

LEAF STOMATAL PARAMETERS OF *IRIS GERMANICA* L. INFLUENCED BY CULTIVAR AND ARBUSCULAR MYCORRHIZAE INOCULATION IN FIELD CONDITIONS, ROMANIA

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Abstract

The aim of this study was to investigate if supplementary inoculation with arbuscular mycorrhizae of *Iris germanica* plants in field conditions has influence on key stomata parameters that are known to determine the maximum leaf diffusive conductance of CO₂ to the site of assimilation as well as water use efficiency. Six *Iris germanica* cultivars ('Black Dragon', 'Blue Rhythm', 'Sultan's Palace', 'Lime Fizz', 'Pinafore Pink', 'Pure As The') were inoculated at planting in autumn with the following arbuscular mycorrhizae fungi species: *Funneliformis mosseae* (*Glomus mosseae*), *Funneliformis geosporus* (*Glomus geosporum*), *Claroideoglomus claroideum* (*Glomus claroideum*), *Rhizophagus intraradices* (*Glomus intraradices*), *Glomus microaggregatum*. Microscopic examination revealed that both inoculated and non-inoculated plants presented AM root colonization after entering in vegetation. Analysis of leaf imprints collected in spring showed that inoculation with arbuscular mycorrhizae determined a decrease in stomatal density but increase of guard cell length. Inoculated plants presented higher potential stomatal conductance index.

Key words: physiological parameters, ornamental geophyte, AMF inoculation, root.

INTRODUCTION

Stomata are involved in regulation of water and carbon dioxide balance of plants. Maximum stomatal conductance to water vapor and CO₂ is associated with stomata density and stomata size (Franks et al., 2017). During the evolution of plants, changes in stomata density and size were linked to fluctuations in atmospheric CO₂ levels (Franks et Beerling, 2009), this is why how future elevated CO₂ levels might affect plants is subject of high attention (Hepworth et al., 2015). In addition, recent studies showed stomata parameters to be reliably linked to drought-resistance in some cultivated monocot plants (Li et al., 2017; Hughes et al., 2017). Current understanding of molecular control of these leaf structures already allowed researchers to modify stomata density by manipulating epidermal patterning factor family of secreted signaling peptides which intervene in regulation of stomata development (Hepworth et al., 2015; Hughes et al., 2017). Previous studies indicate that AMF inoculation could have influence on primary physiological functions of leaves as well. For example, root colonization with AMF species was already

shown to be accompanied by increase in stomatal conductance (Augé et al., 1992; Augé et al., 2008) or density (Chitarra et al., 2016) for several cultivated species. Regarding the effect of AMF on *Iris* plants, it was demonstrated the promoting effect of *Glomus mosseae* on photosynthesis rate (Chen et al., 2014). Also, inoculation with *Diversispora epigaea*, *Glomus aureum*, *Rhizophagus irregularis*, *Rhizophagus clarus* influenced physiological processes of *Iris pseudacorus* plants because it was observed an improved phytoremediation capacity (Węzowicz et al., 2015).

Previously, differences in stomatal density of *Iris* leaves were studied in terms of histological gradients within the leaves of the plant in different light conditions (Pazourek, 1970) or in order to highlight variations between *Iris* species, subspecies or clones (Miljkovi et al., 2013; Ghasemi et al., 2014; Kandemir et Çelik, 2017). Thus, although previous studies were able to put in evidence variations in stomatal parameters in *Iris* plants the influence of other factors was little explored so far.

Iris germanica leaves present epicuticular wax structures as film or platelets with regeneration type following concentric-layer formation and

platelets (Koch et al., 2009). In the genus *Iris* leaf stomata observed is of anomocytic and tetracytic type. In addition, under microscope *Iris* leaf can present papillae on the outer periclinal wall of epidermal cells as well as bulliform cells, while prism-shaped styloids of calcium oxalate occur in idioblasts in the mesophyll and are arranged parallel to long axis of the leaf often being visible through epidermis. Micro-morphology leaf features hold taxonomic importance in genus *Iris* (Wu et Cutler, 1985; Mitić et Pavletić, 1995; Çölgeçen and Tug, 2006; Wang et al., 2010; Kandemir et Çelik, 2017).

In all monocots stomatal development begins with an asymmetric mitosis in a meristemoid-mother cell, which gives rise to a larger stomatal-lineage ground cell and shorter meristemoid that directly forms a guard-mother cell. Each guard-mother cell undergoes symmetric mitosis to form a pair of guard cells that rapidly cease expansion compared with the greater enlargement of pavement cells as leaves grow. Because in monocots with linear leaves and parallel venation, stomata occur in epidermal cell files that develop basipetally from an intercalary meristem (Rudall et al., 2017), there is a histological gradient along the leaf blade.

The aim of the study was to investigate the influence of inoculation in field conditions with a consortium of five AMF species on key stomata characteristics such as stomata density and guard cell length for six *Iris germanica* cultivars.

Results of this study could help in identifying if AM inoculation might optimize basic physiological processes that could help plants cope with increasing atmospheric pollution and drought, both known to influence plant development within the context of current climate trend.

MATERIALS AND METHODS

The experimental field was located in the Agro-Botanical Garden of UASVM Cluj-Napoca Romania, elevation (AMSL) 380-430 m, with average annual temperature 8.1°C and average sum of annual precipitation 635 mm (Index Seminum USAMV Cluj).

The physical-chemical analysis of the soil collected when plants entered vegetation in

spring 2017, conducted at the O.S.P.A. Cluj showed a clay loam soil type with 6.72 pH, low humus level (1.35%) and good NPK supply (N 0.461%, P 68 ppm, K 312 ppm). Sum of basic cations through Kappen method was 20.96 (me/100 g of soil), while hydrolytic acidity through extraction method was 1.92 (me/100 g soil). Basic cation saturation level determined by calculation was 92. Granulometric analysis using Kacinski method was: coarse sand 14.42, fine sand 25.08, dust I 7.95, dust II 13.65 and clay 38.90. Low dose of NPK fertilizer was applied before bloom and wood ash was applied in autumn and spring.

The bifactorial experiment established in October 2016 was organized in randomized blocks with three replicates: factor A - *Iris germanica* cultivars, with six levels (a_1 = 'Black Dragon', a_2 = 'Blue Rhythm', a_3 = 'Sultan's Palace', a_4 = 'Lime Fizz', a_5 = 'Pinafore Pink', a_6 = 'Pure as The') and factor B - the treatment applied with two levels (b_1 = non-inoculated at planting and b_2 = inoculated at planting with 13 grams mycorrhizal products per rhizome). The two commercial mycorrhizal products special destined for use in ornamental plants were imported from U.K. and contained the following AMF species: *Funneliformis mosseae* (*Glomus mosseae*), *Funneliformis geosporus* (*Glomus geosporum*), *Claroideoglomus claroideum* (*Glomus claroideum*), *Rhizophagus intraradices* (*Glomus intraradices*), *Glomus microaggregatum*. In addition to the AMF spores, the products contained excipients such as organic materials, humates and auxins along a few others. Between the three blocks of inoculated plants and the three blocks of non-inoculated plants was ensured 7 m, sufficient to prevent potential cross transport of AM propagules (Powell, 1979), also tools were washed after working with each set of plants. Microscopic examinations were conducted with optical microscope (Figure 1c) ML-4M made in Romania. Images were taken with Bresser camera and microscope (Figures 2, 5). The success of root colonization was confirmed in 2017 under microscope (Figure 2) using the staining technique of Stoian et Florian (2009) and colonization estimation method (<https://www2.dijon.inra.fr/>). Using clear polish and tape method (Palasciano et al., 2005), in

May 2017 were collected leaf imprints from middle segment of the leaf blade surface facing south (Figure 1c).

For each leaf imprint sample, the number of stomata was counted according to Vâtcă et al.

(2007) and presented as density per square mm. Guard cell length was measured with eye piece reticle. Stomata were considered in the field of view only if the entire ostiole was visible.

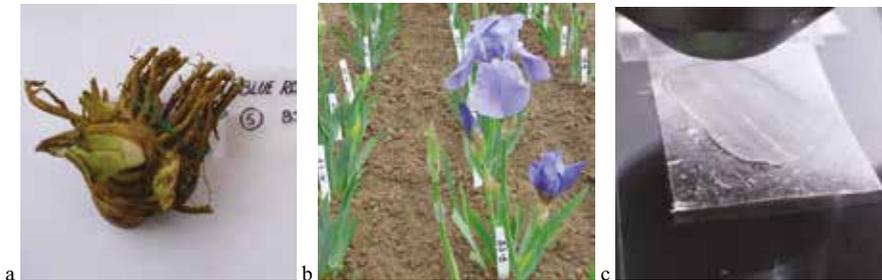


Figure 1. *Iris germanica* 'Blue Rhythm': a) rhizome at planting; b) plants in spring; c) leaf imprint sample

In total for all six *Iris germanica* cultivars, stomata were counted on more than 1900 microscopic fields of view and over 5700 stomata were measured for guard cell length.

Leaf imprints were collected also from several other rhizomatous cultivated species (*Iris pallida*, *Iris pseudacorus*, *Iris sibirica* and *Iris chrysographes*) grown in Agro-Botanical Garden UASVM Cluj-Napoca, for comparison purposes and analyzed similarly.

Based on the microscopic examinations were calculated:

1. $PCI = L^2 \times SD \times 10^{-4}$ (Holland et Richardson, 2009), where PCI = potential conductance index, L = guard cell length (μm), SD = stomata density per 1 mm^2 ;
2. $a\% = [(100 \times mA_3) + (50 \times mA_2) + (10 \times mA_1)]/100$ (Trouvelot et al., 1986), where a% = arbuscule abundance in mycorrhizal parts of the root fragments, based on percentages of *m* rated according to methodology.

Data was analyzed with Microsoft Excel, Mycocalc and Origin.

RESULTS AND DISCUSSIONS

In 2017 at the end of active vegetative growth of plants (end of spring) both inoculated and non-inoculated *Iris germanica* plants presented root colonization confirmed under microscope

that looked rather discontinuous or patchy along root length.

The fresh, washed, and unstained thick roots of first order were white but thinner roots of higher order, were darker in color indicating root colonization causing a darkening of the cortical cells under the pigment released by collapsed arbuscules (Fester et al., 2002). All AMF structures were present: extraradical hyphae, hyphopodium and inner root coils at the entrance points, young and collapsed arbuscules, vesicles (oblong and round) and spores as well as a few loose sporocarps.

Under microscope was identified Arum type proliferation as well as intermediate Arum-Paris type proliferation. Spreading patterns varied slightly among cultivars. Thus, it was noticed in some segments a preponderant linear spreading within the roots of 'Pure As The', arbuscules preferentially along sieve elements in 'Lime Fizz' or in the outer root layers in 'Sultan's Palace', abundant hyphae coils within roots of some 'Black Dragon' plants.

Arbuscules abundance in mycorrhizal parts of root fragments of the six *Iris germanica* cultivars was slightly increased from average $a\% = 44.09$ in non-inoculated plants to $a\% = 44.45$ in inoculated plants, hinting to a higher transfer occurring between plants and arbuscular mycorrhizae fungi following inoculation treatment.

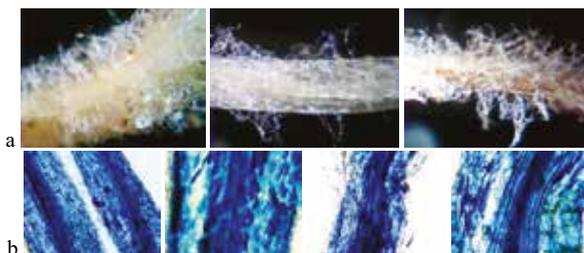


Figure 2. Microscopic view: a) colonized *Iris germanica* roots, some with pigmentation due to apocarotenoids released by collapsed arbuscules; b) roots after staining

In five out of six *Iris germanica* cultivars, stomata density decreased as a result of inoculation with arbuscular mycorrhizae (Figure 3). The only cultivar that presented a slight increase in stomata density due to inoculation was ‘Black Dragon’ from 61.41/mm² in non-inoculated plants to 61.95/mm² in inoculated plants. On average, stomata density decreased in inoculated plants

with about 10 stomata/mm², from an average density of 63.83/mm² in non-inoculated plants to 53.12/mm² in inoculated plants. Highest decrease of stomata density due to inoculation was observed in ‘Sultan’s Palace’ followed by ‘Pinafore Pink’ and ‘Pure As The’. The smallest decrease in stomata density caused by inoculation was found in ‘Lime Fizz’ followed by ‘Blue Rhythm’.

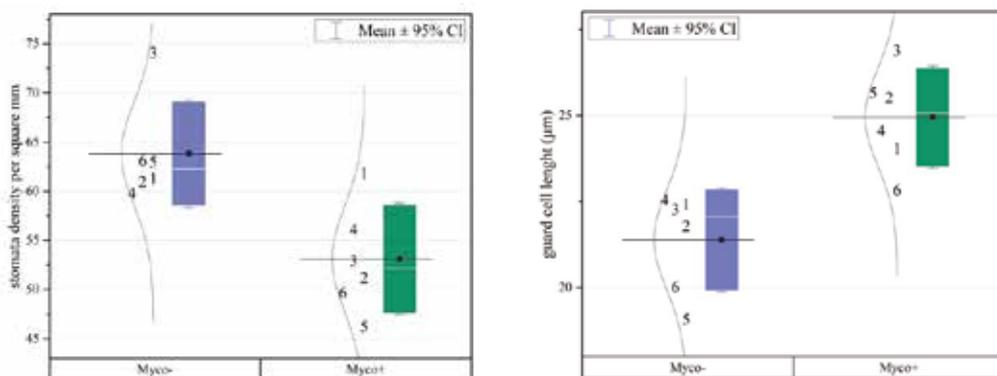


Figure 3. Stomata density (per mm²) and guard cell length (µm) in six *Iris germanica* cultivars: 1) ‘Black Dragon’; 2) ‘Blue Rhythm’; 3) ‘Sultan’s Palace’; 4) ‘Lime Fizz’; 5) ‘Pinafore Pink’; 6) ‘Pure as The’ non-inoculated (Myco-) and inoculated with arbuscular mycorrhizae (Myco+)

In all inoculated *Iris germanica* cultivars guard cell length increased (Figure 3) on average with 3.56 µm. Non-inoculated plants on average had a stomata length of 21.39 µm while inoculated cultivars had an average stomata length of 24.95 µm. The largest increase in guard cell length linked to inoculation was observed in ‘Pinafore Pink’ that had the second highest decrease of stomata density as a result of inoculation followed by ‘Sultan’s Palace’. The slightest increase in guard cell length due to inoculation was observed in ‘Black Dragon’ of only 1.6 µm on average.

Potential stomatal conductance index increased due to inoculation in all studied *Iris germanica* cultivars (Figure 4), from the average of 2.93 in non-inoculated plants to 3.31 in the inoculated plants. The highest increase was observed in ‘Pinafore Pink’ (from 2.30 to 3.06). This cultivar also experienced one of the highest decrease in stomata density and highest increase in guard cell length due to inoculation. Also, it can be noted that non-inoculated plants of ‘Pinafore Pink’ presented the smallest guard cell length among all cultivars studied. Two cultivars presented very similar increase of potential stomata conductance due to

inoculation: ‘Black Dragon’ (from 3.10 to 3.59) and ‘Blue Rhythm’ (from 2.91 to 3.35). Both these cultivars had either a slight decrease in stomata density or a very small increase in stomata density due to inoculation. The least increase in potential stomata conductance index was observed in ‘Pure As The’, from 2.54 to 2.61, followed by ‘Sultan’s Palace’ with an increase from 3.69 to 3.85. The cultivar ‘Lime Fizz’ with yellow flowers, situated between the dark-flowered cultivars and the light-flowered

cultivars, with an increase of PCI due to inoculation from 3.06 observed in non-inoculated plants to 3.40 observed in inoculated plants (Figure 4).

Although stomata density did not increase, on contrary decreased in inoculated plants with one exception, the potential stomatal conductance increased in all studied *Iris germanica* cultivars due to inoculation, because of the increase in guard cell length.

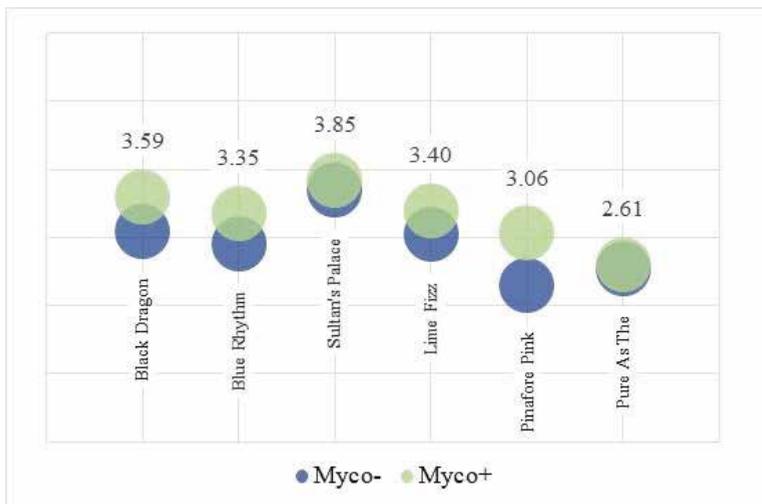


Figure 4. Potential stomatal conductance index (PCI) in six *Iris germanica* cultivars non-inoculated (Myco -) and inoculated (Myco +) with arbuscular mycorrhizae

During the vegetative months prior to flowering and before collecting the leaf imprint samples there was a warm spring with temperatures exceeding 20°C on no less than 17 days during the interval 15 March - 15 May 2017. The sum of precipitations was about 45 mm during same time interval, while the wind had intensities between 3-37 km/h (<https://www.wunderground.com/history>).

Plants were not irrigated and supported a natural water regime. Agro-Botanical Garden of UASVM is situated on the steep side of Someșul Mic river valley within Cluj-Napoca and experiences windy conditions in spring and fall, fact known to increase the transpiration and water loss in plants.

In the given conditions, it can be considered that plants had to find ways to cope with these environmental challenges by optimizing their physiological processes. Hepworth et al. (2015)

citing previous researches mention that plants with low stomata density presented enhanced water use efficiency and reduced transpiration levels that allowed plants to grow larger especially under water restriction conditions. But reduced stomata could also mean lower transpiration. Yet, transpiration is driving the mass flow (Hepworth et al., 2015), fact that would suggest that if stomata density decreases under a certain level it would be expected to be made at the expense of nutrient accumulation. The increased guard cell length ensured the increase of potential stomata conductance index despite of a reduction in stomata density. Plants can rely on mycorrhizae mycelia network for increase uptake of both nutrients and water, but mycorrhizal fungi take in exchange part of the carbon fixed by the plant. Thus, the inoculated plant needs to be able to conduct photosynthesis at optimum levels, and this is perhaps

why the reduction in stomata density was accompanied by increase in stomata length. Since both sets of plants (inoculated and non-inoculated presented root colonization), the observed changes might be linked to inoculated species rather than those already present in the soil; also, some excipients could have played a beneficial role as well, either directly on plant rooting and nutrition or indirectly by stimulating the establishment of symbiose. Micromorphology differences between studied *Iris germanica* cultivars and other rhizomatous

species were observed under microscope on collected leaf imprint samples (Figure 5). Comparing the stomata density and guard cell length of non-inoculated *Iris germanica* plants (Figure 3) with those of *Iris pallida* (Table 1), it can be observed that values for *Iris germanica* are higher. It is known that current *Iris germanica* cultivars are tetraploids (Norris, 2012) and this cultivated species is actually a natural hybrid with diploid *Iris pallida* as one of its ancestors (Lim, 2016).

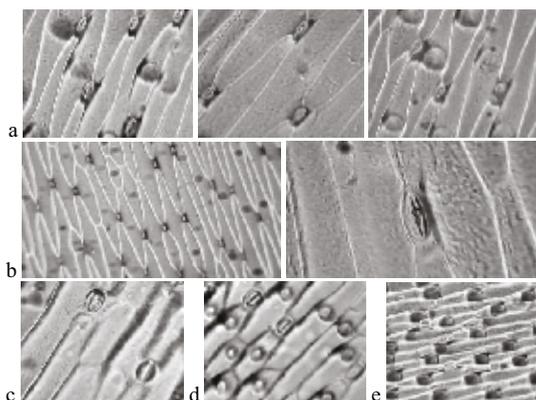


Figure 5. Leaf micromorphology on imprints from cultivated rhizomatous *Iris* species: a) *Iris germanica*; b) *Iris pallida*; c) *Iris pseudacorus*; d) *Iris sibirica*; e) *Iris chrysographes*

Ploidy level influences stomatal characteristics, since guard cell size has been used to predict the haploid level of primitive angiosperms and the extent of polyploidy in the present-day angiosperms (Willmer et Fricker, 1996). On average both inoculated and non-inoculated plants of *Iris germanica* presented longer guard cell length, and polyploidy of *Iris germanica* could explain this characteristic compared to diploid *Iris pallida*. However, the values are too close for a clear indication of ploidy level in *Iris germanica* based on stomata parameters compared to *Iris pallida*, even more since inoculated *Iris germanica* plants had slightly smaller stomata density than *Iris pallida*. Ghasemi et al. (2014) trying to find if stomata parameters can be used in several *Iris* taxa from Iran to predict ploidy level, reached same conclusion that, environmental factors have a strong influence on stomata characteristics that would make this approach less exact in predicting ploidy level in *Iris*.

All three species from subgenus *Limniris* (*Iris pseudacorus*, *Iris sibirica* and *Iris chrysographes*) presented higher stomata density than the two species from subgenus *Iris* (*Iris germanica* and *Iris pallida*).

Iris germanica and *Iris pallida* are native to milder Mediterranean climate, with warm summer (Lim, 2016), and their stomata density and guard cell length have similar values. The lower stomata density compared to the other three species studied (Table 1) can be an indication of their known drought tolerance and their known preferences for dryer substrate (Beresford-Kroeger, 2004). On *Iris germanica* leaves were observed large papillae (no more than one per epidermal cell) while stomata over the leaf veins are present but disposed sparsely. *Iris sibirica* and *Iris chrysographes* originate from colder regions of Eurasia and respectively Asia (White et al., 1997). Under microscope these two present smaller epidermal cells compared to other *Iris* plants from this study and abundant leaf papillae. Both present

smaller guard cell length and high stomata density. It is known that smaller stomata occur at higher frequency, fact that determines the total possible pore area for leaf to be similar (Willmer et Fricker, 1996). *Iris pseudacorus* presents both a large number of stomata per leaf unit area as well as longer guard cells. This

species is used for water gardens and grows best in marshes (White et al., 1997), fact that can be linked to observed stomata parameters that indicates to higher water requirements known that this species has (Jacobs et al., 2011).

Table 1. Stomata parameters in four *Iris* species from Agro-Botanical Garden UASVM Cluj-Napoca

Species	SD (mm ²)	L (μm)	PCI
<i>Iris pallida</i> Lam.	56.33	20.83	2.44
<i>Iris pseudacorus</i> L.	82.11	23.77	4.64
<i>Iris sibirica</i> L.	97.59	17.28	2.91
<i>Iris chrysographes</i> Dykes	72.34	16.13	1.88

SD – stomata density, L – guard cell length, PCI – potential stomatal conductance index

Summarizing the observations and findings of this research, firstly can be suggested that patchy colonization pattern observed in both sets of *Iris germanica* plants (inoculated and non-inoculated) could be due to soil texture, particularly abundant clay component, that also might have caused poorer root ramification too. Previous studies showed that soil texture particularly clay rich soil along lime application are some of the most important factors influencing AMF root colonization in maize, sorghum and peanuts (Carrenho et al., 2007). Also, patchiness characterizes the distribution of mycorrhizal structures of members from genera *Ambispora*, *Archaeospora*, *Diversispora*, *Entrophospora*, *Intraspora* and *Paraglomus*, whereas members of the families *Gigasporaceae*, *Glomeraceae* and *Pacisporaceae* usually present a continuous mycorrhizal distribution along the root (Błaszowski, 2012). This might as well explain the patchy colonization observed.

The different colonization patterns observed in some cultivars can be attributed both to some differences in root architecture between cultivars as well as to possible plant genotype-AMF colonizing specificity but could not be associated to inoculation treatment.

Previously in *Iris pseudacorus* plants grown in pots on sterile substratum and inoculated with *Diversispora epigaea*, *Glomus aureum*, *Rhizophagus irregularis* and *Rhizophagus clarus* obtained from trap cultures was reported typical Arum type spreading (Wężowicz et al., 2012), but in this study conducted in field conditions both Arum and intermediate Arum-Paris type proliferation was identified in *Iris*

germanica cultivars either inoculated or non-inoculated. This comes to show that in field conditions colonizing patterns of plants can be different.

The yellowish tint of different intensities identified in colonized *Iris germanica* roots of higher order appears in response to AM fungi and various carotenoid degradation products (apocarotenoids) released during collapse of arbuscules. These accumulate as hydrophobic droplets in root cortical cells and were identified in many Liliopsida species analyzed. Their function is little known but might have importance in the arbuscule development and function (Fester et al., 2002). This observation indicated that *Iris germanica* plants were colonized during spring. Root samples were collected shortly after the flowering season. Between inoculated and non-inoculated plants was identified only a small difference regarding arbuscules abundance in mycorrhizal parts of root fragments.

A direction for future investigation would be to investigate the seasonal variation of root colonization in order to establish the link between plant phenology and mycorrhiza life cycle.

In five out of six studied *Iris germanica* cultivars, a decrease in stomata density was accompanied by increase in stomata length. These findings are in accordance with previous patterns observed in *Iris* stomata. In two taxa from Turkey, *Iris masia* subsp. *dumaniana* *Iris masia* subsp. *masica* higher number of stomata was linked to smaller length of stomata (Kandemir et Çelik, 2017). Similarly, Ohsumi et al., 2007 found a negative correlation

between stomata density and stomata length in *Oryza sativa* plants, another monocot.

All *Iris germanica* cultivars inoculated with arbuscular mycorrhizae presented increased potential stomatal conductance index (Figure 4). Previous studies showed that *Glomus mosseae* inoculation had a stimulating effect on photosynthesis of *Iris* plants (Chen et al., 2014). Also, leaves of AMF inoculated *Vigna unguiculata* had higher stomatal conductance than those of non-mycorrhizal plants before and after lowering soil water potential (Augé et al., 1992).

In contrast with the results of this study on *Iris* plants, in controlled conditions *Rhizophagus intraradices*-inoculated tomato plants presented significantly increased number of stomata in mature leaves; stomatal density was almost twice that of control tomato plants or *Funneliformis mosseae*-inoculated plants (Chitarra et al., 2016). By comparison, in this study the inoculated *Iris* plants grown in field conditions experienced a reduction in stomata density, maybe as a strategy to reduce water loss. Previous experiments showed that a route towards improving drought tolerance and water use efficiency without significantly affecting photosynthetic capacity or nutrient accumulation by mass flow is a slight reduction in stomata density (Hepworth et al., 2015).

In conclusion can be said that environmental factors influence stomata parameters, but plant genotype also has a strong influence as well. This can be exemplified by two observed phenomena from this study.

First, different *Iris germanica* cultivars did not responded identical to inoculation with arbuscular mycorrhizae, although in all cases there was an increased potential stomatal conductance index. Secondly, different rhizomatous species grown in Agro-Botanical Garden of Cluj retain their ancestral characteristics from adaptation to their habitat of origin. This is most simply explained by the known fact that each species maintains specific growing requirements similar with the environment they evolved in, just as stomata density in different *Iris* species mentioned above can be easily linked to the way their physiological processes were adapted to ecological niche they preferentially occupied. This suggests there might be a certain threshold

up until a certain growing factor (like inoculation) can be used to enhance plant response and its effect cannot guarantee an equal response among plants.

Expression of genes homologous to those involved in the regulation of stomatal development in *Arabidopsis* known as *STOMAGEN* and genes encoding two intercellular signaling factors that act as negative regulators for stomatal development antagonistic to the first one was investigated in developing leaves of AMF inoculated tomato plants.

It was showed that genes EPF1 and EPF2 were significantly modulated only in the presence of AM symbiosis, while *LeEPFL9* transcript levels were correlated with changes in stomata density of *Rhizophagus intraradices*-inoculated plants (Chitarra et al., 2016).

According to the results of this study, supplementary inoculation has influence on two key stomata parameters.

In inoculated plants, on average stomatal density decreased but the length of guard cells increased, possibly hinting to a tendency to balance water use efficiency and increased assimilation rate that ultimately lead to potential stomatal conductance index to increase in all inoculated cultivars.

CONCLUSIONS

On average, stomata density decreased in arbuscular mycorrhizae-inoculated plants with about 10 stomata/mm², at the same time guard cell length increased on average with 3.56 μm. The changes in stomata parameters following inoculation lead to an increase of potential stomatal conductance observed in all studied cultivars.

Both inoculated and non-inoculated plants presented root colonization, with some close values for arbuscule abundance in mycorrhizal parts of root fragments between the two sets of plants.

More studies should be conducted to assess the physiological response of plants to mycorrhiza inoculation in field conditions, in order to further define the practicality of its application for ornamental irises as well as irises destined to obtaining orris oil.

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