ARBUSCULAR MYCORRHIZA OF ENDEMIC AND ENDANGERED PLANTS FROM THE TATRA MTS

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ABSTRACT

The mycorrhizal status of 24 plant species considered as endemic, endangered in Poland and included in the IUCN Red List of Threatened Plants is reported. Selected plants and rhizosphere soil samples were collected in the Tatra Mts (Western Carpathians). Individuals of seriously threatened taxa were obtained from seeds and inoculated with available AM fungal strains under laboratory conditions. AM colonisation was found in 16 plants; 9 species were of the *Arum*-type, 4 – *Paris* and 3 taxa revealed intermediate morphology. The mycelium of the fine endophyte (*Glomus tenue*) and dark septate fungi (DSE) were observed in the material collected in the field. 20 AMF species (Glomeromycota) found in the rhizosphere of the investigated plants were reported for the first time from the Tatra Mts. The results provide information that might be useful for conservation and restoration programmes of these species. Application of AMF in active plant protection projects is discussed.

KEY WORDS: arbuscular mycorrhiza (AM), *Arum*/*Paris*-type, DSE, endemic and endangered plants, conservation.

INTRODUCTION

A great climatic diversity, varied topography, geological formations and soils resulted in the exeptional richness of the Tatras flora. The massif is an endemism center in Europe and the only locality for many plant species (Mirek 1996; Piękoś-Mirkowa et al. 1996). A wide range of natural processes and human activity have had a strong impact on the stability of this high-mountain ecosystem, leading to the destruction of plants' habitats, plant endangerment or even extinction. The preservation of rare and endangered species is the main goal of numerous plant protection projects and is considered as an obligation for a number of countries bound by international agreements (Kaźmierczakowa and Zarzycki 2001). There is an urgent need for interdisciplinary studies on plants of special concern to develop effective methods of their maintenance and propagation. As part of these efforts to better understand the biology and ecology of these species and to improve success of plant conservation actions, we examined the mycorrhizal status of endemics, taxa endangered in Poland and included in the IUCN Red List of Threatened Plants. We also isolated and identified AMF species occurring in the rhizosphere of the investigated taxa. Such research is considered a pre-requisite for making further active plant conservation projects successful (Turnau and Haselwandter 2002; Fuchs and Haselwandter 2004).

Most valuable taxa occurring in the Tatra Mts have been investigated mainly on floristic and phytosociological aspects (Piękoś-Mirkowa and Kaczmarczyk 1990a, b; Piękoś-Mirkowa et al. 1996). However, almost no mycorrhizal investigations have been conducted. First studies concerning mycorrhizae of plants from this mountain range had been carried out in the 1950s by a group of Polish mycologists (Nespiak 1953; Dominik and Nespiak 1954; Dominik et al. 1954a, b; Dominik and Pachlewski 1956). These studies, however, did not include most species presently considered as rare, endemic and threatened. Apart from these investigations, only selected Tatra species cultivated ex situ in the Mountain Botanic Garden in Zakopane have been surveyed (Zubek et al. 2005). Except for the above mentioned studies conducted over 50 years ago, no mycorrhizal assessment of the Tatra endemic and endangered plants have been carried out in the field.

MATERIALS AND METHODS

Site description and field sampling

The material was collected from selected locations in the Polish Tatra Mts (Western Carpathians). The sampling sites and the number of plant specimens were strictly determined in order not to cause additional threat to the taxa. The special permission for rare and endangered plant species collection was obtained from the authorities of the Tatra National Park (TPN). On the whole, 17 taxa were collected in the field during the flowering and early seed formation period (June and July in the 2003). Root systems with soil were carefully excavated intact and transported in plastic bags to the laboratory. Site numbers, plant species collected and the locations of sampling are as follows: **1.** *Cardaminopsis neglecta*, above Czarny Staw Gąsienicowy lake, 1730 m a.s.l., 49°13'33''N, 20°01'36''E; **2.** *Cortusa matthioli*, Dolina Małej Łąki valley, 990 m a.s.l., 49°16'20''N, 19°54'09''E; **3.** *Leucanthemum waldsteinii*, Dolina Ku Dziurze valley, 1100 m a.s.l., 49°16'09''N, 19°56'38''E; **4.** *Melampyrum herbichii*, Dolina Ku Dziurze valley, 1100 m a.s.l., 49°16'09''N, 19°56'38''E; **5.** *Ranunculus pseudomontanus*, above Czarny Staw Gąsienicowy lake, 1630 m a.s.l., 49°13'42''N, 20°01'25''E; **6.** *Knautia kitaibeli*, Wąwóz Kraków ravine, 1115 m a.s.l., 49°14'13''N, 19°52'28''E; **7.** *Knautia kitaibeli*, Wąwóz Kraków ravine, 1115 m a.s.l., 49°14'17''N, 19°52'12''E; **8.** *Delphinium oxysepalum*, Dolina Mułowa valley, 1830 m a.s.l., 49°14'19''N, 19°54'14''E; **9.** *Soldanella carpatica*, *Saxifraga wahlenbergii*, Dolina Litworowa valley, 1845 m a.s.l., 49°14'19''N, 19°54'57''E; **10.** *Soldanella carpatica*, *Saxifraga wahlenbergii*, Mała Dolinka valley, 1275 m a.s.l., 49°15'19''N, 19°56'00''E; **11.** *Sesleria tatrae*, Piec rock in Czerwone Wierchy massif, 1485 m a.s.l., 49°14'53''N, 19°53'21''E; **12.** *Campanula polymorpha*, *Cerastium tatrae*, Ciemniak Mt., 1950 m a.s.l., 49°13'57''N, 19°54'00''E; **13.** *Dianthus plumarius* subsp. *praecox*, *Thymus pulcherrimus*, *Euphrasia tatrae*, Sarnia Skała rock, 1300 m a.s.l., 49°15'12''N, 19°56'30''E.

Plant inoculation under laboratory conditions

Field collection of 7 plant species were impossible due to the rarity of the taxa. Hence the mycorrhizal status of *Artemisia eriantha*, *Astragalus penduliflorus*, *Papaver burseri*, *Pulsatilla slavica*, *Saussurea pygmaea*, *Senecio umbrosus* and *Sibbaldia procumbens* have been surveyed under laboratory conditions. Seeds of these plants were provided by the Mountain Botanic Garden of the Polish Academy of Sciences in Zakopane or collected in the Tatras. The seedlings were obtained on Petri dishes according to available protocols (Piękoś-Mirkowa and Kaczmarczyk 1990a, b). The 4-6 day-old seedlings of each taxa were put in two pots (700 ml) on sterile substrata (A, B) that were inoculated. The substrata, whose characteristics are reported in Table 1, were provided by the Botanic Garden of the Jagiellonian University. *S. pygmaea* seedlings were cultivated on the substratum B, the other species on the substratum A (with an addition of ground limestone). The mixture of available AMF species was used as inoculum; *Glomus claroideum* BEG96, *Glomus constrictum* 262-5 (6) (the collection of C. Walker), *Glomus geosporum* 25-4 (the collection of C. Walker), *Glomus intraradices* E-1-99, BIORIZE Sarl France, *Glomus mosseae* BEG12, *Glomus mosseae* BEG. The cultures were grown for two months under greenhouse conditions and then roots were collected and stained in the same way as the material collected in the field.

Mycorrhizal status assessment

In total, roots of 24 plant species were analysed. Only roots attached to the main root of the plants were used for mycorrhizal assessment (to avoid the possibility of collecting roots from another species). The roots were prepared according to the modified Phillips and Hayman (1970) method. After careful washing in running tap water, the roots were cleared in 10% KOH for 24 hours and subsequently rinsed in water. The material was acidified in 5% lactic acid in water for 24 h, then stained with 0.01% aniline blue in 80% lactic acid for 24 h, and finally stored in 80% lactic acid. The whole procedure was carried out at room temperature. Mycorrhizal colonisation analysis were conducted accorrding to Trouvelot method (Trouvelot et al. 1986). The morphology of AM colonisation, the presence of AM structures (arbuscules, vesicles, coils), and the occurrence of other root endophytes, such as dark septate fungi (DSE) and fungi from the genus *Olpidium* were assessed using the light microscope Nikon Eclipse 800 with Nomarski interference contrast optics. Roots were also analysed for the presence of the fine endophyte [*Glomus tenue* (Greenall) I.R. Hall; Thippayarugs et al. 1999; Dodd et al. 2000], as the fungus has been observed to dominate in some alpine habitats (Haselwandter and Read 1980).

AMF spores isolation and identification

Soil samples were excavated in 13 selected locations in the Tatra Mts, from which the plant material was obtained. Additional soil samples were collected from the natural stands of two seriously endangered plants of special concern, which were cultivated to assess their mycorrhizal status under laboratory conditions. In case of *Pulsatilla slavica*, soil samples were obtained from the immediate vicinity (within 20 cm) of the individuals occurring in a natural population. As *Senecio umbrosus* is considered extinct in the wild in Poland, sampling procedure was carried out in the stand where the species used to grow in the Tatras. The site numbers and the locations of sampling are as follows: **14.** *S. umbrosus*, Szeroki Żleb gully in Chochołowska valley, 1130 m a.s.l., 49°15'06''N, 19°48'12''E; **15.** *P. slavica*, Koryciska Wielkie ravine, 1130 m a.s.l., 49°16'12''N,

TABLE 1. The chemical properties of substrata used in the mycorrhizal status survey under laboratory conditions (see Materials and Methods).

Substrata	pH (H_2O)	N%	C%	Organic matter %	C/N	Contents total in mg 100 g^{-1} of dry soil				Exchangeable cations in mg 100 g^{-1} of dry soil			
						K_2O	P_2O_5	MgO	CaO	K	Na	Mg	Ca
A	6.79	0.43	5.53	9.88	13.40	70.40	12.20	>20.00	960.00	70.00	4.80	16.75	690.00
B	6.78	0.18	4.79	7.70	24.50	6.60	1.00	18.00	490.00	5.00	2.40	15.75	350.00

19°48'27''E. In total, 15 trap cultures were established with the soil samples collected from the rhizosphere of the investigated plants. The soil with root fragments were placed in pots (500 ml) containing sterile substratum (sand: expanded clay 2:1, v/v). The cultures were kept in Sigma sunbags (B7026) under greenhouse conditions using *Plantago lanceolata* L. as a host plant (approximately 20 seedlings per pot). After six months spores were isolated using the wet filtering technique (mesh size 50 µm). 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 (Omar et al. 1979). Slides with isolated spores were deposited in the slide collection of the Department of Plant Pathology, University of Agriculture, Szczecin. Cultures of selected AMF strains are kept under greenhouse conditions in the collections of the Jagiellonian University in Kraków and the University of Agriculture in Szczecin.

RESULTS

AM status and morphology

Arbuscular mycorrhizae with arbuscules, which are the structural and functional criterion of the symbiosis, were observed in 16 of the 24 species examined (67%) (Table 2). In roots of these plants coarse AMF (mycelium diameter above 2 µm) dominated. The fine endophyte, usually considered *Glomus tenue*, characterised by mycelium ca. 1 µm diameter, abundant small vesicles or swellings of a diameter vary-

TABLE 2. Mycorrhizal status of endemic and threatened plants of the Tatra Mts.

Family	Plant species [a]	Category of threat [b]	AM literature status [c]	Number of plants analysed	AM type [d]	AM structures [e]			Other endophytes [f]	
						C	V	FE	DSE	Olp.
Asteraceae	* *Artemisia eriantha* Ten.	LR	3-	5	A	–	+	×	×	×
	Leucanthemum waldsteinii (Sch. Bip.) Pouzar	P-se	2+, 3+	5	A	+	+	+	+	–
	* *Saussurea pygmaea* (Jacq.) Spreng.	CR	2+	10	A	–	–	×	×	×
	* *Senecio umbrosus* Waldst. et Kit.	EW	NS	10	A	–	+	×	×	×
Brassicaceae	*Cardaminopsis neglecta* (Schult.) Hayek	P-se	2-	3	–	–	–	–	–	–
	Dentaria glandulosa Waldst. & Kit.	P-se	3-	5	–	–	–	–	–	–
Campanulaceae	*Campanula polymorpha* Witasek	P-se	3+	8	A	+	+	+	+	–
Caryophyllaceae	*Cerastium tatrae* Borbás	W-e	3-	9	–	–	–	–	+	–
	Dianthus plumarius L. subsp. *praecox* (Kit.) Pawł.	W-e, prot.	2-,3-	5	–	–	–	–	+	–
Dipsacaceae	*Knautia kitaibelii* (Schult.) Borbás	W-se	2+	3	A	+	+	–	+	+
Fabaceae	* *Astragalus penduliflorus* Lam.	CR	NS	10	A	–	–	×	×	×
Lamiaceae	*Thymus pulcherrimus* Schur	P-e	3+	9	A	+	+	+	+	–
Papaveraceae	* *Papaver burseri* Crantz	T-se	2-	15	–	–	–	×	×	×
Poaceae	*Festuca versicolor* Tausch subsp. *versicolor*	P-se	2+	11	P	+	+	+	+	+
	Sesleria tatrae (Degen) Deyl	W-e	3+	10	P	+	+	–	+	–
Primulaceae	*Cortusa matthioli* L.	LR, prot.	2+	5	P	+	–	–	+	–
	Soldanella carpatica Vierh.	W-e	2+, 3+	5	P	+	–	+	+	–
Ranunculaceae	*Delphinium oxysepalum* Borbás & Pax	W-e	NS	3	I	+	+	–	–	–
	* *Pulsatilla slavica* G. Reuss	VU, IUCN, W-e, prot., CEWH	NS	10	I	–	–	×	×	×
	Ranunculus pseudomontanus Schur	P-se	2+	5	I	+	+	+	+	–
Rosaceae	* *Sibbaldia procumbens* L.	VU	1-, 4+	10	A	–	+	×	×	×
Saxifragaceae	*Saxifraga wahlenbergii* Ball	W-e	NS	6	–	–	–	–	–	–
Scrophulariaceae	*Euphrasia tatrae* Wettst.	P-se	NS	6	–	–	–	–	+	–
	Melampyrum herbichii Woł.	P-se	NS	5	–	–	–	–	+	–

[a] Names of plants after Mirek Z., Piękoś-Mirkowa H., Zając A., Zając M. (2002) Flowering plants and pteridophytes of Poland. A Checklist. Asterix (*) indicates that the mycorrhizal status of species was analysed under laboratory conditions.

[b] Category of threat to the taxon in Poland and on a global scale after Kaźmierczakowa R., Zarzycki K. (2001) Polish Red Data Book of Plants: LR – lower risk, CR – critically endangered, EW – extinct in the wild, VU – vulnerable, IUCN – species included in the world list of threatened plant species, CEWH – protection provided by the *Convention on the Conservation of European Wildlife and Natural Habitats*. The legal status of the taxon in Poland after Piękoś-Mirkowa H., Mirek Z. (2003) Flora Polski. Atlas roślin chronionych: prot. – protected plant species. Endemism of the species after Piękoś-Mirkowa H., Mirek Z., Miechówka A. (1996) Pol. Bot. Stud. 12: 1-107: P-e – Pan-Carpathian endemic species, W-e – West-Carpathian endemic species, P-se – Pan-Carpathian subendemic species, W-se – West-Carpathian subendemic species, T-se – Tatra subendemic species.

[c] AM status according to available literature: 1 – Harley J.L., Harley E.L. (1987a) N. Phytol. (Suppl.) 105: 1-102, Harley J.L., Harley E.L. (1987b) N. Phytol. 107: 741-749; 2 – Nespiak A. (1953) Acta Soc. Bot. Pol. 22: 97-125; Dominik T., Nespiak A. (1954) Acta. Soc. Bot. Pol. 22: 753-769; Dominik T., Nespiak A., Pachlewski R. (1954a) Acta Soc. Bot. Pol. 23: 487-504; Dominik T., Nespiak A., Pachlewski R. (1954b) Acta Soc. Bot. Pol. 23: 471-485; Dominik T., Pachlewski R. (1956) Acta Soc. Bot. Pol. 25: 3-26; 3 – Zubek S., Turnau K., Błaszkowski J. (2005) Acta Mycol. 40 (1): 25-41; 4 – Wang B., Qiu Y.L. (2006) Mycorrhiza 16: 299-363; (+) AM present, (-) AM absent, NS – not surveyed.

[d] AM status and morphotype observed in this study: A – *Arum* type, P – *Paris* type, I – intermediate type.

[e] AM structures: C – coils, V – vesicles, FE – Fine endophyte mycelium; (+) present, (-) absent.

[f] Other endophytes: DSE – mycelium of dark septate endophytes; Olp. – resting spores of *Olpidium* spp.; (+) present, (-) absent, (×) – not surveyed because the plants were obtained from seeds under laboratory conditons.

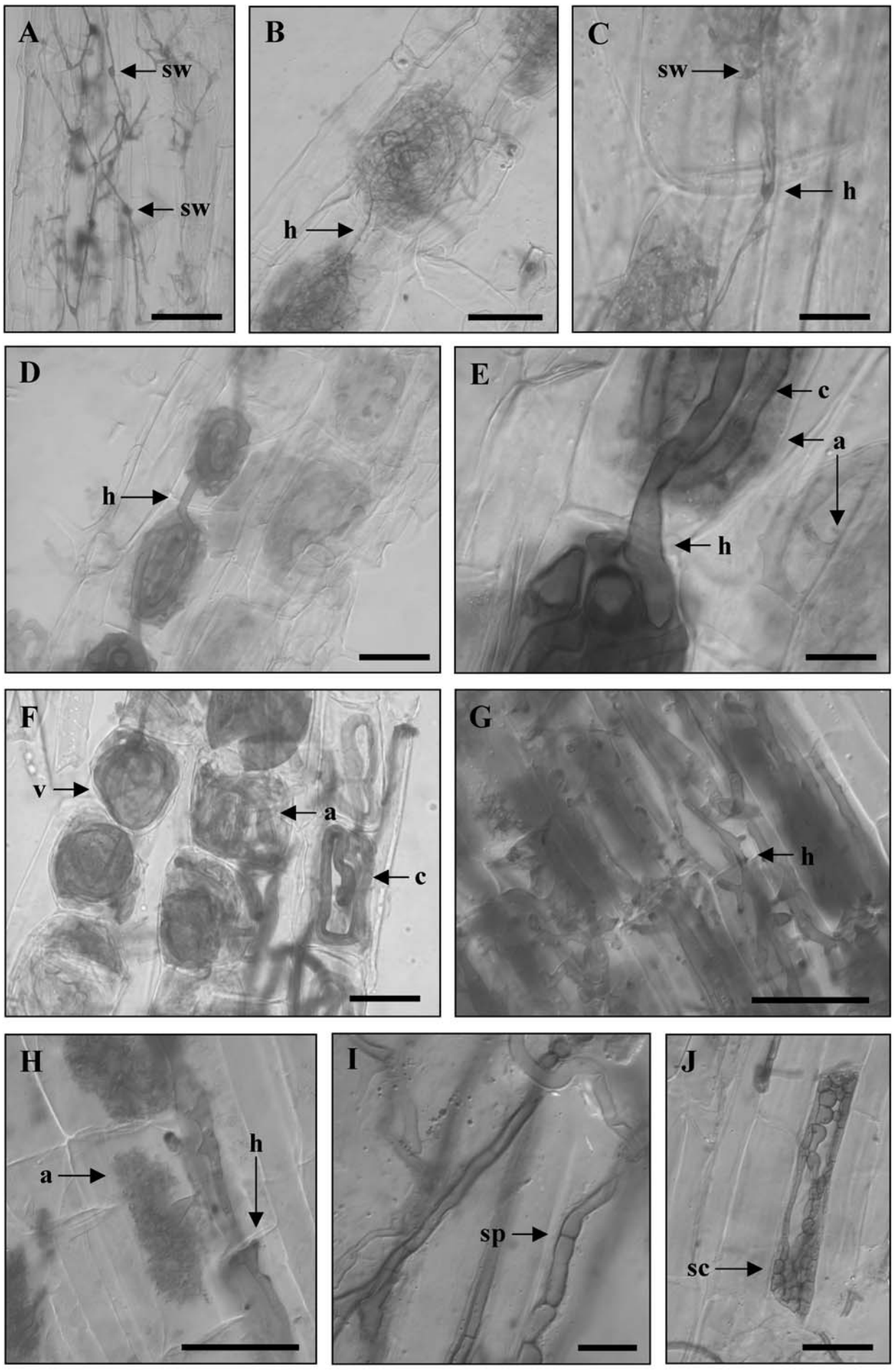
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TABLE 3. AMF species (Glomeromycota) associated with the investigated plants in the Tatra Mts.

Family	Fungal species [a]
Archaeosporaceae	*Archaeospora gerdemannii* (S.L. Rose, B.A. Daniels & Trappe) J.B. Morton & D. Redecker [1, 3, 4, 6, 12, 15]
	Arch. trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker [1, 15]
Acaulosporaceae	*Acaulospora bireticulata* F.M. Rothwell & Trappe [3, 6]
	Ac. capsicula Blaszk. [5, 9]
	Ac. gedanensis Blaszk. [3, 14]
	Ac. paulinae Blaszk. [1, 5]
	Ac. koskei Blaszk. [1, 3]
	Ac. mellea Spain & N.C. Schenck [14]
	Ac. scrobiculata Trappe [5]
	Entrophospora baltica Blaszk., Madej & Tadych [9]
Gigasporaceae	*Scutellospora dipurpurescens* J.B. Morton & Koske [2, 15]
Pacisporaceae	*Pacispora scintillans* (S.L. Rose & Trappe) Sieverd. & Oehl [12, 13]
Glomeraceae	*Glomus aggregatum* N.C. Schenck & S.M. Sm. emend. Koske [7]
	G. caledonium (Nicol. & Gerd.) Trappe & Gerd. [15]
	G. claroideum N.C. Schenck & S.M. Sm. [7-11, 14]
	G. constrictum Trappe [1-11, 13-15]
	G. deserticola Trappe, Bloss & J.A. Menge [6, 15]
	G. fasciculatum (Thaxt.) Gerd. & Trappe emend. C. Walker & Koske [6, 7, 13]
	G. geosporum (Nicol. & Gerd.) C. Walker [6]
	G. macrocarpum Tul. & C. Tul. [9, 15]

[a] Species names after Walker C., Trappe J.M. (1993) Mycol. Res. 97: 339-344. Numbers after species names indicate site numbers (see Materials and Methods) from which the soil samples were collected

ing from 3-9 µm and fan-shaped branches (Fig. 1A), was found sporadically in 5 plants and was rarely observed to form arbuscules in these plants. The exception was *Soldanella carpatica*. In the root systems of this species, *G. tenue* was the only endophyte from Glomeromycota, and formed *Paris*-type colonisation (Fig. 1B-C). No AMF structures were found in roots of eight species belonging to the Brassicaceae, Caryophyllaceae, Papaveraceae, Saxifragaceae and Scrophulariaceae families.

The examined plants from the Poaceae and Primulaceae families showed *Paris*-type colonisation in which neighbouring cortical cells contained hyphal coils without hyphae in the intercellular spaces (Fig. 1B-F). The intermediate AM colonisation was found among representatives of the Ranunculaceae. The fungi colonised roots by growing mainly intracellularly from cell to cell, but the arbuscules were formed terminally as in the *Arum*-type (Fig. 1G-H). Mycorrhizal plants of the families Asteraceae, Campanulaceae, Dipsacaceae, Fabaceae, Lamiaceae and Rosaceae were of the *Arum* morphology (Table 2). AMF hyphae were found mainly in the intercellular spaces of the root cortex, forming arbuscules terminally, one per cortical cell.

DSE

Dark septate endophytes (DSE) were found in 13 of the 17 species collected in the field (76%), however, the percentage of root colonisation was low. The regularly septated hyphae were accompanied sporadically by sclerotia (Fig. 1I-J). The mycelium stained with anilin blue or remained brownish. DSE were observed in the cortex together with AMF, and were also detected in the roots of non-mycorrhizal species from the Caryophyllaceae and Scrophulariaceae families (Table 2). In both cases, however, they were not abundantly developed.

AMF spores isolated from trap cultures

Spores of 20 species were extracted from the trap cultures established with the soils collected from the Tatra Mts (Table 3). AMF spores were found in all pots, indicating that the propagules of Glomeromycota were present in the rhizosphere of all examined plants, including those that were found to be non-mycorrhizal in this study. Spores of *Archaeospora gerdemannii*, *Glomus claroideum* and *G. constrictum* were most frequently isolated from the pot cultures. In contrast, *Acaulospora mellea, Ac. scrobiculata, Entrophospora baltica, G. aggregatum*, *G. caledonium* and *G. geosporum* were detected in single cultures.

DISCUSSION

The use of soil microorganism consortia to support plant growth have been proposed in agriculture (Vestberg et al. 2002), horticulture (Hamel 1996), and in the restoration strategies of destroyed habitats (Leyval et al. 2002; González-Chávez et al. 2006; Turnau et al. 2006). The application of rhizosphere microbiota, especially arbuscular mycorrhizal fungi (AMF), in the conservation programmes

Fig. 1. A-J – Light micrographs of endophytes observed in the roots of rare and endangered plant species of the Tatra Mts.
A – Fine endophyte (*Glomus tenue*) mycelium in the cortex of *Leucanthemum waldsteinii*; sw – swollen hyphae, scale bar – 50 µm.
B-C – *G. tenue* colonisation of *Paris*-type in the cortex cells of *Soldanella carpatica*; sw – swollen hypha, h – hyphae growing intracellularly from cell to cell, bars: B – 50 µm, C – 40 µm.
D-E – Coarse AMF colonising *Cortusa matthioli* root (*Paris*-type); h – hyphae growing intracellularly, a – arbuscules formed on the coils (c), bars: D – 50 µm, E – 25 µm.
F – *Paris*-type of AM infection in the cortex of *Festuca versicolor* subsp. *versicolor*; v – vesicle, a – arbuscule, c – coil, bar – 50 µm.
G-H – *Pulsatilla slavica* – AM colonisation of intermediate type; intracellular growth of hyphae (h) and terminally formed arbuscule (a), bars – 50 µm.
I-J – Septate hypha (sp) and sclerotium (sc) of DSE in the cortex of *Knautia kitaibelii*, bars: I – 15 µm, J – 50 µm.

of threatened taxa has been also highlighted (Gemma et al. 2002; Turnau and Haselwandter 2002; Fisher and Jayachandran 2005). Some research clearly showed that AMF enhance nutrient uptake and growth of endangered plants (Barroetavena et al. 1998; Fisher and Jayachandran 2002; Panvar and Vyas 2002). Both in maintaining species composition and reintroduction of rare and threatened taxa, knowledge of plant interactions below ground is a necessity (Eriksen et al. 2002). In our research we recognised the mycorrhizal status of 24 plant species of a high priority, i.e. considered as endemic, listed on the Polish Red Data Book of Plants, endangered on the global scale, and protected in Poland. The present studies provide important basic information concerning the mycorrhization of valuable taxa. They enable the selection of plant species for which the use of AMF might be possible in restoration attempts. However, this research must be followed up by investigating plant-fungus interactions and possible dependency. Only on the basis of detailed research, successful methods of AMF application in conservation programmes can be developed (Fisher and Jayachandran 2005).

Two main structural classes of AM symbiosis, the *Arum*- and *Paris*-type (Smith and Read 1997), were observed in roots of the investigated plants. An intermediate type was also detected among representatives of the Ranunculaceae family. It is well recognised that the AM morphology may depend both on the plant and fungal identity (Cavagnaro et al. 2001; Dickson 2004; Kubota et al. 2005). Nevertheless, some recent research indicates that the issue of AMF colonisation patterns is more complex. It has been suggested that e.g. the ecology of the plant and environmental factors may have an impact on AM morphology (Yamato 2004; Ahulu et al. 2005). Recent studies have also revealed several exceptions to the main morphological types, such as the occurrence of paired arbuscules in roots of *Linum usitatissimum* L. (Dickson et al. 2003) or atypical *Paris* morphology in the Mediterranean species *Similax aspera* L. (Bedini et al. 2000). Numerous intermediate structural classes of AM symbiosis have been described as well (Dickson 2004; Yamato 2004; Ahulu et al. 2005). This suggests that studies on AM morphology of species from different genera and families are needed to analyse mechanisms involved in forming particular AM colonisation types. Moreover, Smith and Smith (1997) suggested that differences in the fungal pattern of root colonisation may influence physiology of the symbiosis. Hence the information of AM morphology seems to be useful for further research concerning practical application of AMF in active plant conservation projects.

The mycelium of fine endophyte (*Glomus tenue*) was detected in roots of 6 plant species collected from the Tatras. However, the root length occupied by this fungus was low. Only in roots of *Soldanella carpatica*, where coarse AMF were not observed, the mycelium was abundant. This suggests that *G. tenue* may be the main root coloniser only when there is no competition with other Glomeromycota species. Its role might be important at higher altitudes, where coarse AMF are not present. The fungus has commonly been observed in the Alps, and has been shown to become dominant above 3000 m above sea level (Read and Haselwandter 1981).

20 AMF species revealed in the rhizosphere of rare and endangered plants collected from 15 locations of the Tatra Mts were recorded for the first time in this region. Functioning soil microbiota, including AMF, is considered crucial for successful revegetation attempts (Haselwandter 1997; Turnau and Haselwandter 2002). Hence the knowledge of AM status and AM fungi occurring in rhizosphere is necessary to prepare a soil environment as natural as possible for plants to be established in introduction or reintroduction projects. AMF strains from the habitats of endangered taxa maintained in trap cultures can serve as a ready-to-use inoculum source for restoration programmes (Turnau and Haselwandter 2002; Fuchs and Haselwandter 2004).

Dark septate endophytes (DSE) are root colonisers coappearing with mycorrhizal fungi in a variety of ecosystems, especially in arctic and alpine habitats (Haselwandter and Read 1980; Read and Haselwandter 1981; Jumponen 2001). They were also found in roots of several plants surveyed in this study. However, the percentage of root length occupied by DSE mycelium was low. On the basis of our observations it is impossible to conclude on the influence of this group of endophytes on plants. In order to clarify the degree of benefit that these plant species may derive from DSE, experimental research is needed. Nevertheless, since some strains of these endophytes have been shown to form mutualistic associations (Haselwandter and Read 1982), and therefore in some cases classified as mycorrhizal (Jumponen 2001), they should not be neglected in ecological studies. Similarly to AM fungi, they may strongly improve active protection projects of rare and threatened taxa. The application of DSE could be important in case of plants which are not colonised by AMF, e.g. species from the Caryophyllaceae and Scrophulariaceae examined in this study.

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