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Arbuscular mycorrhizal and dark septate endophyte associations of medicinal plants

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Abstract

Arbuscular mycorrhizal fungi (AMF) and dark septate endophyte (DSE) associations were studied in 36 medicinal plant species from 33 genera and 17 families, collected from the Botanical Garden of the Jagiellonian University in Kraków. Arbuscular mycorrhiza (AM) was found in 34 species (94%); 26 were of the *Arum*-type, 4 – *Paris* and 4 taxa revealed intermediate morphology. The abundance of AMF hyphae in roots varied with particular species, ranging from 2.5% (*Helianthus tuberosus*) to 77.9% (*Convallaria majalis*). The mycelium of DSE was observed in 13 plant species (36%), however, the percentage of root colonization by these fungi was low. Spores of 7 AMF species (Glomeromycota) were isolated from trap cultures established from rhizosphere soils of the investigated plants: *Archaeospora trappei* (Archaeosporaceae), *Glomus aureum*, *Glomus caledonium*, *Glomus claroideum*, *Glomus constrictum*, *Glomus mosseae*, *Glomus versiforme* (Glomeraceae). Our results are the first detailed report of root endophyte associations of the plant species under study. Moreover, the mycorrhizal status of 14 plant species is reported for the first time.

Keywords: arbuscular mycorrhiza (AM), AM morphology, *Arum*-type, dark septate endophytes (DSE), Glomeromycota, mycorrhizal status, *Paris*-type

Introduction

Over 35000 medicinal plant species are used in medicine in different regions of the world [1]. To meet the increasing demand of plant material used in herbal industry, cropping of numerous species is presently carried out. In order to develop effective methods of biomass production and obtain high quality plant material, recent research emphasis is on exploring soil microorganisms beneficial for plant performance. Among them the most important are arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). In several studies, AMF and DSE have been found to enhance plant growth, photosynthetic activity, phosphorus content, act antagonistically towards soilborne fungal pathogens, and modify the concentration of plant metabolites [2-17]. For this reason, the recognition of mycorrhizal status, monitoring of soil fungi and selection of beneficial microbial consortia to inoculate cultivated medicinal plants could be of particular value.

Poland is one of the leaders among European countries in the production of plant material for herbal industry [18-20]. Nevertheless, investigations on symbiotic microorganisms have been rarely conducted in Poland so far. First and, to our best knowledge, the only one study to date on the mycorrhizal status of medicinal plants conducted Zubek and Błaszkowski [21]. Furthermore, also few experimental studies concerning the influence of AMF on selected medicinal plant species have been carried out recently by Sawilska et al. [22], Jurkiewicz et al. [15], and Zubek et al. [16]. The aim of the present study was to examine the AMF diversity and the fungal root colonization of selected species, both introduced and native to the flora of Poland, from the collection of the Jagiellonian University Botanical Garden in Kraków. We focused on the evaluation of mycorrhizal status and the degree of AMF and DSE root colonization as well as the characterization of morphotypes of arbuscular mycorrhiza (AM) of these plant species. The research broadens the knowledge of ecology of the investigated plants.

Material and methods

Sample collection

The material was collected from the section of medicinal plants of the Jagiellonian University Botanical Garden in Kraków. The garden holds a collection of approximately 200 medicinal plant taxa (E. Nowotarska personal communication, 2010). The selection of plant species was done mainly on the basis of their presence in Polish Pharmacopoeia [23], the importance of species for herbal industry (based on the amount

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of plant raw material obtained in Poland for pharmaceutical purposes; [18]), and the legal status of taxa in Poland [24]. Moreover, the species of unknown mycorrhizal status and AM morphology were selected for investigations. Thirty six species were collected during the flowering and early seed formation period in June 2009 (see Tab. 1). In the case of each species, three samples were collected. Whole plants were excavated and cleaned mechanically from soil. Roots were washed in running tap water and then subjected to staining procedure. Rhizosphere soils were collected for the establishment of AMF trap cultures.

Root staining and the assessment of fungal colonization

Roots were prepared according to the modified Phillips and Hayman [25] method. Roots were cleared in 10% KOH for 24 h and then rinsed in tap water. The material was then acidified in 5% lactic acid in water (24 h), stained with 0.05% aniline blue in 80% lactic acid (48 h), and finally stored in 80% lactic acid until analyzed. The whole procedure was performed at room temperature (approx. 22°C). Root fragments (ca. 1 cm long; 15-30 fragments per sample) were mounted on slides in glycerol:lactic acid (5:1) and squashed using coverslides.

Fungal root colonization was assessed using Nikon Eclipse 80i light microscope with Nomarski interference contrast optics and a digital camera with a panel for image analysis. AMF colonization was identified on the basis of aseptate hyphae of irregular diameter, growing (***i***) intercellularly, forming arbuscules terminally in cortical cells (*Arum*-type of AM morphology), (***ii***) intracellularly with arbuscules developed on coils in cortical cells (*Paris*-type) or (***iii***) forming intermediate types [26-28]. Fine endophyte [usually considered *Glomus tenue* (Greenall) I.R. Hall [26,29]] AM-type colonization was counted separately from coarse AM-type colonization. The fine endophyte was identified on the basis of the following characteristics: mycelium ca. 1 µm in diameter, deep blue stained hyphae, the presence of small vesicles or swellings and fan-shaped branches [21,26,30,31]. The method proposed by Trouvelot et al. [32] was followed for the assessment of AM development. The parameters evaluated were: mycorrhizal frequency (*F*), relative mycorrhizal root length (*M*), and relative arbuscular richness (*A*). An estimate of mycorrhizal frequency (*F*%) is given as the ratio between root fragments colonized by AMF mycelium and the total number of root fragments analysed. The relative mycorrhizal root length (*M*%) is an estimate of the amount of root cortex that is mycorrhizal relative to the whole root system. Arbuscule abundance (*A*%) is an estimate of arbuscule richness in the whole root system [32]. DSE colonization was identified on the basis of regularly septate hyphae, usually dark pigmented, with facultatively occurring sclerotia [21,30,31,33,34]. In the case of DSE colonization, the frequency of DSE mycelium occurrence in roots (F_{DSE}%) was estimated as detailed above for AMF. Additionally, the frequency of occurrence of resting spores of fungi from the genus *Olpidium* (F_{Olp}%) was assessed [21,30].

Establishment of AMF trap cultures

Soil samples were excavated from the rhizosphere of all investigated plant species. In the case of each species, three subsamples were collected and mixed together. For the trap culture establishment, the soils with root fragments (ca. 100 g of fresh soil) were placed into 9 × 12.5 cm plastic pots (500 ml) containing autoclaved commercially available coarse-grained sand (grains 1.0-10.0 mm in diam. – 80.50%; grains 0.1-1.0 mm in diam. – 17.28%; grains <0.1 mm in diam. – 2.22%). *Plantago lanceolata* L. was used as host plant. On the whole, 36 trap cultures were established. The cultures are maintained under greenhouse conditions in the AMF collection of the West Pomeranian University of Technology, Szczecin.

AMF spores extraction and identification

Seven months after the establishment of trap cultures, AMF spores were extracted using wet sieving and decanting method [35]. Morphological properties of spores and their subcellular structures were determined in material mounted in a drop of polyvinyl alcohol/lactic acid/glycerol (PVLG) and in a mixture of PVLG/Melzer's reagent (4:1, v/v) on a slide [36]. Identification of AMF spores was performed using Olympus BX51 light microscope. Fungal species names were after Walker and Trappe [37]. The slides with isolated spores were deposited in the slide collection of the Department of Plant Protection, West Pomeranian University of Technology, Szczecin.

Determination of soil characteristics

For soil chemical analyses (Tab. 2), five soil samples excavated at the site were mixed and analysed as bulk sample. The total phosphorus content was determined in ammonium lactate extraction according to the Egner-Rim method, total nitrogen by the Kjeldahl method, and total carbon by the Tiurin method [38,39].

Results

AM status and morphology

Arbuscular mycorrhizae with arbuscules, which are the structural and functional criterion of the symbiosis, were found in 34 out of 36 investigated plant species (94%). AM structures were not found in the roots of *Glycine max* and *Veronica urticifolia*. The abundance of arbuscular mycorrhizal fungi (AMF) in roots varied with particular species, ranging from 2.5% (*Helianthus tuberosus*) to 77.9% (*Convallaria majalis*; Tab. 1). In roots of all mycorrhizal plants coarse AMF (hyphae diameter above 2 µm) dominated (Fig. 1). The fine AM endophyte (*Glomus tenue*; Fig. 1) was found sporadically in 2 plant species (5.9%) – *Pimpinella anisum* and *Verbena officinalis*, and was observed to form arbuscules only in *P. anisum* (Tab. 1).

The AM of 26 plant species was of the *Arum* morphology (Tab. 1). Hyphae were observed mainly in the intercellular spaces of root cortex, forming arbuscules terminally in cortical cells (Fig. 1). Four species were characterized by *Paris*-type colonization in which neighbouring cortical cells contained hyphal coils, without hyphae in the intercellular spaces (Fig. 1). The intermediate AM colonization was found in 4 plant species from Apiaceae family (Tab. 1).

DSE colonization

DSE were found in 13 plant species (36%; Tab. 1). The single hyphae, accompanied sporadically by sclerotia (Fig. 1), were found in rhizodermis and outer cortical cells. The mycelium was brownish or stained with aniline blue. Single DSE hyphae were also detected on the root surface. In the old roots of several species, which were not included in the assessments of AMF and DSE colonization, DSE mycelium was abundant.

Tab. 1 Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) associations of medicinal plant species collected from the Botanical Garden of the Jagiellonian University.

			AM		Coarse AMF[e]				Other endophytes[f]		
Family	Plant species[a]	Medicinal use[b]	literature status[c]	AM type[d]	*F*	*M*	*A*	F_V	F_{FE}	F_{DSE}	F_{Olp}
Acanthaceae	*Acanthus longifolius* Host non Poir. (= *A. mollis*)[1]		NS	A	97.8	51.9	36.6	14.1	-	-	-
Apiaceae	*Conium maculatum* L.[7]		1+	I3, I4	88.1	29.8	23.3	9.7	-	32.9	-
	Levisticum officinale W.D.J. Koch[15]	PP, 2	NS	I1, I3, I4	95.0	56.0	44.5	-	-	5.0	5.0
	Peucedanum ostruthium (L.) W.D.J. Koch[36]		1+	P	98.0	56.6	56.3	-	-	2.0	-
	Pimpinella anisum L.[19]	PP	NS	I3, I4	87.7	30.3	24.4	16.7	59.8	23.3	1.8
	Pimpinella major (L.) Huds.[20]		1+/–	I3, I4	89.3	17.8	21.1	-	-	10.7	24.1
Apocynaceae	*Vinca minor* L.[27]	p-prot.	1+	A	95.2	64.2	41.3	55.6	-	-	-
Asclepiadaceae	*Vincetoxicum hirundinaria* Medik.(= *V. officinale*)[35]		2+	A	68.4	12.5	9.0	-	-	-	-
Asteraceae	*Achillea millefolium* L. s.str.[2]	PP, 1	1+/–, 3+	A	71.0	37.3	23.2	2.1	-	-	31.8
	Arnica chamissonis Less.[6]		NS	A	88.0	61.3	46.1	4.8	-	-	-
	Grindelia robusta Nutt.[13]		NS	A	96.0	53.9	42.2	4.8	-	-	-
	Helianthus tuberosus L.[5]		NS	A	17.5	2.5	1.0	2.2	-	-	33.7
	Tanacetum parthenium (L.) Sch. Bip.[28]		1–	A	68.4	20.2	14.4	-	-	-	3.8
	Taraxacum officinale F.H. Wigg.[29]	1	1+	A	75.5	19.6	13.2	-	-	-	15.9
	Tussilago farfara L.[31]	1	1+/–	A	51.2	14.1	11.9	-	-	-	45.5
Boraginaceae	*Anchusa officinalis* L.[4]		NS	A	25	8.4	4.2	-	-	-	25.0
Convallariaceae	*Convallaria majalis* L.[8]	p-prot., 3	1+/–	A	95.4	77.9	69.4	65.1	-	-	-
Fabaceae	*Glycine max* (L.) Merr.[11]	PP	1+	NM	-	-	-	-	-	-	13.3
Lamiaceae	*Prunella vulgaris* L.[23]		1+/–, 3+	A	88.8	53.6	44.3	-	-	-	-
	Salvia sclarea L.[24]	PP	NS	A	69.6	19.2	18.6	2.8	-	-	10.3
	Salvia verticillata L.[25]		NS	A	63.8	14.1	9.3	2.2	-	-	2.2
	Teucrium botrys L.[10]		NS	A	97.6	58.7	47.1	4.7	-	6.8	22.6
	Teucrium chamaedrys L.[14]		1+	A	90.9	46.4	29.9	-	-	-	8.8
	Thymus serphyllum L. emend. Fr.[30]		1+	A	86.4	38.0	37.1	-	-	-	-
Linaceae	*Linum usitatissimum* L.[16]	PP	1+/–	A, A1	13.3	4.7	4.5	-	-	-	8.0
Papaveraceae	*Papaver rhoeas* L.[18]	PP	1+/–	P	74.4	57.8	57.8	-	-	-	23.3
Plantaginaceae	*Plantago major* L. s.str.[32]		1+	A	89.0	57.7	40.8	-	-	-	11.0
Primulaceae	*Primula veris* L. (= *P. officinalis*)[22]	PP, p-prot.	1+/–	P	33.2	16.5	16.0	-	-	2.4	-
Rosaceae	*Agrimonia eupatoria* L.[3]	PP	1+	A	87.5	43.7	29.5	27.8	-	47.7	-
	Potentilla erecta (L.) Raeusch.[21]	PP	1+/–	A	100	47.1	33.2	24.1	-	-	3.7
	Sanguisorba officinalis L. [26]	PP	1+	A	91.3	36.3	18.4	8.3	-	4.8	28.3
Scrophulariaceae	*Digitalis lutea* L.[9]		NS	A	94.5	41.0	37.4	-	-	18.1	32.0
	Gratiola officinalis L.[12]	prot.	NS	A	80.3	49.8	43.1	-	-	-	-
	Veronica urticifolia Jacq. (= *V. latifolia*)[34]		NS	NM	-	-	-	-	-	5.0	3.3
Solanaceae	*Physalis alkekengi* L.[17]		NS	P	92.4	71.4	70.4	42.9	-	18.3	22.0
Verbenaceae	*Verbena officinalis* L.[33]	PP	1+	A	100	72.2	38.5	16.8	3.5	5.5	32.8

[a] Plant species names according to Mirek et al. [60] except for *Arnica chamissonis* and *Salvia sclarea* which followed Anioł-Kwiatkowska [61]. Numbers after plant species names indicate collection sample number and trap culture number (see also Tab. 3). [b] Medicinal use and other information concerning plant species; PP indicates that the plant species is included in the 8th edition of Polish Pharmacopoeia [23]. Numbers (1-3) indicate the amount of plant raw material obtained per year in Poland for herbal industry (based on data from the years 1995-1999), after Jambor [18]. 1 – 50-100 tons; 2 – 10-50 tons; 3 – 5-10 tons. The legal status of the taxon in Poland after Piękoś-Mirkowa and Mirek [24]. prot. – protected plant species; p-prot. – species partially protected. [c] AM status according to available literature. The presented information is based mainly on the checklist by Wang and Qiu [40] and is updated with the data published thereafter: 1 – [40], 2 – [53], 3 – [62]. "+" – AM present; "–" – AM absent; NS – not surveyed. [d] AM status and morphotype (according to Dickson [27]) observed in this study. A – *Arum* type; A1 – *Arum* type with paired arbuscules in adjacent cells; P – *Paris* type; I – intermediate types: I1 – intercellular hyphae with terminally formed arbuscules in inner cortex and intracellular hyphae in outer cells; I3 – intracellular hyphae with arbuscules and intercellular hyphae; I4 – intracellular hyphal coils, intracellular arbusculate coils and intercellular hyphae. NM – nonmycorrhizal. [e] Coarse AMF; mycorrhizal parameters (%; mean, $n = 3$). *A* – relative arbuscular richness; *F* – mycorrhizal frequency; F_V – frequency of the occurrence of vesicles; *M* – relative mycorrhizal root length. [f] Frequency of the occurrence of other endophytes (%; mean, $n = 3$). F_{FE} – fine AM endophyte (*Glomus tenue*) mycelium; F_{DSE} – mycelium of dark septate endophytes; F_{Olp} – resting spores of *Olpidium* spp.

Other fungal endophytes

The resting spores of *Olpidium* spp. (Fig. 1), which were stained with aniline blue, were found in 23 plant species. The frequency of occurrence of these structures varied from 1.8 to 45.5% (Tab. 1). The percentage of root infection was low (data not shown); the single resting spores were found in rhizodermis and outer cortex.

AMF diversity

Spores of 7 AMF species from 2 families were isolated from the 36 trap cultures established from the rhizosphere soils collected in the garden: *Archaeospora trappei* (R. N. Ames & Linderman) J. B. Morton & D. Redecker emend. Spain (Archaeosporaceae), *Glomus aureum* Oehl & Sieverd., *Glomus caledonium* (T. H. Nicolson & Gerd.) Trappe & Gerd., *Glomus claroideum* N. C. Schenck & S. M. Sm., *Glomus constrictum* Trappe, *Glomus mosseae* (T. H. Nicolson & Gerd.) Gerd. & Trappe, and *Glomus versiforme* (P. Karsten) S. M. Berch. (Glomeraceae). AMF spores were found in all examined trap cultures. The fungi most frequently found were *G. claroideum* and *G. mosseae*, which were isolated from 27 and 24 cultures, respectively. These species usually sporulated abundantly in the cultures (Tab. 3). In contrast, *G. aureum* and *G. versiforme* were detected only in single cultures. Additionally, one unidentified *Glomus* species (spore morphotype) was isolated (Tab. 3).

Tab. 2 The chemical properties of soil collected from the section of medicinal plants of the Jagiellonian University Botanical Garden (see "Material and methods").

pH					Total content mg 100 g^{-1} of dry soil			
(H_2O)	N (%)	C (%)	Organic matter %	C/N	K_2O	P_2O_5	MgO	CaO
7.1	0.2	2.9	5.1	12.3	18.8	45.0	31.0	761.6

Discussion

In this paper, we present the detailed report of both AMF and DSE association of 34 medicinal plant species. The mycorrhizal status of 14 plant species is reported, to our best knowledge, for the first time (see Tab. 1). The presence of AM in 11 plant species was confirmed, consistent with previous literature data. Nine plant species were recognized in our studies as colonized by AMF, being reported earlier as either mycorrhizal or devoid of AMF. *Tanacetum parthenium* was found to form AM, whereas in earlier research was observed to be nonmycorrhizal [40]. Our investigations add data to the knowledge of mycorrhizal status and ecology of medicinal plant species. Furthermore, as it was recognized for rare and endangered plants, studies on mycorrhizal associations may also be important in the context of further investigations, regarding ecological restoration, preservation and propagation [30,41-45]. It could be crucial in the case of four species investigated in our research that are under legal protection in Poland (see Tab. 1). There is also the aforementioned possibility of AMF application in the cropping system of several investigated species in order to improve biomass production and the quality of plant material obtained for herbal industry [5,6,8,10,12,13,16,21,46]. In such attempts, basic studies, like a mycorrhizal status survey, are considered as a prerequisite for further investigations [21,46].

We found AMF spores in all trap cultures, even the ones established from the rhizosphere soils of *Glycine max* and *Veronica urticifolia*, which were found to be nonmycorrhizal in our studies. As AMF, in general, are believed to colonize roots of a wide range of plants [28], the lack of functional need for mycorrhizal association in these particular edaphic conditions seems to be the reason for the absence (*G. max* and *V. urticifolia*) or low (*Anchusa officinalis*, *Helianthus tuberosus*, *Linum usitatissimum*) AM colonization of these species rather than the absence of AMF propagules in the soil. Nevertheless, there is increasing evidence for some degree of physical and functional specificity in the symbiosis [28,47-49]. Therefore, if some AMF are required for particular plant species, the lack of compatibile fungal symbionts may be the reason for the lack or lower root colonization. Furthermore, *G. max* was found to be mycorrhizal in earlier studies [40], which seems to support

Tab. 3 Species of arbuscular mycorrhizal fungi (Glomeromycota) isolated from trap cultures established from rhizosphere soils of the investigated medicinal plant species.

	Frequency of spore occurrence in trap cultures[a]																																		
Fungal species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Archaeospora trappei													1										1		4											
Glomus aureum			1																																	
Glomus caledonium																							1												4	
Glomus claroideum	4	4	4	4	3	2		4	1	4		3		4		2	1		4		2	3	4	4	2	4	3		4	4	4	1		1		2
Glomus constrictum	1					1																														
Glomus mosseae		2	2				4	1	1		4		3		4	3		4		3	2	4				2	3	1	1	2	2	4	4	2	1	2
Glomus versiforme															1																					
Glomus sp. 178													2	1					1	2							1									

[a] Numbers from 1 to 36 indicate plant numbers and trap culture numbers (see Tab. 1) from which the fungal species were isolated. The numbers 1-4 indicate the frequency of spore occurrence of particular species in the trap cultures. 4 – spores most frequently isolated; 1 – spores least frequently isolated.

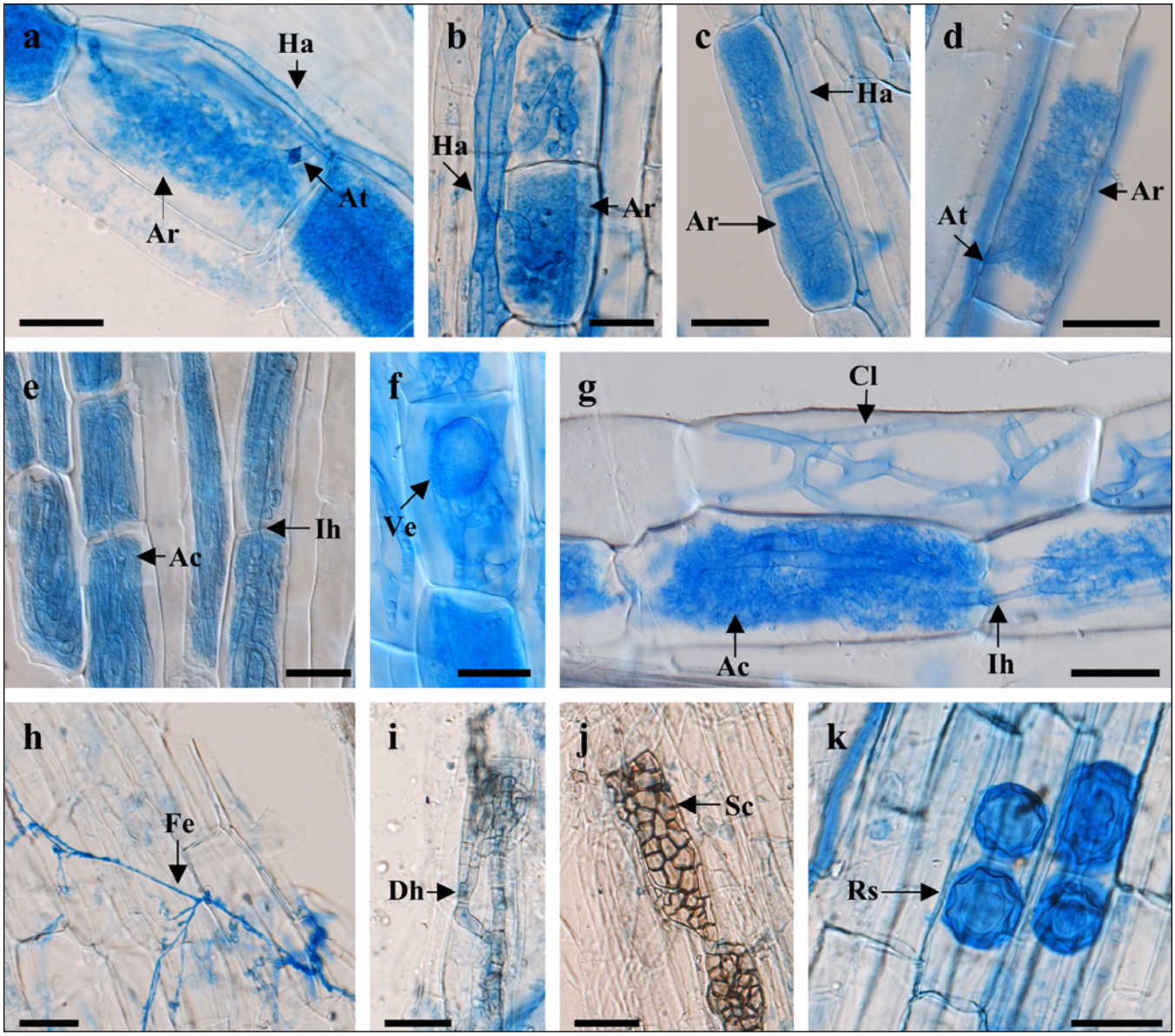

Fig. 1 **a-k** Endophytes in the roots of investigated medicinal plant species (Nomarski interference contrast optics). **a-d** Coarse AMF mycelium in the cortex of **a** *Arnica chamissonis*; **b** *Grindelia robusta*; **c** *Prunella vulgaris*; **d** *Gratiola officinalis* (*Arum*-type). **e-g** *Paris*-type of AM in **e** *Primula veris* and **f,g** *Physalis alkekengi*. **h** Fine endophyte (*Glomus tenue*) mycelium (*Arum*-type) in the root cortex of *Pimpinella anisum*. **i,j** DSE hyphae and sclerotium in the outer cortex of *Pimpinella anisum* root. **k** Resting spores of *Olpidium* sp. in the rhizodermis of *Helianthus tuberosus*. Ac – arbuscules formed on coils; Ar – terminally formed arbuscules; At – arbuscule trunks; Cl – coils; Dh – DSE hyphae; Fe – fine endophyte mycelium; Ha – hyphae growing intercellularly; Ih – coarse AMF hyphae growing intracellularly from cell to cell; Rs – resting spores; Sc – sclerotium; Ve – vesicle formed inside a cortical cell. Scale bars: 25 μm.

both of these possibilities.

It is widely recognized that plant species differ in the pattern of AMF root colonization. Host plants have main control over AM morphology, however, a fungal identity and different environmental factors may also have an impact on the structural classes of AM [27,28,50-53]. In our investigation, 26 plant species representing Acanthaceae, Apocynaceae, Asteraceae, Boraginaceae, Convallariaceae, Lamiaceae, Linaceae, Plantaginaceae, Rosaceae, and Scrophulariaceae showed *Arum*-type. The results are in accordance with other studies concerning AM morphology, where exclusively or mostly *Arum*-type was detected in these families. However, also *Paris* and intermediate patterns of AM colonization were observed in our survey. It confirms earlier observations where *Paris*, intermediate and *Arum* colonization was found among different species belonging to Apiaceae, Asclepiadaceae, Primulaceae, Solanaceae, and Verbenaceae [11,21,30,44,53,54].

In the case of *Achillea millefolium*, *Convallaria majalis*, *Plantago major*, *Primula veris*, *Taraxacum officinale*, *Vinca minor*, and *Vincetoxicum hirundinaria*, the morphotypes found in the present study were consistent with previous observations [53]. *Linum usitatissimum* was found to form typical and paired arbuscules of *Arum*-type, whereas previous investigations reported intermediate, *Paris* pattern or paired arbuscules of *Arum*-type in this species [53,55]. To our best knowledge, the AM morphology is characterized for the first time in the case of all other studied species and for Papaveraceae family.

Spores of 7 AMF species were identified in the trap cultures. Except for *G. aureum*, the other species have been frequently isolated from different soils and have a worldwide distribution [56]. Some of fungal isolates are maintained in the cultures and may serve as an inoculum source for the aforementioned

experiments concerning plant-fungus interactions. Moreover, one unidentified *Glomus* species (spore morphotype) was found in the cultures. However, on the basis of the material isolated so far, the exact identification was not possible. Extraction and identification of spores further multiplied in the trap cultures may enable taxonomical classification of this isolate.

Dark septate endophytes (DSE) were rarely found in roots of the investigated medicinal plant species. DSE represent a taxonomically and ecologically diverse group of fungi. Their interactions with plants range from parasitic to symbiotic, depending on fungal species, host plants and environmental conditions [33]. Some strains were found to be beneficial to plant performance [14,17,57-59]. For example, in the studies by Zijlstra et al. [58] on *Deschampsia flexuosa* L., three DSE isolates were reported to enhance the shoot nitrogen content of seedlings. DSE were also found to colonize roots of Chinese medicinal plant – *Saussurea involucrata* Kar. et Kir. ex Maxim. The authors inoculated *S. involucrata* and the species displayed enhanced performance when the individuals were colonized by DSE [14,17]. Wu and Guo [14] proposed a biotechnological application of DSE in the future cropping system of *S. involucrata*. Similarly to AMF, DSE may play an important role in improving plant performance, especially in the case of taxa which are rarely or not colonized by AMF. The mechanism of interaction between DSE and their hosts is complex, thus experimental research to reveal the impact of this group of fungi on the plants investigated in our studies is necessary.

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