infection burden of leaf in this rootstock had a drastic increase in season III (111 isolated colonies) in compare with the first two seasons. Collectively, twig was found as the most infected tissue compartment in Korponay rootstock while leaf samples had the lowest infection burden in this host. The most frequent species was *Alternaria* sp.1 (222 isolated colonies) that was detected on all samples during season III: 69 colonies isolated from leaf samples (RF=62%), 97 colonies isolated from twigs (RF=63%), and 18 colonies isolated from roots (RF=21%), but only was obtained from root samples in season II (38 isolated colonies, RF=37%) and was observed on neither of the tissue samples during season I. In contrast, *Alternaria* sp.2 was detected in season I on leaves (13 isolated colonies, RF=68%), twigs (66 isolated colonies, RF=62%), and on roots (4 isolated colonies, RF=10%), and in season II on leaf (4 isolated colonies, RF=80%) and twig (43 isolated colonies, RF=63%), but had no occurance in season III. From CEMANY rootstock, a total of 592 colonies were isolated and identified during three sampling periods. In season I and season III, the largest number of isolated colonies was obtained from twigs (97 isolated colonies and 134 isolated colonies, respectively), while in season II, root samples had the highest infection burden (91 isolated colonies). In the first two seasons, leaf samples showed a very low infection burden (7 isolated colonies and 2 isolated colonies, respectively) but in season III a total of 107 colonies were isolated from leaves of this rootstock. *Alternaria* sp.1 was the most frequent species (151 isolated colonies) obtained only from root samples (26 isolated colonies, RF=29%) in season II, but from all tissue samples in season III (23 isolated colonies from leaf, RF=21%, 84 isolated colonies from twig, RF=63%, and 18 isolated colonies from root, RF=37%). This fungus was not observed in season I. the second most frequent species, *Alternaria* sp.2 (127 isolated colonies), was detected in leaves (4 isolated colonies, RF=57%), twigs (67 isolated colonies, RF=69%, and in roots (3 isolated colonies, RF=37%) during the season I and in leaf (2 isolated colonies, RF=100%) and in twig (51 isolated colonies, RF=75%) but not from root during the season II. Although in general, twig samples from Magyar rootstocks had the highest infection burden but in season II the largest number of colonies was isolated from root samples (75 isolated colonies). The number of isolated colonies was comparatively low in leaf samples during the first two seasons (11 isolated colonies and 5 isolated colonies, respectively), while 81 colonies were isolated from this tissue in season III. *Alternaria* sp.2 was the most frequent fungus isolated from

this rootstock (152 isolated colonies). This species was found only in root (30 isolated colonies, RF=40%) in season II, but was isolated from all tissue compartments (35 isolated colonies from leaf, RF=43%, 65 isolated colonies from twig, RF=68%, and 8 isolated colonies from root, RF=26%) during season III. *Alternaria* sp.2 was not detected on Magyar rootstock in season I. with a slightly lower abundance, *Alternaria* sp.1 was the second most frequent species on this rootstock isolated in season I from leaf (7 isolated colonies, RF=64%), twig (87 isolated colonies, RF=67%), and from root (7 isolated colonies, RF=33%) and in season II from leaf (2 isolated colonies, RF=40%), and from twig (49 isolated colonies, RF=73%) and had no occurance in root during this season.

From *Prunus avium*, a rootstock with high prevalence in Hungarian niche (Vadcseresznye) was selected for the present study. According to the results, a total number of 372 fungal endophyte colonies were isolated from tissue samples of Vadcseresznye rootstock. The infection burden in the first season was relatively low as 30 colonies from twig samples and only 4 colonies from root samples were isolated and leaf samples showed no infection during season I. The rate of infection had an increase during the second and the third seasons. In season II, the largest number of colonies was isolated from roots (80 isolated colonies) but this amount reduced to 65 isolated colonies in season III. *Alternaria* sp.1 and *Alternaria* sp.2 were the most frequent species among other identified endophytes associated with Vadcseresznye rootstock; however the pattern of tissue colonization and temporal occurrence of these species showed a significant difference. A total of 407 colonies were isolated from Prob rootstock (*Prunus fruticosa*) during three sampling periods, while the highest infection burden was detected on twig in all seasons (109, 66, and 50 isolated colonies, respectively). In the first season 75 colonies were isolated from root samples, but the infection burden drastically decreased in root as in season III only 19 colonies were obtained from this tissue. On contrary, the number of isolated colonies in leaf was the lowest during the first two seasons (6, and 5 isolated colonies, respectively) but in season III increased to 37 isolated colonies, showing a temporal change in infection burden of leaves in this rootstock. The most frequent species was *Alternaria* sp.2 (125 isolated colonies) which was observed in season I on leaf (5 isolated colonies, RF=83%), twig (72 isolated colonies, RF=66%), and on root (4 isolated colonies, RF=5%), and in season II on leaf (5 isolated colonies, RF=100%) and on twig (39 isolated colonies, RF=59%). This species was not detected on root during the second season and had no occurrence in season III.

Gisela6 rootstock, known as a hybrid rootstock of *Prunus cerasus* and *Prunus canescens*, was also examined regarding the association of endophytic fungi on different tissue compartments in the present study. As a sum, 407 colonies were isolated from this rootstock and the highest infection burden was observed in twig samples during all seasons (109, 66, and 50 isolated colonies, respectively). Infection burden showed a gradual decrease in root and sudden increase in leaf from season I to season III. In the first season, 75 colonies were isolated from root samples but this amount was 40 isolated colonies in season II and 19 isolated colonies in season III. Contrarily, the number of isolated colonies from 6 in season I ascended to 37 in season III for leaf samples. The most frequent species in Gisela6 rootstock was *Alternaria* sp.1 (72 isolated colonies) which was detected only in root (7 isolated colonies, RF=32%) during season II, but had occurrence on all tissues: 30 isolated colonies (RF=71%) in leaf, 21 isolated colonies (RF=55%) in twig, and 14 isolated colonies (RF=42%) in root, during the third season.

3.2. Endophytic fungi associated with *Capsicum annuum* L.

The impact of environment and cultivation conditions on endophytic assemblages associated with both cultivars, 'Hó' and 'Kárpia', were investigated in plants cultivated in greenhouse as well as in open field. As in sweet cherry, we observed that the potentially pathogenic *Alternaria* genus occurred most frequently in pepper, members of this genus were detected in every organ. In addition to *Alternaria* 4 further morphotypes were very common: *Cladosporium, Verticilium*, *Acremonium* and the yet unidentified morphotaxa. From the root we also isolated *Plectosphaerella*, *Colletotrichum, Paecilomyces*, *Penicillium* and *Fusarium* strains, while in the shoot *Cladosporium*, *Acremonium, Chaetomium* and *Lewia* strains were identified. Our results indicate that greenhouse-grown and field-grown peppers harbour different endomycota. We also observed differences between individual plant parts and seasonal difference in the samples of the same plant. Plant parts of the cultivar 'Hó' usually showed higher colonisation rate by endophytic fungi than those of 'Kárpia'. As expected, colonization rates were higher in field-grown plants and in older organs. Highest colonisation frequency was found in old leaves and in stalks of fruits.

3.3. Potential antagonistic activities of the identified edophytic fungi

All identified taxa were subjected to a pilot study to investigate their potential antagonistic activity against two selected plant pathogens, *Agrobacterium tumefaciens* and *Monilia laxa* using

dual-culture method on Malt yeast-Extract Agar (MYEA) and Potato-Dextrose Agar (PDA) media. Due to the aftermath of MYEA cultures, the pilot study yielded that examined fungi species have remarkably more vigorous growth on PDA. Thus this culture medium was chosen for further investigation about anti-pathogenic traits of isolated fungal endophytes. Furthermore, amidst 27 taxa included in antagonistic activity test, 7 species showed competent colony expansion and development in dual-cultures with pathogens: *Botrytis cinerea*, *Alternaria* sp.1, *Rhizopycnis vagum*, *Epicoccum nigrum*, *Embellisia* sp., *Fusarium oxysporum* and *Ceratobasidium* sp1. Therefore, recently mentioned endophytes were applied for dual-culture with pathogens and other species were omitted from the test. Primary evaluation of antagonistic effect between endophytes and the selected pathogens demonstrated the potential ability of the candidate fungi species to inhibit the radial growth of both *Monilia laxa* and *Agrobacterium tumefaciens* on PDA cultures. Inhibition of growth was assayed by calculating growth rate (GR) and growth inhibition (GI) indices and results were tested regarding the significance of the differences by a one-way ANOVA test. As a conclusion, a decline in growth rates of both *Monilia laxa* and *Agrobacterium tumefaciens* was observed in all dual-cultures after the incubation period (15 days), however neither endophytic fungus showed any defect in growth rate while confronting the pathogen in compare with control samples. According to the growth rate, *Monilia laxa* had comparatively less radial extension toward horizontal and diagonal dimensions but such a difference was not found in vertical growth of the pathogen. Expansion of *Agrobacterium tumefaciens* colonies was dominated in all measured dimensions while confronting the endophyte in dual-cultures. *Alternaria* sp.1 had the strongest antagonistic effect on *Monilia laxa* while *Fusarium oxysporum* showed a relatively feeble effect on this pathogen particularly against vertical extension of the colonies. In compare with other endophytes, *Fusarium oxysporum* had also weaker antagonistic effect on growth of *Agrobacterium tumefaciens* although other fungi showed almost the same growth inhibitory effect in dual-cultures with this pathogen. In general, restriction in colony growth of *Monilia laxa* was significant (P=0) in horizontal and diagonal directions but no difference was found in vertical growth of the pathogen colonies in deal with endophyte in dual-cultures. Changes in horizontal growth of this pathogen ranged from relatively weak when cultured with *Rhizopycnis vagum* (mean±variance=1.39±0.17 mm) to the most regressed growth in dual-culture with *Botrytis cinerea* (mean±variance=0.68±0.01 mm) on PDA. The inequality in growth inhibition effect between nominated endophytes was less significant concerning their ability to diminish diagonal growth of

Monilia laxa colonies. Although *Botrytis cinerea* showed a relatively strong inhibitory effect on diagonal growth (mean±variance=0.92±0.03 mm) of the pathogen, differences between other endophytes was not significant. No significant difference was found between endophytic fungi regarding their capability to inhibit vertical growth of *Monilia laxa* on PDA. Horizontal growth of *Agrobacterium tumefaciens* had the maximum regression while confronted *Ceratobasidium* sp.1, *Embellisia* sp., and *Alternaria* sp.1 on the media and the mean value of horizontal regression of the pathogen colonies was equal in dual-cultures with mentioned fungi (mean± variance= 0.56± 0.003 mm). In contrast, *Rhizopycnis vagum* had the weakest effect on horizontal extension of the pathogen (mean±variance=0.71±0.022 mm). As well, this fungus had relatively weaker effect on diagonal (mean±variance=0.66±0.006 mm) and vertical (mean±variance=0.71±0.01 mm) growth of *Agrobacterium tumefaciens* in compare with other fungi, however variance of radial growth inhibition toward diagonal and vertical dimensions had no significant difference between dual-cultures of this pathogen with selected endophytic fungi.

4. Discussion

As an overall, the present study provided valuable data regarding biodiversity and impact of determinant factors on composition of fungal endophyte communities that attribute symbiotic micro-flora of widely used cherry rootstocks in the region. It has been demonstrated that endophytic fungi can profoundly influence different aspects of plant pathophysiology and actively participate bio-mechanisms by which plant growth and regeneration is controlled. Thus understanding the structure of endophyte assemblages is required for further approaches in order to improve the agro-economic status of the fruit production and to find novel applications of these microorganisms and their metabolites in horticultural, or even medicinal, studies (Rodriguez *et al*., 2009). Results of the present study were applied for an analytical assessment to explicate the composition of fungal assemblages on sweet cherry and pepper in Hungary. Moreover, the antagonistic activity of isolated endophytes with vigorous growth on PDA was assessed *in vitro* against two common plant pathogens. Such data could be useful for further investigation about the effect of these symbionts in pathophysiology of the host and their contribution in host resistance against pathogens. As a sum, 26 morpho-taxa were classified among a total of 6587 isolated colonies from cherry rootstocks. The majority of identified species (24 species) associated with cherry rootstocks were from the phylum *Ascomycota*. Only two species of the phylum

Basidiomycota were isolated from cherry rootstocks. From *Ascomycota,* the most diverse species belonged to *Sordariomycetes* while *Dothideomycetes* were the most frequent fungi isolated from cherry rootstocks. *Sordariomycetes* were mostly *Hypocreales* (5 species), but this class also included *Phyllachorales* (1 species), *Sordariales* (1 species), *Diaporthales* (1 species), and *Xylariales* (2 species). The most abundant fungi (*Alternaria sp.1* and *Alternaria sp.2*) (Paleosporales) along with other less frequent fungi: *Davidiella sp.* (Capnodiales), *Macrophomina phaseolina* (Botryosphaeriales), *Epicoccum nigrum* and *Rhizopycnis vagum* belonged to class *Dothideomycetes,* however taxonomy of the last two species is not thoroughly assigned yet (Incertae sedis). Such a structure of endophytic fungi assemblages is identical to formerly suggested endophytic association with woody plants and implies the potential contribution of class 2 endophytes in symbiotic micro-flora of cherry rootstocks (Faeth and Sullivan 2003). *Basidiomycota* are reported to have rare evenness in plants. According to the results, two species of Agaricomycetes (*Ceratobasidium sp.1* and *Ceratobasidium sp.2*) were the less abundant fungi identified on examined cherry rootstocks. As well, two species of Agaricomycetes (*Ceratobasidium sp.1* and *Ceratobasidium sp.2*) were the less abundant fungi identified on examined cherry rootstocks. The most diverse fungi in orchestration of endophytic communities associated with cherry rootstocks were *Fusarium sp*. Such a result may imply the ability of this genus to colonize host tissue with no detectable symptom, however these fungi are normally considered as plant pathogens (Reininger and Sieber 2012) (Kaplan *et al*., 2013). Some endophytes isolated from cherry rootstocks have formerly been shown to be beneficial to the host. *Acremonium sp.,* and *Epicoccum nigrum,* as two widespread fungi have been described as endophytes with ability to improve the host resistance against pathogens (Marasco *et al*., 2012) (Mandyam *et al*., 2013). The most striking outcome of this study was the isolation and identification of two fungi species on the leaf of cherry rootstocks (only during season I) which according to the best of our knowledge have not been reported so far as resident endophytes of leaves at least in temperate climate. *Rosellinia sp.,* (Sordariomycetes) and *Pyronema sp.,* (Pezizomycetes) were found only in leaves in the first sampling period and had no occurrence on other tissues in that season or on all examined specimens during the next two seasons, suggesting that these fungi may colonize the host incidentally in a tissue-specific manner in early stages of the foliar growth.

All cherry trees subjected to the present study, were selected from an orchard nursery. Thus such a sampling procedure did not allow analyzing the biodiversity of endophytes in different habitats.

Nonetheless, by considering the regional ecosystem of the sampling locality and other factors like quality of soil and environmental conditions, results of this study not only demonstrate the status of endophytic fungi infection in cherry rootstocks throughout the state but also can be extended to a broader geographical range in Central and Eastern Europe.

According to the results, endophytic communities associated with rootstocks belonged to *Prunus mahaleb L*, were comparatively more diverse and distinctive species had higher occurrence on these rootstocks during a particular sampling period, although the general feature of endophyte assemblages on cherry rootstocks showed more tendencies to vary depending on the host organ and seasonal changes than the host species. Perhaps less diverse endophytic assemblages were observed on some rootstocks like Vadcseresznye or Gisela6, but disparities in infection rates and species richness in different tissues of these individual hosts during three sampling periods suggested mild impact of host species in composition of endophyte communities and their diversity in cherry rootstocks. Consequently, it remains to be elucidated whether the association of endophytic fungi can favor vegetative growth, precocity, adaptability to different environmental conditions, disease tolerance, and productivity of the examined cherry rootstocks. Furthermore, the probability of host-endophytes interaction to switch from mutualism to antagonism and changes in transmission mode of the fungi under the influence of host physiology and other stresses is to be studied. Infection burden on the host tissues and diversity of endophytic fungi showed more correlation to the organ where they were colonized and the time when tissue specimens were obtained. However, some degree of tissue-specificity was detected in distinctive fungi, but colonization pattern and distribution of endophytes encompassed the pivotal role of temporal changes in host physiology that more likely can induce alteration in the feature of these symbionts communities. The first and the third sampling procedures were fulfilled in early autumn time, with almost one year interval between two periods, prior to defoliation of the trees, when the second sampling period was carried on in mid spring during the flowering time. Differences concerning the infection rate and composition of endophytes assemblages between these two periods perhaps demonstrate the effect of annual changes and aging process in the host on biodiversity of fungal endophytes. Nevertheless, host species may have an impact on endophyte divergence, more probably based on the differences in growth habit, vegetation characteristics and their ability to maintain efficient adaptivity to environmental stresses. Temporal changes of infection rate in twigs were similar in all rootstocks and exhibited high colonization and isolation rates in season I and

season III, however these indices comparatively had decline in season II. On the other hands, the number of isolated taxa had no difference between the first two seasons and rose during the last sampling period. Altogether these findings indicate that biodiversity of endophyte infection in the twig is more likely under the influence of annual biochemical and physiological changes in this organ (Ahlholm *et al*., 2002). It can be assumed that aged twigs are preferentially colonized by endophytes that travel through water and nutrient transportation system from the root to foliage during the time of plant vegetative growth. Moreover, twigs may be less vulnerable to endophyte infection probably due to the physiological processes occur during simultaneous vegetative and reproductive growth of the host. As for leaves, results showed a remarkable increase in infection burden during season III in compare with the first two seasons. Perhaps such findings indicate that the infection burden and diversity of fungal endophyte flora on leaves of cherry rootstocks differs in accordance to the aging process in foliar tissues but has less correlation to the annual changes (Khan *et al*., 2012). In addition, tendency of some isolated fungi to exclusively colonize the leaf and occurrence of other taxa that as well were accommodated in twigs and roots can highlight the existence of air-born fungi in the studied ecosystem, although soil-born endophytes can infest the leaf via water and nutrient transportation system. As a matter of fact, these outcomes can be confirmed by existing knowledge about aspects of endophytic fungi biodiversity in terrestrial plants. Former studies have also suggested that species composition of endophyte assemblages and infection frequencies vary according to host species, site characteristics, such as elevation, exposure, and associated vegetation, tissue type; and tissue age (Arnold *et al*., 2003) (Khan *et al*., 2012).

In our experiments 42 endophytic fungal strains were successfully identified in pepper. They belong to 19 genera: *Alternaria*, *Cladosporium*, *Penicillium*, *Acremonium*, *Chaetomium*, *Fusarium*, *Lewia*, *Arthirinium*, *Cercospora*, *Colletotrichum*, *Galactomyces*, *Myrothecium*, *Paecilomyces, Plectosphaerella*, *Pyrenochaeta*, *Rhizopycnis*, *Verticillium* and *Xylaria*. 10 of these genera were also showed to be present in pepper by Paul *et al*., (2011): *Alternaria*, *Aspergillus*, *Bionectria*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Plectosphaerella* and *Xylaria*. We also identified *Acremonium* sp. as a quite frequently occurring endophyte, but this was not found by the Korean research group. On the other hand, 11 of their strains were not positively identified in our experiments up to now. This discrepancy may originate

from the completely different geographical region of cultivation, but also from the fact that neither Paul and coworkers' studies nor ours cover the whole range of the endophytes.

In the present study, seven endophytic species with more vigorous growth on the media were selected for dual cultures with *Monilia laxa* and *Agrobacterium tumefaciens*. As it was demonstrated by results, all species showed a range of inhibition and growth factors of pathogens in all plates were collectively impaired in compare with controls. In all plates, endophytic fungi had higher radial growth rate and their confrontation with pathogens had not only no influence on colony expansion and development but also in the case of some species induced improved growth rate to the endophyte. Presumably, being obliged competing other microorganisms inflicts enhanced growth and intensifies colony development of the endophytic fungi in order to acquire more nutrient resources and extend the life span of the species (Li *et al*., 2007). Such an inference may explain a possible mechanism by which endophytic fungi induce immunity to the host as they contend other microorganisms in colonized tissues and inhibit the growth of potential pathogens. Many fungal endophytes produce secondary metabolites and some of these compounds are antifungal and antibacterial which strongly inhibit the growth of other microorganisms including plant pathogens (Hellwig *et al*., 2002). Arguably, fungal endophyte association with host plants can shift between pathogenic and symbiotic under the particular circumstances (Kaplan *et al*., 2013). It is assumed that horizontally transmitted fungi may primarily generate parasitism, but in case emergence of infection by other microorganisms, apply mechanisms to eliminate the rival and sustain normal status of the host through life-history trade-offs with growth/survival. Thus they can perpetuate their dominance in the adapted niche that in turn indirectly confers benefit to the host and decreases the pathology of diseases (Arnold *et al*., 2003). Although screening of antimicrobial activity of endophytic fungi isolated from cherry rootstocks was performed by using In Vitro assays, such a life-history could be evolved for some species. For instance, *Botrytis cinerea* had a comparatively high antagonistic effect on pathogen growth in dual cultures, whereas it has been rather introduced as a pathogenic fungus in different hosts. These types of fungi probably tend to colonize the host tissue as parasites and rapidly occupy the ecological niche and leave no space for pathogen which would be the main reason that fungal endophytes inhibit pathogen infection in plants (Petrini *et al*., 1992). Such a property of fungal endophyte along with the host reactions against endophytes (i.e. secretion of lignin and other cell-wall deposits to limit the endophyte growth which as a shelter protects endophyte from pathogen invasion) and their capability to

parasitize pathogen organisms (hyperparasite endophytes) can be considered as the ecological effects of endophytic fungi on plant resistance to pathogens (Petrini *et al*., 1992) (Grosch *et al*., 2006).

5. Novel scientific achievements by the present study

Based on the assigned goals and analytical approaches of the present study, our findings were entirely unique as there has been no study as comprehensive and descriptive as ours on the biodiversity of fungal endophytes associated with the examined hosts in Hungary as well as in the Central Europe yet. The most important highlights of our achieved data are listed as follow:

1. The present study for the first time showed the phenotypic and phylogenic interrelations of endophytic fungi assemblages grafted on *Prunus Sp.*

2. Biodiversity of isolated endophytes was analyzed on different rootstocks of *Prunus Sp.* By application of statistical indices such as Simpson's diversity index and Shannon-Weaver index which could reliably demonstrate the host-dependent features of identified fungi colonized these species.

3. Results of the present Study were also analyzed according to the tendency of isolated fungal endophytes to colonize a particular anatomical compartment in order to represent the tissue-specific traits of these symbionts on the examined host species.

4. Based on the sampling procedures, we were also able to assess the impact of temporal changes on the biodiversity of isolated endophytic fungi associated with the examined host species.

5. All the above mentioned analyses were cumulatively considered to indicate the significant variations in host-specific and tissue-specific characteristics of isolated fungal endophytes according to the temporal changes in the sampling area.

6. We also examined the impact of harboring tissue, the host species and cultivation conditions on fungal endophytes assemblages associated with *Capsicum annuum* L.

7. Results of this study for the first time showed the antagonistic effects of fungal symbionts colonizing cherry rootstocks on growth of potential pathogens in vitro. As efficiently applied criteria, the growth rate toward three dimensions and the inhibitory effect of fungus-pathogen dual cultures were defined for all assays and the results were comprehensively discussed.

6. References

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7. Most important publications from the subject of the dissertation

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N Haddadderafshi, T Pósa, G Péter, L Gáspár, M Ladányi, K Hrotkó, N Lukács and K Halász (2016) Characterization of community structure of culturable endophytic fungi in sweet cherry composite trees and their growth-retarding effect against pathogens. *Acta Biologica Hungarica* (in press) IF: 0.589 (2014/2015)

Journals without IF

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