

Seasonal arbuscular mycorrhiza colonization dynamic displays genotype-specific pattern in *Iris sibirica* L.

Ioana CRIȘAN¹, Andrei STOIE^{1,2*}

¹University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, 3-5 Manastur Street, 400372 Cluj-Napoca, Romania; crisan.ioana@outlook.com;

²University of Agricultural Sciences and Veterinary Medicine, Agro-Botanical Garden (CLA), 3-5 Manastur Street, 400372 Cluj-Napoca, Romania; andrei.stoie@usamvcluj.ro (*corresponding author)

Abstract

Arbuscular mycorrhiza (AM) is a widespread symbiotic association between plants and Glomeromycota fungi, that brings nutritional-derived benefits for phytobiont. Influence of plant breeding on arbuscular mycorrhiza susceptibility is a topic of current interest that can have many practical implications. Insights into whether new cultivars have a lower mycorrhizal potential, are critical for optimization of AM use. Aim of this research was to conduct a comparative assessment of AM colonization across a phenophase gradient in two *Iris sibirica* genotypes: one displaying the wild traits versus a modern reblooming cultivar with double flowers. Analysis showed that both *Iris sibirica* genotypes developed *Paris*-morphotype. Results indicated that on average the genotype with simple flowers had a higher AM colonization frequency (84.44 ± 2.15) compared to the new cultivar with double flowers (52.22 ± 6.09). Significant influence was exercised both by genotype ($p < 0.001$) as well as by phenophase ($p = 0.0013$), over colonization frequency. The genotypes displayed contrasting colonization dynamics: highest AM frequency level occurred in spring for the genotype with simple flowers, and in autumn for the one with double flowers. Results suggest that host metabolic state has regulating role over functionality of established AM-symbiotic association according to plant nutritional requirements, while fungi might also respond to increased or decreased carbon flux in the plant, associated with geophyte phenology.

Keywords: cultivar; flower; phenology; root; symbiosis; wild

Introduction

Iris sibirica L. is a perennial herbaceous plant with rhizomes, native to Eurasia. It thrives in moist soil and tolerates a mildly acidic to alkaline substrate, growing both under semi-shade as well as in full sun conditions. It is considered one of the finest ornamentals for herbaceous border as well as margins of streams, ponds, and lakes (Grey-Wilson, 1997). Wild *Iris sibirica* can be found growing sporadically in *Molinion*, *Molinio-Arrhenatheretea* phytocoenosis throughout Romania (Crișan *et al.*, 2017). *Iris sibirica* leaves and rhizomes have medicinal properties (Kovalev *et al.*, 2017) while flowers are a source of natural dyes (Kaššák and Kuli, 2014). Also, these plants present phytoremediation capacity for heavy metals (Wang *et al.*, 2018), excess of nutrients (Yang *et al.*, 2017) and some pharmaceutical pollutants (Tejeda and Zurita, 2020).

Received: 29 Oct 2020. Received in revised form: 29 Dec 2020. Accepted: 12 Jan 2021. Published online: 19 Jan 2021.

From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal will use article numbers in place of the traditional method of continuous pagination through the volume.

For *Iris sibirica* there were fewer breeding programs compared to bearded irises. Many of the *Iris sibirica* found in cultivation today are tetraploids (Crişan *et al.*, 2017), while interspecific hybrids are not uncommon in gardens, sometimes leading to confusion (Grey-Wilson, 1997; Kaššák, 2013). Traditionally, series *Sibericae* from section *Limniris* of the genus *Iris*, has been divided in two subseries, first one containing Siberian irises with $2n=28$ that includes *I. sibirica* and *I. sanguinea*, as well a second one known as Sino-Siberian irises with $2n=40$ (Boltenkov *et al.*, 2020). For a long time, it has been known that *Iris sibirica* produces fertile hybrids with species from first subseries as well as unfertile hybrids with some species from the second one (Grey-Wilson, 1997). However, a recent study suggests that species *I. sanguinea* and *I. typhifolia* are in fact conspecific with *I. sibirica* explaining also the overlapping morphological traits (Boltenkov *et al.*, 2020). The breeding trend for irises, including *I. sibirica*, has been the obtaining of cultivars with wide, ruffled tepals displaying high variation in colours and patterns (Crişan *et al.*, 2017). Cultivated diploid sibs can be distinguished by their delicate stems and narrow leaves, while tetraploids have thicker stems and larger flowers (Kaššák, 2013).

Arbuscular mycorrhiza (AM) is a beneficial partnership for 72% of vascular plants with fungi from phylum Glomeromycota, that bring many nutrition-related advantages for their host (Brundrett and Tedersoo, 2018). It was estimated that 1000 times more plant species are involved in this symbiosis than fungi species, pointing to low specificity of the mycobiont (Hodge *et al.*, 2010). It is considered that cultivated plants have a lower dependency on AM compared to their wild counterparts (Kokkoris *et al.*, 2019). This is supported by the fact that average mycorrhiza dependency for field crops was shown to be 44%, for forage crops 56%, while for wild grasses and forbs 70%. Also, perennials appear to exhibit a higher mycorrhiza dependency than annuals (Tawaraya, 2003). However, it is important to mention that these were inferred based on studies conducted on a rather limited number of plant species and particularly on *Arum*-type cultivated plants whereas little attention was given in older works to functional implications of the morphotype diversity across species, as one can see from data available at the time for analysis to Tawaraya (2003). Recent authors remark the poor understanding and knowledge gaps existing in defining more precise the factors that determine symbiotic outcomes for the host plant (Kokkoris *et al.*, 2019). These have important implications, because they hinder the optimization of AM use, due to unpredictability of their effectiveness to enhance traits of interest. A common question heard today is whether during plant breeding history, cultivars developed a lower symbiotic susceptibility compared to their wild counterparts, making them less likely to benefit substantially from AM (Hohmann and Messmer, 2017; Jacott *et al.*, 2017; Liu *et al.*, 2020).

This paper attempts to bring a contribution to the understanding of genotype significance for endomycorrhizal dynamics, adopting a comparison approach by using cultivated *Iris sibirica* displaying wild-traits versus modern-traits.

Aim of this research was to investigate arbuscular mycorrhiza potential in two cultivated *Iris sibirica* genotypes: one displaying wild sib traits (simple flower, thin stem, and narrow leaves) and a novel, reblooming cultivar (*Iris sibirica* 'Concord Crush') displaying modern flower traits such as double flowers and wide tepals.

Two objectives were defined:

- identification of natural susceptibility of the genotypes to arbuscular mycorrhiza
- description of seasonal colonization pattern associated with genotype and phenology.

Materials and Methods

The experimental plot was located in the Agro-Botanical Garden of University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, Romania. Rhizomes were planted in rows, one year prior (2018) to ensure that plants were well established and well-rooted in 2019 when samples were collected for analysis. Space between rows was 40 cm and between plants per row 20 cm. Rhizomes were watered only until rooting. No fertilizer was applied. Weed control was performed mechanically and manually.

The experiment was organized according to a bifactorial design.

Factor A with two levels represented by two *Iris sibirica* genotypes:

a₁ – *Iris sibirica* with simple flowers (Figure 1A) and one blooming/season, from the *Iridaceae* Collection of the Agro-Botanical Garden USAMV Cluj-Napoca (Index Seminum, 2020);

a₂ – *Iris sibirica* ‘Concord Crush’ with double flowers (Figure 1B) and rebloomer; this cultivar was created by Bauer and Coble and registered in 2009 (NGA, 2020).

Factor B with three levels represented by contrasting plant phenophase and corresponding to the three months when roots were collected (2019):

b₁ – flowering (V - May);

b₂ – post-anthesis (VII - July);

b₃ – prior to dormancy (IX - September).



Figure 1. The two *Iris sibirica* genotypes studied: (1A) with simple flowers, (1B) ‘Concord Crush’ with double flowers (Original)

Root samples were collected at 2 months intervals: 15 May, 15 July and 15 September 2019 in order to document colonization level during contrasting metabolic states of the plant.

Root samples were stained immediately after collecting, following method of Vierheilig *et al.* (1998). Stained roots were mounted on glass slides and observed at magnification 100× - 400× under Optika brightfield microscope equipped with condenser 0.90 N.A.

Microscopic evaluation was conducted according to methodology of Trouvelot *et al.* (1986) and performed on sets of 30 root segments of 1 cm long in triplicate. In total were analysed under microscope 540 roots (2 genotypes × 3 phenophase × 30 roots × 3 replicates). Arbuscular mycorrhizal indicators were obtained using MycoCalc software (INRA, 2001).

Colonization indicators obtained were:

F% = frequency of mycorrhizal colonization,

m% = intensity of colonization in mycorrhizal parts of root fragments,

M% = intensity of mycorrhizal colonization in root system.

Statistical analysis was conducted by applying two-way ANOVA and Duncan test.

Soil in the Agro-Botanical Garden has clay-loam texture, neutral soil pH, low humus level but good NPK content (Crişan *et al.*, 2019).

During the experimental year (2019), the last spring frost occurred in April when plants were in shoot growth phenophase and the highest precipitation levels were registered in May when plants were in bloom. The highest temperature was registered in July (34°C) when plants were less metabolically active (Figure 2A). By comparing the average annual temperature for 11 years (2008-2018) was observed that the experimental year was 10.78% warmer and 26.23% drier than the average for the past decade (Figures 2A, 2B).

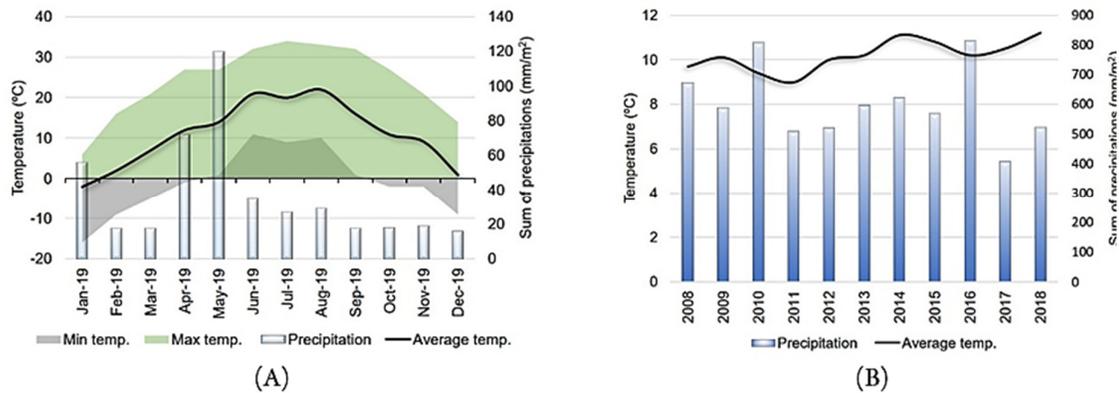


Figure 2. Climatic conditions during: (2A) the experimental year 2019, (2B) previous decade 2008-2018. Cluj-Napoca climate database (2019a, b) (<https://en.tutiempo.net/climate>, <https://www.wunderground.com>)

Results

In the experimental year, phenological stages of *Iris sibirica* were: the start of shoots emergence in April, flowering in May and lasting until early summer only for the cultivar with double flowers, summer resting between June to August for both, fruit formation over the summer months only for the genotype with simple flowers, growth occurring again in September for both genotypes, leaf senescence and dormancy onset in October, dormancy from November until March. In the climate of Cluj-Napoca both genotypes maintained green foliage over the summer, but the rebloomer did not produce flowers again in autumn, because the autumn was short with cool nights, and frost occurred starting with October (Figure 2A).

Analysis of variance revealed that genotype exercised a highly significant influence only on frequency of colonization (Table 1) explaining 65% of variance registered by this indicator (Figure 3). Phenophase exercised a significant influence on both frequency of colonization as well as intensity of colonization in mycorrhizal parts of root fragments (Table 1). Phenophase also explained over 70% of the variance registered by intensity indicators (Figure 3).

Table 1. Influence of experimental factors and their interaction on arbuscular mycorrhiza (AM) frequency (F%) and intensity (m%, M%) of colonization for *Iris sibirica* (2019, Cluj-Napoca)

AM	Factors and interaction	Mean square	F	p	Sig.	s ²
F%	Genotype (G)	4672.54	112.96	<0.001	***	451.35
	Phenophase (P)	505.47	12.22	0.0013	**	
	Interaction (G × P)	746.54	18.04	0.0002	***	
m%	Genotype (G)	2.32	0.39	0.5404	ns	17.94
	Phenophase (P)	30.11	5.14	0.0244	*	
	Interaction (G × P)	86.12	14.71	0.0006	***	
M%	Genotype (G)	5.31	1.46	0.2494	ns	8.42
	Phenophase (P)	7.56	2.08	0.1670	ns	
	Interaction (G × P)	39.57	10.91	0.0020	**	

Note: Two-way ANOVA; p > 0.05 (n.s.), p < 0.05 (*), p < 0.01 (**), p < 0.001 (***)

Interaction between genotype and phenophase exercised a significant influence on all three mycorrhizal indicators analysed (Table 1).

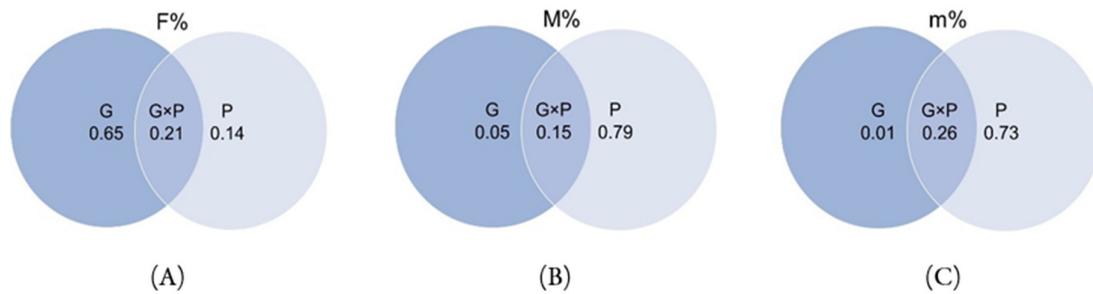


Figure 3. Venn diagrams expressing partitioning of variance (values η^2): share of factors (G=genotype, P=phenophase) and their interaction to overall variance for (3A) AM frequency of colonization, (3B) AM intensity in root system and (3C) AM intensity in mycorrhizal parts of root fragments

Average frequency of AM colonization in 2019 was significantly higher for the *Iris sibirica* genotype with simple flowers compared to the one with double flowers, while differences between the two genotypes for intensity of colonization in root fragments and root system were not significant (Table 2). This suggests that *Iris sibirica* with simple flowers presented a higher number of colonization sites in the root system possibly due to a higher susceptibility compared to the genotype with double flowers, but at the colonization sites the distribution of mycorrhizal structures inside cortex tissue was similar for the two genotypes.

Table 2. Combined effect of experimental factors on arbuscular mycorrhiza arbuscular mycorrhiza (AM) frequency (F%) and intensity (m%, M%) of colonization for *Iris sibirica* (2019, Cluj-Napoca)

Experimental factors		Colonization indicators \pm SE		
Genotype	Month	F%	m%	M%
Simple flower	V	87.78 \pm 4.84 a	7.72 \pm 2.52 b	6.59 \pm 1.82 ab
	VII	80.00 \pm 0.00 ab	4.64 \pm 1.09 bc	3.69 \pm 0.89 b
	IX	85.55 \pm 4.01 a	6.46 \pm 1.61 b	5.45 \pm 1.26 ab
Double flower	V	30.00 \pm 3.33 d	1.40 \pm 0.23 c	0.43 \pm 0.11 c
	VII	57.78 \pm 4.84 c	13.38 \pm 0.73 a	7.80 \pm 1.08 a
	IX	68.89 \pm 2.94 bc	6.20 \pm 1.02 b	4.24 \pm 0.62 b
Simple flower	Overall 2019	84.44 \pm 2.15	6.28 \pm 1.02	5.24 \pm 0.81
Double flower	Overall 2019	52.22 \pm 6.09	6.99 \pm 1.78	4.16 \pm 1.12

Note: Different letters between values denote significant differences (Duncan test, $p < 0.05$)

The seasonal pattern of colonization was different between the two genotypes:

- *Iris sibirica* genotype with simple flowers presented highest AM frequency and intensity in May, values decreased in July and increased again in autumn (Table 2).

- *Iris sibirica* with double flowers presented lowest frequency of colonization in spring. Frequency increased in July and reached highest level in September. However, intensity of colonization although was also lowest in spring, it reached the highest levels in July, not in autumn (Table 2).

AM colonization morphology in both *Iris sibirica* genotypes corresponded to *Paris*-type (Figure 4).

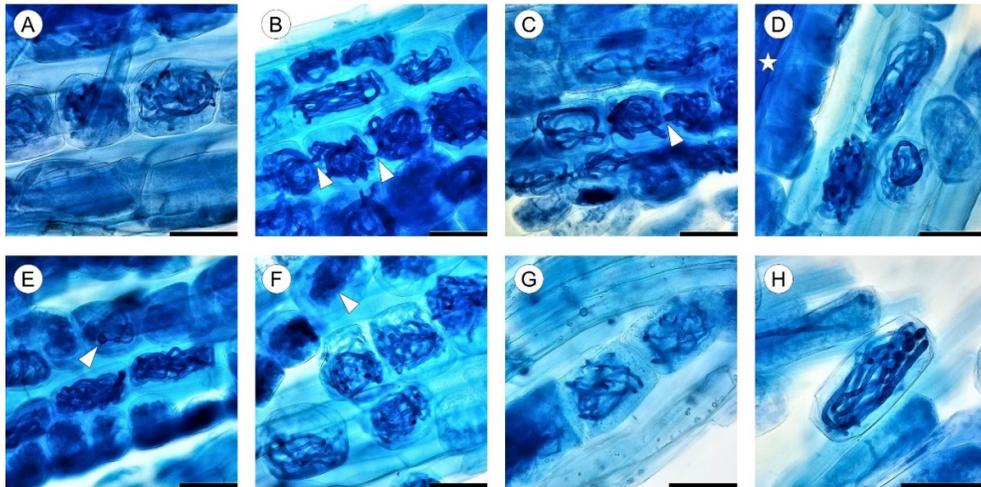


Figure 4. Arbuscular mycorrhiza morphology in *Iris sibirica* stained roots: (4A-H) typical *Paris* morphotype displaying intra-cellular hyphae coils, scale bar=50 μm ; (4B, 4C) arrows - hyphae passing point from one cell to another; (4D) star - localization of vascular bundle in root; (4E) arrow - swelling of intracellular hyphae as incipient stage of spore or vesicle formation; (4F) arrow - digested endomycorrhiza structures; (4A-F, 4H) *I. sibirica* with double flowers ('Concord Crush'), (4G) *I. sibirica* with simple flowers

Discussion

The mycorrhiza pattern observed can be associated with phenology and with corresponding plant metabolic state. Thus, *Iris sibirica* with double flowers presented a gradual increase of colonization frequency towards autumn because it is a rebloomer and in a milder climate it would produce flowers again in autumn. Thus, higher frequency of colonization in September could signify increased nutrient requirements. By comparison, *Iris sibirica* with simple flowers followed the typical spring geophyte phenology (as of the wild *I. sibirica*) by entering summer resting at post-anthesis, fact also reflected by decreased frequency of colonization over the warm months accompanied by yellowing of the very tip of the leaves.

Based on these results, it can be deduced that firstly there might be a relationship between the metabolic activity of the plant and the level of root colonization. This would further infer that plant has a role in regulating the functionality of an established symbiotic mechanism according to nutritional requirements, while fungi might also respond to increased or decreased carbon flux in the plant. Despite the altered phenology through plant breeding of rebloomers a synchronization between AM frequency and metabolic state of the plant can be observed just as in the case of the genotype displaying wild traits.

Similar AM *Paris*-morphotype identified in this study for *Iris sibirica* was reported before in *Iris stolonifera* (Zubek *et al.*, 2011) and *Iris germanica* (Crişan *et al.*, 2019). This morphotype is characterized by spreading of hyphae from cell to cell, without development between cortical cells (Dickson, 2004). This type has been the least studied and therefore less is known about nutrient transfer between symbionts under *Paris* morphotype compared to *Arum*-type, with some interesting possibilities proposed recently (Giesemann *et al.*, 2019). The morphotype is controlled mainly by plant genotype, particularly restrictiveness of spaces between cells and to some extent by fungi identity as well (Dickson, 2004; Dickson *et al.*, 2007). Adaptation of the host plant to specific environments might have played an evolutive role in the development of *Paris* morphotype by certain groups of plants (Giesemann *et al.*, 2019). Spreading of mycorrhiza inside roots in the case of *Paris*

morphotype is slow compared to *Arum* type (Cavagnaro *et al.*, 2008; Giesemann *et al.*, 2019), and this might have an influence on the temporal dynamic of the colonization.

The synchronization between colonization dynamic and phenology observed in *Iris sibirica* from this study, could be explained at least in part by geophytic lifestyle of the host plant. Because arbuscular mycorrhizal fungi take up to 20% of assimilated C from the host plant (Konvalinková *et al.*, 2017), and since geophytes function under an efficient system of resource allocation by having to partition carbon fixed through photosynthetic activity between symbiont, host metabolic activity and storage organ, it was suggested that geophytes might be more sensitive to carbon expenditure (Crişan *et al.*, 2018). Below-ground C partitioning by the plant is expected to vary during growing season (Ven *et al.*, 2019), but the role played by AM in C allocation by the host according to phenophase could hold the keys to better understanding in-plant C dynamics, particularly in geophytes. And for this reason, it is proposed that geophytes might be suitable model plants for the study of these mechanisms.

Arbuscular mycorrhiza interaction was not generally considered during plant breeding programs (Jacott *et al.*, 2017). The fact that *Iris sibirica* genotype displaying wild traits had higher susceptibility to AM colonization compared to the new cultivar in this study is in accordance with studies on other plant species. Thus, a study has shown a decrease of mycorrhizal responsiveness in modern wheat cultivars compared to older ones (Zhu *et al.*, 2001). Also, domesticated legumes were shown to have fewer symbionts, including AM than wild ones (Liu *et al.*, 2020). These have prompted recent authors to stress the need to no longer ignore the relation between soil microbial players, such as arbuscular mycorrhiza and plants, in order to ensure that new created cultivars, or genotypes taken in cultivation are equipped to benefit from ecosystem services these microorganisms can provide (Hohmann and Messmer, 2017; Jacott *et al.*, 2017).

Conclusions

On average *Iris sibirica* displaying wild traits had higher frequency of AM colonization (84.44 ± 2.15) compared to the new cultivar with double flowers (52.22 ± 6.09). However, in both cases the pattern was synchronized with plant metabolic state respectively with phenophase.

We propose that mycorrhizal susceptibility to be taken into consideration in plant breeding programs. *Iris sibirica* developed *Paris*-morphotype of colonization, similar to *I. stolonifera* and *I. germanica* reported in literature. Considering this is the least studied AM morphotype the results bring a contribution to the understanding of colonization dynamics for *Paris*-type plants.

Authors' Contributions

Conceptualization: IC, Formal analysis: IC; Investigation: IC; Resources: AS; Supervision: AS; Writing - original draft: IC; Writing - review and editing: AS. Both authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Brundrett MC, Tedersoo L (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* 220:1108-1115. <https://doi.org/10.1111/nph.14976>
- Boltenkov E, Artyukova E, Kozyrenko M, Erst A, Trias-Blasi A (2020). *Iris sanguinea* is conspecific with *I. sibirica* (Iridaceae) according to morphology and plastid DNA sequence data. *PeerJ* 8:e10088. <https://doi.org/10.7717/peerj.10088>
- Cavagnaro T, Smith FA, Lorimer MF, Haskard KA, Ayling SM, Smith SE (2008). Quantitative development of *Paris*-type arbuscular mycorrhizas formed between *Asphodelus fistulosus* and *Glomus coronatum*. *New Phytologist* 149:105-113. <https://doi.org/10.1046/j.1469-8137.2001.00001.x>
- Cluj-Napoca climate database (2019a). Retrieved 2020 October 20 from <https://www.wunderground.com>
- Cluj-Napoca climate database (2019b). Retrieved 2020 October 20 from <https://en.tutiempo.net/climate>
- Crișan I, Vidican R, Stoian V, Stoie A (2017). Wild *Iris* spp. from Romanian meadows and their importance for ornamental plant breeding. *Romanian Journal of Grassland and Forage Crops* 16:21-32. <https://www.cabdirect.org/cabdirect/abstract/20183119145>
- Crișan I, Vidican R, Stoian V, Sandor M, Stoie A (2018). Arbuscular mycorrhizae of five summer geophytes from Cluj county. *Scientific Papers Agronomy Series* 61(1):61-66. <http://www.uaiasi.ro/revagrois/PDF/2018-1/paper/11.pdf>
- Crișan I, Vidican R, Olar L, Stoian V, Morea A, Ștefan R (2019). Screening for changes on *Iris germanica* L. rhizomes following inoculation with arbuscular mycorrhiza using Fourier transform infrared spectroscopy. *Agronomy* 9:815. <https://doi.org/10.3390/agronomy9120815>
- Dickson S, Smith FA, Smith SE (2007). Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza* 17:375-393. <https://doi.org/10.1007/s00572-007-0130-9>
- Dickson S (2004). The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytologist* 163:187-200. <https://doi.org/10.1111/j.1469-8137.2004.01095.x>
- Giesemann P, Rasmussen HN, Liebel H., Gebauer G (2019). Discreet heterotrophs: green plants that receive fungal carbon through *Paris*-type arbuscular mycorrhiza. *New Phytologist* 226:960-966. <https://doi.org/10.1111/nph.16367>
- Grey-Wilson C (1997). Series Sibericae. In: White B, Bowley M, Brearley C *et al.* (Eds). *A guide to species irises their identification and cultivation*. Cambridge University Press, Cambridge, United Kingdom, pp 142.
- Hodge A, Helgason T, Fitter AH (2010). Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* 3(4):267-273. <https://doi.org/10.1016/j.funeco.2010.02.002>
- Hohmann P, Messmer MM (2017). Breeding for mycorrhizal symbiosis: focus on disease resistance. *Euphytica* 213:113. <https://doi.org/10.1007/s10681-017-1900-x>
- Index Seminum (2020). Hortus Agro-Botanicus Napocensis, Academic Pres, pp 7-8.
- INRA (2001). Mycocalc software. National Institute for Agricultural Research Dijon. Retrieved 2020 October 22 from <https://www2.dijon.inrae.fr/mychintec/Mycocalc-prg/download.html>
- Jacott CN, Murray JD, Ridout CJ (2017). Trade-offs in arbuscular mycorrhizal symbiosis: disease resistance, growth responses and perspectives for crop breeding. *Agronomy* 7:75. <https://doi.org/10.3390/agronomy7040075>
- Kaššák P (2013). Screening of presence of the chosen anthocyanin colorants in the *Limniris* group Irises. Preliminary Communication of 8th Croatian & 8th International Symposium on Agriculture, Dubrovnik, Croatia, pp 383-387. http://sa.agr.hr/pdf/2013/sa2013_p0409.pdf
- Kaššák P, Kuli M (2014). Dyeing potential of the *Iris sibirica* L. flowers. 2nd Annual International Interdisciplinary Conference, Azores, Portugal. AIC Proceedings 2:372-380. <http://www.ejournal.org/index.php/esj/article/view/4163/3999>
- Kokkoris V, Hamel C, Hart MM (2019). Mycorrhizal response in crop versus wild plants. *PLoS One* 14(8): e0221037. <https://doi.org/10.1371/journal.pone.0221037>

- Konvalinková T, Püschel D, Řezáčová V, Gryndlerová H, Jansa J (2017). Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. *Plant and Soil* 419(1-2):319-333. <https://doi.org/10.1007/s11104-017-3350-6>
- Kovalev V, Mykhailenko O, Krechun A, Osolodchenko T (2017). Antimicrobial activity of extracts of *Iris hungarica* and *Iris sibirica*. *Annals of the Mechnikov Institute* 2:57-64.
- Liu A, Ku YS, Contador CA, Lam HM (2020). The Impacts of domestication and agricultural practices on legume nutrient acquisition through symbiosis with rhizobia and arbuscular mycorrhizal fungi. *Frontiers in Genetics* 11:583954. <https://doi.org/10.3389/fgene.2020.583954>
- NGA (2020). The National Gardening Association. Plant Data Base. Retrieved 2020 October 23 from <https://garden.org/plants/view/232413/Siberian-Iris-Iris-Concord-Crush/>
- Tawaraya K. (2003). Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition* 49(5):655-668. <https://doi.org/10.1080/00380768.2003.10410323>
- Tejeda A, Zurita F (2020). Capacity of two ornamental species (*Iris sibirica* and *Zantedeschia aethiopica*) to take up, translocate, and accumulate carbamazepine under hydroponic conditions. *Water* 12:1272. <https://doi.org/10.3390/w12051272>
- Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986). Estimation of vesicular arbuscular mycorrhizal infection levels. *Proceedings of the 1st European Symposium on Mycorrhizae*, Dijon, France.
- Ven A, Verlinden MS, Verbruggen E, Vicca S (2019). Experimental evidence that phosphorus fertilization and arbuscular mycorrhizal symbiosis can reduce the carbon cost of phosphorus uptake. *Functional Ecology* 33(11):2215-2225. <https://doi.org/10.1111/1365-2435.13452>
- Vierheilig H, Coughlan AP, Wyss URS, Piché Y (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64:5004-5007. <https://doi.org/10.1128/AEM.64.12.5004-5007.1998>
- Wang Y, Lv N, Mao X, Yan Z, Wang J, Tan W, Li X, Liu H, Wang L, Xi B (2018). Cadmium tolerance and accumulation characteristics of wetland emergent plants under hydroponic conditions. *RSC Advances* 8:33383-90. <https://doi.org/10.1039/c8ra04015j>
- Yang J, Qi Y, Li H, Xu G (2017). Comparison of nitrogen and phosphorus purification effects of different wetland plants on eutrophic water. *ICAEESEE IOP Conf. Series: Earth and Environmental Science* 113:012042. <https://iopscience.iop.org/article/10.1088/1755-1315/113/1/012042>
- Zhu Y-G, Smith SE, Barritt AR, Smith FA (2001). Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant and Soil* 237(2):249-255. <https://doi.org/10.1023/a:1013343811110>
- Zubek S, Nobis M, Błaszczkowski J, Mleczko P, Nowak A (2011). Fungal root endophyte associations of plants endemic to the Pamir Alay Mountains of Central Asia. *Symbiosis* 54:139-149. <https://doi.org/10.1007/s13199-011-0137-z>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Norulae Scientia Biologicae* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.