Frequency of Dark Septate Endophytes Colonization in *Iris germanica* L.

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SHORT COMMUNICATION

Abstract

Dark septate endophytes (DSE) are a group of fungi from phylum Ascomycota that develop inside healthy root tissue of a wide range of plants from diverse habitats. They play a role in nutrient acquisition and survival of their hosts in limiting conditions. Aim of this research was to identify the seasonal occurrence of dark septate endophytes across a cultivar gradient in urban conditions from Cluj-Napoca, Romania. Root samples from six *Iris germanica* cultivars were collected in spring and autumn. Microscopic assessment was conducted on 2160 root segments. Average DSE frequency in roots of *Iris germanica* was 14.58%. Analysis of variance revealed that influence exercised by the cultivar was not significant ($p=0.37$), but the interaction between cultivar and phenophase explained 53.97% of overall variance. DSE were identified in all six cultivars, indicating either to a similar susceptibility of the host genotype or lack of specificity of the fungal endophyte. Unravelling the functional roles of these fungi could contribute to a better understating of plant-fungi interactions in anthropic environments.

Keywords: rhizosphere; genotype; fungi; season; root.

INTRODUCTION

Dark septate endophytes (DSE) are a widespread group of sterile or conidial fungi classified in phylum Ascomycota, that develop characteristic microsclerotia and melanized mycelia, colonizing asymptotically healthy root tissue of a wide variety of plant species (Berthelot et al., 2020). From phylogenetic point of view, DSE are diverse and belong to various orders such as: Helotiales, Xylariales, Pleosporales, Sordariales, Hypocreales and Chaetothyriales (Berthelot et al., 2019). DSE can be met in woody and herbaceous plants and have high occurrence in disturbed ecosystems (Spagnoletti and Giacometti, 2020). DSE are believed to establish the link between plant roots and soil biologic crust (Zhang et al., 2020). Their relationship with plants can range from mutualism to parasitism (Berthelot et al., 2019). Resilience of these fungi is suggested by their occurrence in heavy metal contaminated soils that were shown to host species from genera *Cadophora* sp., *Leptodontidium* sp., *Exophialapisciphila*, and *Phialophora* sp. (Spagnoletti and Giacometti, 2020). Soil microbiota from urban space can have a beneficial role through ecosystem services they provide with cascading impact, such as supporting stability and sustainability of vegetation assemblages and promoting urban biodiversity. As consequence it is having long term effect on human well-being and health (Mills et al., 2020; Abrego et al., 2020). Because of this, soil microorganism-plant interactions in urban environments are worth to be studied and monitored for a better understanding of the dynamics that governs these relationships in anthropized environments. *Iris germanica* L., belongs to family

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Iridaceae and is a widespread cultivated geophyte species with a variety of utilizations: ornamental and medicinal (Crişan and Cantor, 2016; Crişan et al., 2019a), as source of natural dyes (Crişan et al., 2018), for phytoremediation (Song et al., 2020). It is a species cultivated throughout urban gardens from Cluj-Napoca and highly appreciated for colorful and large flowers that bloom in late spring. As showed in a previously published work (Crişan et al., 2020), *I. germanica* in the edaphoclimatic conditions of Cluj-Napoca displayed highly significant spring-autumn variation of arbuscular mycorrhiza (AM) colonization. This study explores spring-autumn dynamic for DSE in *I. germanica* and prospects a possible overlap between AM and DSE colonization pattern for this plant species.

Aim of the research was to identify the occurrence of dark septate endophytes across a cultivar gradient in local, urban conditions.

MATERIALS AND METHODS

Experiment was carried out in a green space from western part of Cluj-Napoca city, Romania (DD: 46.766569, 23.545294.), and was organized according to a bifactorial design: Factor A – the cultivar with 6 levels represented by the following cultivars ‘Black Dragon’ (black flowers), ‘Blue Rhythm’ (blue flowers), ‘Sultan’s Palace’ (red flowers), ‘Lime Fizz’ (yellow flowers), ‘Pinafore Pink’ (pink flowers) and ‘Pure As The’ (white flowers); Factor B the phenophase with 2 levels – shoot growth (May 2018) and debut of senescence (September 2018). Rhizomes (108) were planted in 6 randomized blocks during the previous year (2017) to ensure plants were well established by the time roots were collected. Roots were stained according to Vierheilig et al. (1998), cut into 1 cm long pieces and examined at 400× under Optika microscope equipped with condenser 0.9 NA (Figure 1). Frequency of colonization was expressed as relative number of root segments with DSE structures divided by the total number of root segments analyzed. Frequency was determined for sets of 30 root segments in 6 replicates for each cultivar both in spring and autumn (18 plants/cultivar were sampled). In total were assessed 2160 root segments.

Data was analyzed with two-way ANOVA using software Origin by OriginLab.

Figure 1. Experiment setup: (a) *Iris germanica* plants in spring and autumn; (b) cultivars at bloom; (c) root collecting and samples processing for microscopy (Original).

The soil type in Cluj-Napoca is luvisol (Stănilă and Dumitru, 2016) and at the experimental location the soil had clay-loam soil texture, pH 8.05, humus 3.24% and macronutrient supply content of 0.129% Nitrogen, 224 ppm Phosphorus, 304 ppm Potassium (Crişan et al., 2020).

During interval between planting of rhizomes (July 2017) and last root sampling (September 2018), warmest month was August both in 2017 and 2018 and coldest ones were January and February 2018. Maximum and minimum temperatures were 37°C registered in August 2017 and respectively -17°C registered in March 2018. Highest level of precipitation occurred in June 2018, at post-anthesis (Figure 2a). Average annual temperature in 2018 was 10.89% higher than the average for ten years (2008-2017), while precipitation level registered in 2018

was 14.29% lower than the last ten-year average (Figure 2b). This indicated that the experimental year (2018) when roots were collected was drier and warmer than the average for past decade.

Figure 2. Climatic conditions: (a) during planting and collecting last samples; (b) during previous decade 2008-2018 (<https://en.tutiempo.net>, <https://wunderground.com>).

RESULTS AND DISCUSSIONS

Average DSE frequency in roots of *I. germanica* was 14.63% (SE±1.84) in spring and 14.54% (SE±1.65) in autumn. Analysis of variance revealed that the influence exercised by the cultivar was not significant ($p=0.37$), nor the one exercised by the phenophase ($p=0.97$) (Table 1). However, the interaction between cultivar and phenophase explained over half of the variance registered by frequency of DSE colonization ($\eta^2=0.53$).

Table 1. Analysis of variance for DSE root colonization in *Iris germanica* (2018)

Factors	<i>F</i>	<i>p</i>	Significance
Phenophase (P)	0.0014	0.970	n.s.
Cultivar (C)	1.0957	0.372	n.s.
Interaction (P × C)	1.2848	0.282	n.s.

Note: Two-way ANOVA, $p > 0.05$ (n.s.)

Differences between cultivars in regards with the average DSE colonization frequency were not significant. Between 11.67% and 18.89% of *I. germanica* root segments analyzed in 2018 presented DSE structures (Table 2).

Table 2. Mean frequency (±SE) of DSE root colonization in six *Iris germanica* cultivars (2018)

Cultivar	Mean DSE frequency (F%)	Variance (s^2)
'Black Dragon'	18.89±4.03	194.61
'Blue Rhythym'	18.06±2.70	87.79
'Sultan's Palace'	13.06±2.23	59.51
'Lime Fizz'	11.67±1.99	47.47
'Pinafore Pink'	13.89±4.02	193.60
'Pure As The'	11.94±2.30	63.55

Analysis of the spring-autumn dynamic for root colonization of each cultivar indicated that in four out of six cultivars the frequency of DSE colonization decreased towards autumn (Figure 3). Thus, between spring and autumn, DSE frequency decreased in roots of the cultivars 'Black Dragon' and 'Sultan's Palace' but increased an average of 24-50% relative to spring in roots of cultivars 'Blue Rhythm', 'Lime Fizz', 'Pinafore Pink' and 'Pure As The'. On average, the cultivars with dark-colored flowers (black, blue, dark red) had higher levels of DSE frequency in spring (between 15-25%) compared to the ones with light-colored flowers (yellow, pink, white) that presented a DSE frequency between 10-11% in April (Figure 3). DSE structures as observed under microscope can be seen in Figure 4.

Figure 3. Seasonal dynamic of dark septate endophytes (DSE - Ascomycota) frequency (F%) for *Iris germanica* in IV - April, IX - Sept. 2018.

Figure 4. Dark septate endophytes (DSE - Ascomycota) in *Iris germanica* stained and squashed roots: (a-b) stars indicate microsclerotia; (b) arrowheads indicate presence of characteristic dark melanized septa inside hyphae, scale bar=50 µm (Original).

Arbuscular mycorrhiza colonization frequency reported for *I. germanica* in same conditions, also displayed a higher average level in spring (AM F% = 78.15) and lower level in autumn (AM F% = 63.80) (Crişan et al., 2020). However, there are two main differences between AM and DSE colonization frequency reported. Firstly, as shown here, DSE colonization frequency was lower (<15%) while AM frequency was much higher (>50%) as shown in previous work (Crişan et al., 2020). Secondly, phenophase exercised a highly significant influence on AM frequency ($p < 0.001$) (Crişan et al., 2020) while the influence of phenophase was not significant on DSE frequency, as was demonstrated in this study. This aspect might suggest a different synchronization of DSE vs. AM spreading with plant phenology and respectively with the metabolic state of the host. Based on these first observations, it is proposed more in-depth research to be conducted in relation with these aspects in the future.

According to previous research on herbaceous plants in field conditions, frequency of DSE colonization can vary widely between species but for comparison purposes are proposed three colonization frequency classes: first one $DSE \leq 10\%$ considered a low colonization level, $DSE = 11-49\%$ considered a middle level of colonization and

respectively DSE $\geq 50\%$ considered a high colonization level. These classes can conveniently group plants into categories based on DSE frequency, as follows:

- a) Low colonization frequency levels (DSE $\leq 10\%$) were reported for some common cultivated plants such as *Echinacea purpurea*, *Mentha × citrata*, *Origanum majorana* (Zubek and Błaszczowski, 2009), *Levisticum officinale*, *Verbena officinalis* (Zubek et al., 2010), a highly invasive plant - *Reynoutria sachalinensis* (Gucwa-Przepióra et al., 2016) or some alpine plants: *Homogyne alpina*, *Soldanella carpatica* (Zubek et al., 2009), as well as some ornamentals *Helianthus annuus*, *Liatris spicata*, *Tanacetum vulgare*, *Cosmos bipinnatus*, *Zinnia elegans* (Crişan et al., 2019b).
- b) Medium frequency of colonization levels (DSE=11-49%) were reported for several *Solidago* species and some wild and invasive plants (Gucwa-Przepióra et al., 2016), some cultivated medicinal plants such as *Melissa officinalis*, *Salvia officinalis*, *Digitalis lanata*, *Althaea officinalis*, *Calendula officinalis* (Zubek and Błaszczowski, 2009), in *Physalis alkekengi* and two *Pimpinella* species (Zubek et al., 2010), as well as in three common garden ornamentals *Ageratum houstonianum*, *Coreopsis tinctoria*, *Rudbeckia hirta* (Crişan et al., 2019b).
- c) High frequency of colonization levels (DSE $\geq 50\%$) were reported for some common cultivated herbaceous plants such as *Thymus vulgaris*, *Borago officinalis*, *Lavandula angustifolia* and *Cichorium intybus* (Zubek and Błaszczowski, 2009) as well as for garden ornamentals *Aster novi-belgii* (Gucwa-Przepióra et al., 2016), *Tagetes erecta* and *Aster dumosus* (Crişan et al., 2019b).

Determination of DSE frequency for plants across diverse habitats can serve as basis for a comprehensive comparative analysis that could lead to the identification of potential patterns correlated with conditions, taxa, genotype, or any other variables that cannot emerge outside a broader perspective on plant-DSE association. Punctual experiments such as this may constitute a piece in a larger puzzle that could pave the way to a better understanding of the conditions and factors that govern the interaction of these fungi with plants, and eventually how different the nature of this relationship is compared to other beneficial root fungal colonizers such as fine root endophytes or mycorrhizal fungi.

CONCLUSIONS

This paper reports DSE presence in *Iris germanica* from urban conditions of Cluj-Napoca, Romania. Results showed that average DSE frequency in roots of *I. germanica* was 14.63% in spring and 14.54% in autumn, with little variation between cultivars. The overall frequency pattern for DSE does not overlap with the one identified for AM under same conditions, fact that suggest to either a different life-strategy of these fungi or to the different nature of their interaction with plants. Compared to data from literature, DSE level obtained in this study situates within a middle range of colonization.

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Conflicts of Interest

The author declares that she does not have any conflict of interest.

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