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Mycorrhizal status of several Quercus species in Romania (*Quercus cerris, Q. frainetto, Q. robur*) and the optimization perspective of growth conditions for in vitro propagated plants transplanted in the field

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Abstract. There is an increasing interest for important tree species conservation in the context of climate change, anthropogenic pressure and invasion of alien tree species. A key factor in the survival of trees is represented by the mycorrhizal association. The success of micropropagated trees also depends on the acquisition of mycorrhizal mutualists. Ectomycorrhizal roots samples from several *Quercus* species (*Q. cerris, Q. frainetto, Q. robur*) were examined for mycorrhizal morphotypes' characterization. The samples were collected during the vegetation season from stands located in Southern and North-Western Romania. 30 morphotypes of active mycorrhizae were identified with *Cenococcum geophilum* Fr. (*Ascomycota*) as dominating morphotype. Previous studies on somatic embryogenesis in *Q. robur* and *Q. frainetto* demonstrated the utility of in vitro techniques in obtaining plants from these recalcitrant seed producing species, considered at risk in various areas of the country, due to increasingly stressful conditions. The success rate of the acclimatization process depends on the mycorrhization performed either artificially, in the laboratory, either naturally, in the field. *Ex situ* mycorrhization solutions are considered as less costly, yet efficient alternative to improve the ex vitro survival of micropropagated plants or endangered tree species or for those with economic importance, in vitro propagation is an important conservation tool combined with the acquisition of appropriate mycorrhizal mutualists.
Keywords ectomycorrhizae, morphotypes, *Quercus robur, Quercus cerris, Quercus frainetto.*

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Introduction

Quercus species form ectomycorrhizas with several fungal partners. The actual associations vary in time and space, according to tree and stand age, to successional moment and environmental factors. The diversity of the fungal partners is shedding light on biocomplexity - properties emerging from the interplay of behavioral, biological, chemical, physical and social interactions that affect, sustain or are modified (Michener et al. 2001) by tree-fungi associations. Mycorrhizal association is to be considered the elementary functional unit of the forest ecosystem and one of the problems at work in the frame of biocomplexity namely, the interaction roles of mycorrhizal fungi, plants and soil resources in carbon and nutrient transfer (NSF-Biocomplexity 1999). Also, mycorrhizae have been recognized as ecosystem level functional systems, involved in nutrient turn-over, producing a profound alteration of root chemistry, architecture and biology of the organisms living in the rhizoplane (Langley & Hungate 2003).

From theoretical point of view, the diversity of mycorrhizal morphotypes raises questions on the niche partition, community assemblage and rarity vs. commonness of particular morphotypes. From a practical point of view, the selection of a particular morphotype, in order to enhance the acclimatization success of micropropagated plants, is a major goal in obtaining quickly and efficiently tree seedlings by combining *in vitro* micropropagation and mycorrhization. Forests are nitrogen limited, most of this element is organically bounded and mycorrhizal fungi get access to it (Bonfante 2003).

One of the possible explanations on the diversity of the morphotypes is the insurance hypothesis (Yachi & Loreau 1999). The hypothesis is based on the intuitive idea that increasing biodiversity insures the ecosystems against declines and their functioning caused by environmental fluctuations. Also, mycorrhizal associations are diffuse and non-specific in the most associations, multi-host fungi dominating temperate terrestrial ecosystems (Selosse et al. 2006). Playing a central role in tree nutrition and connectivity, mycorrhizal fungi are to be considered keystone species in forest ecosystems.

The microenvironment of roots, especially of the assimilative roots, is highly heterogenous, shaping the interaction with different mycobionts.

Both assimilative roots and mycelia are of modular nature. Their relative autonomy is a partial explanation for the multiple mycobiotic mutualists, relating to the same phytobiont namely the tree. Modularity has evolved to explore and exploit a patchy environment, fungal mycelia being organized as networks and functioning whole organisms. One of the network properties is the possibility of reconfiguration to adapt to new conditions. Plants are connected by mycorrhizal networks.

The niche represented by the tree is partitioned among the mycorrhizal mycobionts and other organisms, mutualists as helper bacteria and an extended array of pathogens. Apparently, the fungal species are interchangeable according to the neutral community theory (Kelly et al. 2008).

There are several approaches in studying mycorrhizal diversity (Trudell, 2003): mushrooms surveys (the oldest method of investigation), morphotyping and the use of molecular markers, such as DNA fingerprinting. Mushroom surveys are still at use, although the list of mycobionts in a particular environment is incomplete, due to the rarity of most of mycorrhizal partners, due to unpredictable fruiting or due to the fact that many mycobionts do not produce fructifications. Morphotyping is based on the observed fact that different combinations of plants and fungi produce different looking ectomycorrhizas. A wide range of macroscopical and microscopical characteristics are used to describe morphotypes. Although by morphotyping actual identity of the

fungal partner is not always assigned, still it offers valuable information upon the actual active mycorrhizas is obtained. An almost complete record of the existing mycorrhizas in a particular site is produced including also the inactive mycobionts.

Facing the threat of extinction for many tree species of the world, we are challenged to find new breeding methods to preserve plant biodiversity. *In vitro* micropropagation is one of alternatives. Maximum benefits can be obtained combining the micropropagation with mycorrhization with an appropriate fungal partner on appropriate substrate (Azcon-Aguilar & Barea 1997). Mycorrhization of *in vitro* propagated plantlets has a positive impact on them in terms of post-transplanting performance (Rai 2001).

The aim of the present study was to: (i) assess mycorrhizal diversity in terms of morphotypes under different conditions (Southern, Western and North-Western Romania) in several *Quercus* species (*Quercus cerris* L., *Q. frainetto* Ten., *Q. robur* L.), (ii) find the similarities in mycorrhizal status (morphotypes) of trees vegetating under different site and geographical conditions, (iii) identify the most frequently found morphotype in order to recommend it for the the mycorrhization of microprapagated plantlets of *Q, robur* and *Q. frainetto.*

Methods

Site locations. Locations of root samples were selected in various forest stands from different forest districts in Southern, Western and North Western Romania (Table 1).

Forest stands are dissimilar with respect to site conditions and stressful factors. For instance, Western and North-Western sites (Dobreşti, Tinca, Oradea, Radna, Buteni) locations are assigned to hilly forest types, natural as well as plantations, exception being Tinca, situated in the high Miersig plain. The forest stands investigated in Southern part (Giurgiu, Comana, Ştefăneşti, Vlăsia) are assigned to broadleaved mixed forests, dominated by *Q. cerris* and *Q. frainetto*, occasionally with *Q. robur* (Ştefăneşti). Sampling was performed during growing seasons, in the period 1996-2001.

The climate of North-Western and Western Romania is of a particular type, displaying sub-Mediterranean and Atlantic influences,

Table 1 Site locations

County	Forest District	Longitude	Latitude
Ilfov	Giurgiu	43° 56' 07.50" N	25° 56' 15.88" E
	Brăneşti	44° 42' 52.54" N	26° 21' 07.36" E
	Vlăsia	44° 42' 29.47" N	26° 00' 14.42" E
	Ştefăneşti*	44° 20' 10.75" N	25° 10' 38.92" E
	Vlădicească	44° 39' 47.65" N	26° 06' 48.25" E
	Băneasa	44° 31' 52.92" N	26° 00' 48.00" E
	Snagov	44° 42' 52.54" N	26° 08' 58.26" E
Călăraşi	Bărăganu	44° 25' 30.68" N	27° 36' 08.10" E
Bihor	Dobreşti	46° 50' 50.42" N	22° 08' 42.89" E
	Oradea	47° 06' 11.71" N	21° 55' 25.94" E
	Tinca	46° 46' 33.15" N	21° 55' 25.02" E
Arad	Radna	46° 07' 02.98" N	21° 40' 37.75" E
	Buteni	46° 21' 46.20" N	22° 08' 42.89" E
Giurgiu	Mihai Bravu	44° 39' 47.65" N	26° 04' 02.44" E
	Comana	44° 09' 47.50" N	26° 08' 28.42" E
Teleorman	Slăveşti	44° 20' 10.75" N	25° 10' 38.92" E

Table 2 Mycorrhizal morphotypes identified in *Quercus robur, Q. cerris* and *Q. frainetto* in forest stands from southern and north-western Romania

Code	Host	Location	Color	Branching	Length of mycorrizal system (mm)	Distance between consecu-tive branches (mm)	Mantle, color, texture	Form of the apex, diameter (mm)	Rhizomorphs and emergent hyphae	Texture of the rhizomorphs and emergent hyphae
M1	*Quercus cerris*	Ştefănăneşti 08.1997	Black-brown	Sympodial, bent and pinnate	1.8-9 constricted at base	0.2-0.4	Wooly, white	M1	*Quercus cerris*	smooth ramified
M2	*Quercus cerris Quercus robur*	Slăveşti 08.1997 Buteni Oradea. et. al.	Yellow- whitish, with rusty spots and visible brown cell contour	Monopodial, loose	4-5 brown and constricted base	0.4	Smooth with rare emergent hyphae, greasy with cystids	M2	*Quercus cerris Quercus robur*	smooth
M3	*Quercus frainetto*	Slăveşti 08.1997	orange	Sympodial pinnate with curved apexes, loose, clavate branches	3.5	0.4-0.5	Smooth, with hyaline rhizomorphs	M3	*Quercus frainetto*	smooth
M4	*Quercus cerris*	Vlăsia 08. 1996	Dull brown, later, deep brown with a purple tinge	Pinnate, compact, with bent apexes	2	0.2	Wooly, white with silvery areas	M4	*Quercus cerris*	smooth
M5	*Quercus cerris*	Vlădiceasca 08. 1996	Orange-yellowish with rusty spots, loose	Dichotomous, uneven	3	0.8-1	Wooly, white, abundant	M5	*Quercus cerris*	smooth
M6	*Quercus cerris*	Ştefăneşti 08.1998	Yellow, with orange-brown base	Repeatedly dichotomous, with bent apexes	3	0.3-0.4	Shinning white, dense wooly texture	M6	*Quercus cerris*	smooth
M7	*Quercus cerris*	Băneasa 06.1996	Black-brown, greasy with cystids	Not branched	0.5-3		rough	M7	*Quercus cerris*	smooth
M8	*Quercus frainetto*	Vlădiceasca 06.1996	White-yellowish	Coralloid, loose, palmetti type	4	0.3-1.5	White, conspicuous. Radiating hyphae with brownish tinge under the mantle	M8	*Quercus frainetto*	smooth ramified
M9	*Quercus frainetto*	Comana., 09.1996	Yellow-orange to dull brown	Sympodial, pinnate, loose with elongated axe	5-6	0.7-1	Cottony, silvery-white	Round 0.4	White rhizomorphs	Hairy, branched, emerging at right angles

Table 2 (continuation)

Code	Host	Location	Color	Branching	Length of mycorrizal system (mm)	Distance between consecu-tive branches (mm)	Mantle, color, texture	Form of the apex, diameter (mm)	Rhizomorphs and emergent hyphae	Texture of the rhizomorphs and emergent hyphae
M10	*Quercus cerris*	Vlăsia 10.1996	Amber yellow with brown spots on apexes	Monopodial, bent apexes, bumpy	3	0.1-0.2	smooth	Round 0.3-0.4	none	none
M11	*Quercus cerris*	Tinca 04. 1997	Dirty white	Monopodial, loose, rare	4	0.3	Wooly, white with silvery areas	Round 0-7-1	Long emergent hyphae	smooth
M12	*Quercus cerris*	Tinca 04. 1997	Yellow-brownish with branch base and apexes brown	Sympodial uneven, loose (up to 80 branches/ system)	8-10	0.5-1	Velvety, hyaline	Round or acute 0.5-1	none	none
M13	*Quercus cerris* and *Quercus frainetto*	Tinca 04. 1997	Shinning black with reddish tinge, constricted at base	Pinnate, Sympodial, loose	3-11	1	Smooth, shinning with greasy shine	Clavate, discolored 0.3-3	none	none
M14	*Quercus cerris*	Tinca 10. 1997 Brăneşti 06.1997	Dirty white	Monopodial, loose, pedicelated, bent apexes	2.5	0.1-0.3	Shinning white, wooly, dense	Clavate 0.3	none	none
M15	*Quercus cerris*	Snagov 06.1997	White-yellowish or pink	Pinnate, sympodial, bent apexes	2.2-4.2	0.3	Shinning white, short wooly, dense	Round or clavate 0.3-0.4	Fan ramified white rhizomorphs	smooth
M16	*Quercus cerris Quercus robur*	Dobreşti 07.1998, Buteni 06.2000	Chocolate brown with discolored apexes, bent	Sympodial, uneven or coralloid,	3	0.3-0.5	Rough, greasy	Round and digitiform at the beginning 0.4-0.6	Brown rhizomorphs emerging near the apex	smooth
M17	*Quercus cerris*	Dobreşti 07.1998	Orange to brown, discolored apex, older parts brown, basal constriction	Sympodial, pinnate, dense	5	0.4	Smooth with rough areas or velvety	Round or clavate 0.3-0.4	Fan shaped brown rhizomorphs emerging from base to apex	smooth

Table 2 (continuation)

Code	Host	Location	Color	Branching	Length of mycorrizal system (mm)	Distance between consecu-tive branches (mm)	Mantle, color, texture	Form of the apex, diameter (mm)	Rhizomorphs and emergent hyphae	Texture of the rhizomorphs and emergent hyphae
M18	*Quercus cerris* + *Russula virescens*	Mihai Bravu 08.1997	Sulfur yellow	Sympodial, pinnate, sinuous apexes	2	0.2	Smooth with sulfur yellow granulations	Round 0.3-0.4	Emerging hyphae, right angles	smooth
M19	*Quercus cerris*	Dobreşti 07.1998	Black-purplish to jet black	Monopodial	3	0.1-0.3	velvety	Round 0.4-0.6	Brown-black rhizomorphs	reticulated and smooth
M20	*Quercus frainetto*	Tinca 09.1998	Rusty-black with a pinkish tinge	Sympodial, basal constriction	11	0.5-1	Rough without conspicuous hyphae	Round to clavate 0.3	none	none
M21	*Quercus cerris, Querus robur*	Dobreşti 07. 1998 Buteni 06.2000	Yellow. Orange	Coralloid	6	0.2-0.3 or less	Velvety, shinning white, relatively rare	Round or relatively acute 0.2-0.4	White, shinning, smooth	smooth
M22	*Quercus cerris* + *Russula atropurpurea*	Giurgiu, Mihai Bravu 08. 1997	Pale yellow with rusty spots and granulation dissolving in KOH 10%	Monopodial, pinnate, loose, sinuous branches	19-29	1.2-2	Smooth with granular areas	Round 0.3-0.4	none	none
M23	*Quercus frainetto*+ *Xerocomus chrysenteron*	Giurgiu, Mihai Bravu 08. 1997	yellow	Monopodial, pinnate, loose	1-4	0.4	Wooly, abundant, dense	Round some with rusty spots 0.3-0.4	Ramified rhizomorphs, interconnected	hairy surface
M24	*Quercus cerris*+ *Lactarius quietus*	Bărăganu 08.1997	Orange to brown with white laticiferous hyphae	Coralloid or pinnate	5	0.5-0.8	Smooth, rare emerging hyphae (also laticiferous hyphae)	Round 0.3-0.6	Orange rhizomorphs	smooth and branched
M25	*Quercus cerris* + *Scleroderma citrinum*	Bărăganu 08.1997	Blackish brown with white tinge	Monopodial, pinnate, sinuous apexes	1.5-2.2	0.5	White-yellowish with silvery areas, wooly	Round 0.2-0.3	Rare emergent hyphae	smooth

Table 2 (continuation)

Code	Host	Location	Color	Branching	Length of mycorrizal system (mm)	Distance between consecu-tive branches (mm)	Mantle, color, texture	Form of the apex, diameter (mm)	Rhizomorphs and emergent hyphae	Texture of the rhizomorphs and emergent hyphae
M26	*Quercus frainetto*	Radna 10.1998	Yellow-dull brown	Repeatedly monopodial, pyramidal aspect, bent apexes	0.7-1	0.2-0.4	farinaceous	Round 0.3-0.4	Smooth, ramified rhizomorphs	smooth
M27	*Quercus cerris, Quercus robur*	Radna 10.1998, Oradea 2000	White-yellowish, near hyaline	Monopodial to coralloid, dense, sometimes solitary, basal constriction	0.8-1	0.3-1	Wooly, dense	Round 0.3-0.4	Fan shaped rhizomorphs	hairy
M28	*Quercus cerris* + *Amanita mairei, Quercus robur*	Dobreşti 07.1998 Buteni 06.2000 Oradea 06.2000	Yellow-orange	Dichotomous to coralloid	4-8	0.1-1	Velvety, rare, shinning white	Round, slightly bent 0.2-0.3	Rare rhizomorphs emerging at right angles	smooth
M29	*Quercus robur*	Buteni 06. 2000, Oradea 06.2000	White-yellowish with rusty areas	Pinnate pyramidal	4-6	0.2-0.4	Granular and spiky due to cystids yellowish	Round, hyaline 0.3-0.4	Bundles of short emerging hyphae	smooth
M 30	*All species* + *Cenococcum geophilum*	All locations	Jet black with sclerotia	Unbranched or Monopodial pinnate	1-2.5		Stringy and shiny due to emerging black hyphae, grainy	Round, in older parts, black	Black, rigid emanating hyphae Sclerotia present: black, spherical, grainy, 2 mm, hard and brittle, coated with soil particles	smooth

wetter and milder during the winter. In South, climate is temperate continental, with pronounced temperature extremes and prolonged drought during the summer months. Southern forest stands are located in plains, frequently exposed to summer drought (Giurgiu - Mihai Bravu, Brăneşti, Vlăsia - Buriaşu, Experimental Station Ştefăneşti, Bărăgan, Vlădiceasca, Slăveşti, Snagov, Comana). Comana and Mihai Bravu are located an the Neajlov river delta. The stand age varied between 60 and 90 years.

Stressful conditions affecting the trees from different investigated locations were assigned to groups: (i) soil pollution from an oil extraction plant (Tinca), (ii) several years of recurring drought (Bărăgan), (iii) multiple sources of city pollution in the city of Oradea, (iv) chronic foliar disease produced by *Erysiphe alphitoides* (Griffon & Maubl.) U.Braun & S.Takam and infestations produced by gall insects-*Dryomyia circinans* (Giraud) (Dobreşti) and *Cynips quercus-folii* (Linnaeus) (Oradea), (v) defoliations produced mainly by *Lymantria dispar* (Linnaeus) (Tinca).

Root processing protocol. Mycorrhizal system is defined as a lateral ramification and all its tributary apices from a sustaining suberified root, total length varying between 1 and 5 cm. During the research mycorrhizal root systems were studied according to this definition, pieces of 10-20 mm being cut and investigated for alive and declining mycorrhizal apices.

Blocks of soil of 5 x 5 x 5 cm were excavated in the rhizosphere of selected tree host species (*Quercus* spp.), in the area of horizontal crown projection, three trees of the same species per stand. The blocks were wrapped

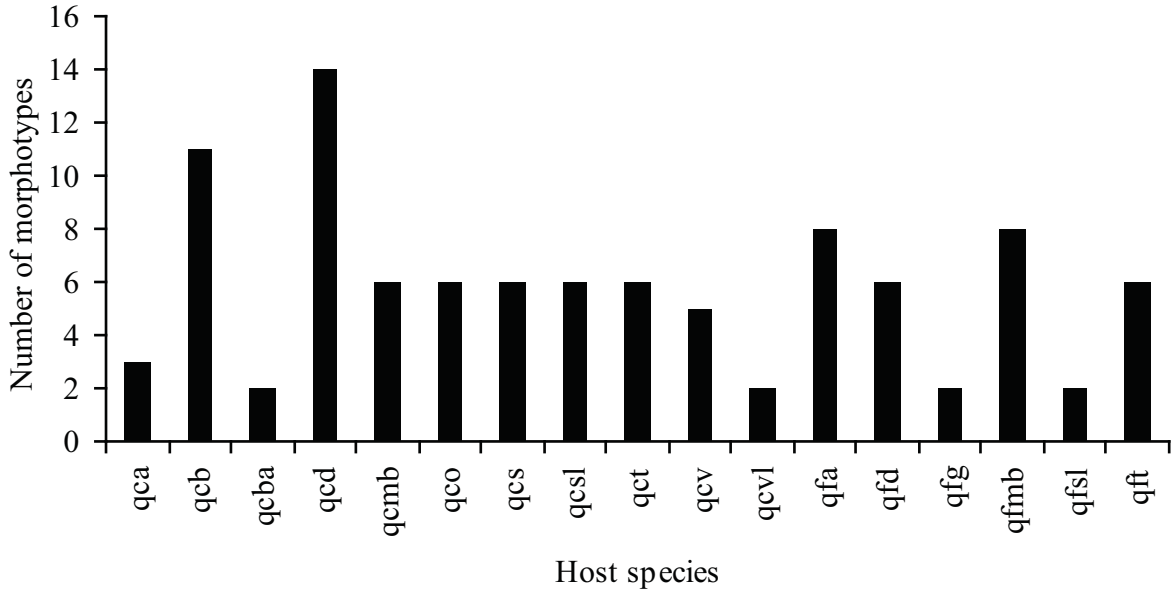

Figure 1 Number of mycorrhizal morphotypes by host (*Quercus cerris, Q. frainetto, Q. robur*) and location

Notation: qca - *Quercus cerris*, Arad; qcbr - *Quercus cerris*, Bărăgan; qcba - *Quercus cerris*, Băneasa; qcd - *Quercus cerris*, Dobreşti; qcmb - *Quercus cerris*, Mihai Bravu, qco - *Quercus cerris*, F.D. Oradea, qcsl - *Quercus cerris*, Slăveşti; qcs - *Quercus cerris*, Ştefăneşti; qct - *Quercus cerris*, Tinca; qcv - *Quercus cerris*, Vlădiceasca; qcvl - *Quercus cerris*, Vlăsia; qfa - *Quercus frainetto*, Arad (Radna); qfd- *Quercus frainetto*, Dobreşti; qfg - *Quercus frainetto*, F.D. Giurgiu (Comana); qfmb - *Quercus frainetto,* Mihai Bravu; qfsl - *Quercus frainetto,* Slăveşti; qft - *Quercus frainetto*, Tinca; qrb - *Quercus robur,* Buteni; qro - *Quercus robur,* city of Oradea; qcb - *Quercus cerris*, Buteni.

Table 3 Proportion of mycorrhizal apices in root samples (15 mycorrhizal systems selected at random) col lected during the vegetation season from several *Quercus* species, different locations (1996-2001)

Tree species, location and date	Proportion of mycorrhizal apices (%)
Q. cerris, Ştefăneşti (08.1996)	43.5
Q. cerris, Mihai Bravu (10.1996)	42.0
Q. cerris, Bărăganu (10.1996)	85.0
Q. frainetto, Comana (10.1996)	86.0
Q. cerris, Tinca (10.1997)	20.0
Q. frainetto, Radna (10.1998)	30.0
Q. frainetto, Tinca (09.1998)	32.4
Q. cerris, Tinca (09.1998)	31.4
Q. frainetto, Mihai Bravu (10.1996)	46.0
Q. cerris, Dobreşti (07.1998)	46.3
Q. frainetto, Dobreşti (09.1998)	13.1
Q. cerris, Dobreşti (07.1998)	67.6
Q. cerris, Oradea (09.1998)	67.6
Q. robur, Buteni (07.2000)	78.0
Q. robur, city of Oradea (06. 2001)	33.0

in paper and brought to the laboratory. The samples, corresponding to one tree merged in one composite sample subjected to further processing. Roots were carefully washed in tap water using sieves, placed in Petri dishes of 9 cm and observed under stereomicroscope cleaning meanwhile the adhered soil with fine brushes and needles. The descriptions of the morphotypes are based on macroscopic characters following the adapted protocol after Agerer (1987-2002). Actual mycorrhizas were confirmed microscopically by the existence of the Hartig net and mycorrhizal mantle (Nylund et al., 1982).

Quantitative analysis. Comparison of the mycorrhizal status (relative frequencies of active mycorrhizal apices) in 5 root samples taken from *Quercus cerris* at Dobreşti was performed by means of One-Way ANOVA. A previous test of variance homogeneity (Bartlett) confirmed the lack of significant differences with respect to the variances among samples. Also Tukey test of pair-wise multiple comparisons was performed in order to detect any significant differences in mycorrhization frequency in the 5 samples set collected from *Quercus cerris* roots at Dobreşti. This location was selected to perform the specified statistical analyses due to the fact that it corresponds to the highest diversity of morphotypes found at any location, at a particular moment.

The association status of different morphotypes with *Coenococcum geophilum* Fr.*,* most frequently encountered morphotype, was assessed using Yule coefficient of association**.** Yule's Q coefficient of association is a symmetric measure, based on the difference between concordant (meaning both absences *a*, and both presences, *d*) and discordant (meaning absence-presence data, *b* and presence-absence data, *c*) data pairs (Singh, 2004).

$$Q = (ad-bc)/(cd+bc)$$

The coefficient takes values between -1 and 1: -1 corresponds to a complete exclusion of the species, 0 corresponds to random associations and 1 to constant associations. *Q* statistic defines null relationship as statistical independence.

Hierarchical cluster analysis of tree species and locations, with regard to mycorrhizal morphotypes, was performed after the calculation of the Sørensen similarity index. The similar-

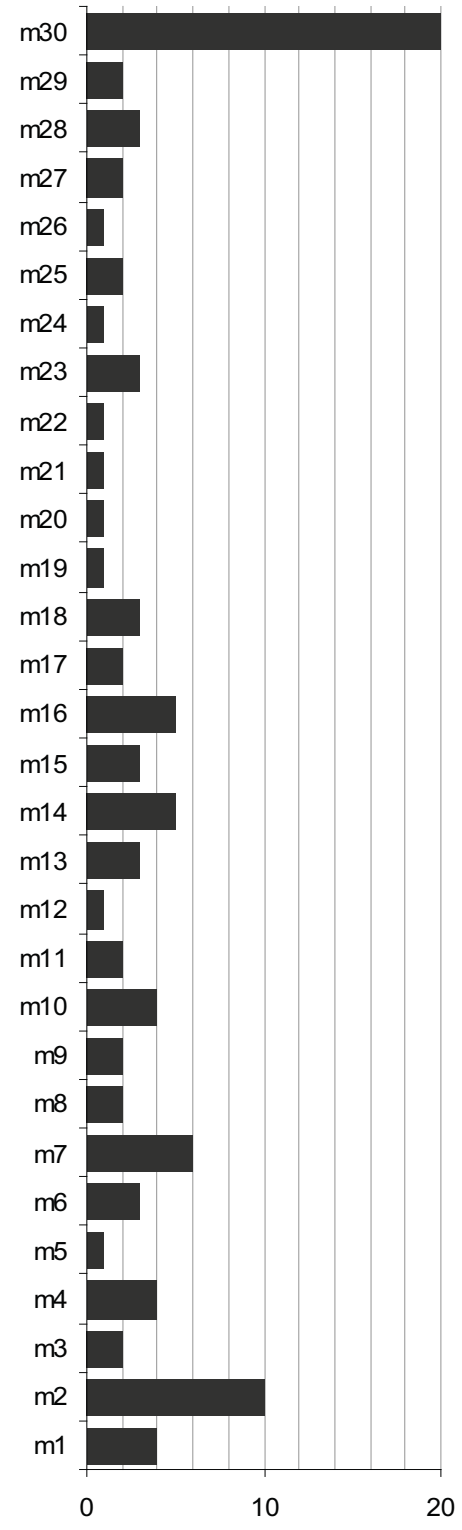

Figure 2 Frequencies of mycorrhizal morphotypes in root samples of *Quecus cerris, Quercus frainetto* and *Quercus robur* in several locations in Southern and North-Western Romania

ity-dissimilarity matrix was used for clustering by means of group average method. Statistical tests were performed using KyPlot program.

Results

There were identified 30 morphotypes of ectomycorrhizae, corresponding to *Quercus robur, Quercus cerris* and *Quercus frainetto* in Southern, Western and North-Western locations, presented in Table 1. The original descriptions (with the exception of *Cenoccocum geophilum* described by Agerer in 1987), are presented in Table 2.

The investigation of the maximum number of morphotypes active a certain moment in the rhizosphere of the host is 14 (Fig. 1) which is the real instantaneous value of morphotype richness (*Q. cerris* at Dobreşti).

The described morphotypes include only 6 cases of identified mycobionts (*Amanita mairei* Foley*, Scleroderma citrinum* Pers.*, Russula atropurpurea* (Krombh.) Britzelm*, Lactarius quietus* (Fr.) Fr., *Russula virescens* (Schaeff.) Fr.*, Boletus chrysenteron* Bull.) based on mycelial continuity between mycorrhizal mantle and the mycelium at the base of the carpophores. Easily recognizable *Cenoccocum geophilum* Fr. is also included in morphotype descriptions. Mantles of mycorrhizas with species of *Lactarius* as mycobionts (as examined on cross sections of fine roots) exhibit characteristic laticifers and pigments which are also found in the structure of the sporocarps. Those of *Rusulla* spp. exhibit distinctive cystidia and sulfovaniline reactive cells (Kernaghan and Currah 1997).

The analysis of frequency distribution of the various morphotypes (Fig. 2) shows that the dominating type is *Cenococcum geophilum* (M30) that is present in all locations. Next most frequent morphotype is M2 described in *Q. robur* and *Q. cerris* at Buteni, Oradea and Slăveşti but found in almost all locations less frequently than *C.geophilum*.

Table 4 The Yule coefficient of association of *Cenococcum geophilum* with several other mycorrhizal mor photypes in *Quercus cerris*, Dobreşti

Association	Yule coefficient of association
C. geophilum + M10	-0.8004
C. geophilum + M17	-0.1287
C. geophilum + M8	0.2564
C. geophilum + M3	0.2467
C. geophilum + M11	-0.0943

C. geophilum associates either randomly with other morphotypes in the same mycorrhizal system (values close to 0), or excludes other morphotypes (value close to -1 in the association with M10) according to our results, a pattern consistent with its dominating position. It is also the most frequent and broad spectrum host associated mycorrhizal type as other authors report (Moser et al. 2005).

The comparison of the mycorrhizal status (relative frequencies of active mycorrhizal apices) among 5 samples of roots from *Quercus cerris* at Dobreşti by of one way ANOVA resulted in no significant differences at $p > 0.05$. Also Tukey test for pair-wise multiple comparisons didn't reveal any significant differences between samples.

Søresen similarity index (Table 4) calculated in order to compare locations and *Quercus* species (within site and between sites) show maximum values in the following cases: *Quercus frainetto* at Tinca with *Quercus cerris* at Oradea (0.72), *Quercus robur* at Buteni with *Quercus robur* in the city of Oradea (0.61), *Quercus cerris* and *Quercus frainetto* at Mihai Bravu (0.57).

The analysis of the dendrogram resulted from the ordination of Sørensen similarity matrix, comparing different sites and tree hosts with regard to identified morphotypes (Fig. 3), reflects the association between similar sites within same geographical and meso-climatic area. Within same location, different host species are highly similar with respect to their mycorrhizal associates, such as *Q. cerris* and *Q. frainetto* at Dobreşti or Tinca (South-West at Mihai Bravu).

A distinct cluster is formed by hosts located in forest stands from Southern Romania where these are vegetating in the plain and are exposed to temperate-continental climate with harsh winters and dry summers. Another distinct cluster is formed by hosts vegetating in stands from Mihai Bravu, in the Neajlov river delta, characterized by wetter soil conditions as compared to other Southern locations. However, *Q. frainetto* from Comana, a nearby location, to Mihai Bravu clusters together with other Southern locations characterized by drier soil conditions. North Western locations cluster, together presents same pattern of association with their mycobionts, including as location Tinca and Oradea. *Quercus robur* is highly dissimilar to other host species with regard to associated mycobionts and clusters separately. Recurrent associations, in this case of ectomycorrhizal mophotypes, correspond to fundamental properties of the interaction between species and their physical (site) and biotic environment (hosts) (Legendre & Legendre, 1998).

Previous studies (Timofte 2007) reported the possibility to obtain micropropagated plantlets of *Q. robur* and *Q. frainetto* by means of somatic embryogenesis initiated from acorns. The embryos can be converted to plantlets which, theoretically, can be outplanted.

Discussion

The energy flow through the mycorrhizal networks represents a major food chain in the forest trophic web. The network represented by

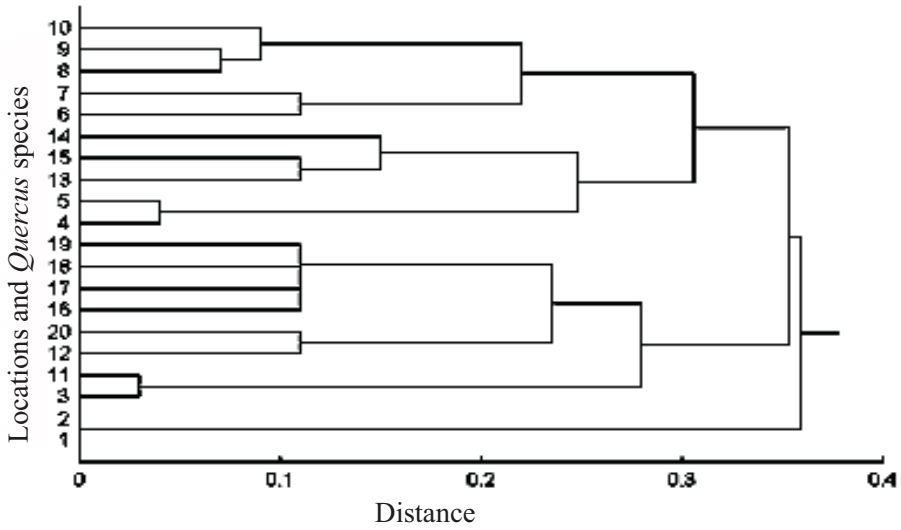

Figure 3 Cluster Analysis of mycorrhizal morphotypes associated with *Quercus cerris, Q. frainetto* and *Q. robur* using Sørensen Similarity Index and Average Linkage Method.
Notation: 1 - *Quercus robur*, Buteni; 2 - *Quercus robur,* Oradea; 3 - *Quercus cerris,* Brăneşti; 4 - *Quercus frainetto,* Mihai Bravu; 5 - *Quercus cerris,* Mihai Bravu; 6 - *Quercus frainetto,* Dobreşti; 7 - *Quercus cerris,* Dobreşti; 8 - *Quercus cerris,* Tinca; 9 - *Quercus frainetto,* Tinca; 10 - *Quercus cerris,* Oradea; 11 - *Quercus cerris,* Arad; 12 - *Quercus frainetto,* Arad (Radna); 13 - *Quercus cerris,* Ştefăneşti; 14 - *Quercus cerris,* Vlăsia; 15 - *Quercus cerris,* Slăveşti; 16 - *Quercus frainetto*, Slăveşti; 17 - *Quercus cerris*, Vlădiceasca; 18 - *Quercus cerris*, Băneasa; 19 - *Quercus frainetto*, Comana (Giurgiu); 20 - *Quercus cerris,* Bărăgan.

mycelia and assimilative roots associated in mycorrhizae operates in allocations and reallocations of nutrients, water, in blocking the potential root infection courts relying on the diversity of mycobionts, each performing a different job. The identified 30 morphotypes, associated with species of *Quercus,* are assigned differently, depending on geographical distribution of hosts, on health status (there are fewer root active apices in trees vegetating under stressful conditions) and host species.

The niche partitioning among several mycobionts is possible because the ectomycorrhizal fungi differ in their tolerance to water stress and temperature extremes, in their ability to take up different nutrients, their resistance to pathogens (Jones et al. 1998). The instantaneous richness value, for morphotypes active at a certain moment on the same host species is far less than the total richness of the existing mycobionts, associated with a host, as can be revealed by molecular methods. During the investigations of the present study, the maximum number of active morphotypes associated to a host species, in a particular location, was of 14 (*Quercus cerris* at Dobreşti), a fact that is concordant with the hypothesis of redundant species as functional insurance (Yachi & Loreau, 1999). There is a sequential mobilization of mycorrhizal fungi from the common species pool, active within a particular time window.

The degree of mycorrhization differs among trees, parts of the root system, and phenologically. Our findings revealed a fluctuation in frequencies of mycorrhizal apices between 13.1% and 86% during the same period.

One of our findings during the study is the dominance of *C. geophilum* morphotype, a result shared with other similar studies. Gerhardt et al. (2007) found that this is the dominating morphotype in 80% of the investigated *Quercus rubra* stands also the most abundant mycorrhiza forming mycobiont on the roots of *Quercus garryana* (Valentine et al. 2004). *C. geophilum* is a pioneer stage mycorrhizal partner but, also a late stage, being present

in seedling mycorrhizae as well as in mature trees' roots (Piggott 1982, Kranabetter & Wylie 1998) associating with 122 host species (Trappe 1964). The outer layer of the mantle consists of thick walled cells, resembling the gelatinous walls of many lichenicolous fungi. When wet, these walls become gelatinous, providing an environment for water storage, which is of capital importance during the drought episodes. This pecularity gives an advantage to seedlings possessing *C. geophilum* mycorrhiza (Piggot 1982). Being ubiquitous by nature, *C. geophilum* links plants in a common mycorrhizal network (Valentine at al. 2004). It is an early stage and also a late stage associate in seral succession of the forests. Late stage fungi, such as *Lactarius* spp. and *Russula* spp. are infrequent on roots and the inoculation produces via root connections, rather than by mycelial fragments or spores as in early stage mycorrhiza (Jones et al. 1998).

The association of different morphotypes is more or less random, with respect to closely related hosts in the same location and show relatively high similarity between locations related to the same host species. Still the null hypothesis of random association is to be carefully verified on larger sets of data. The general trend is a variation of morphotypes, according to geographical factors (Southern versus North-Western Romania) and also to related hosts allocation (*Quercus* spp.). There is an evidence of within site partition of the same morphotypes among *Quercus* hosts and similarity of sites from same geographical area. It is worth to mention the dissimilarity in morphotype allocation in *Q. robur* as compared to *Q. cerris* and *Q. frainetto.*

Stressful conditions affect trees in urban areas, a fact reflected in their mycorrhizal status, and in lower frequency of mycorrhizal apices. Also, carpophore production is a seldom event in urban areas (Danielson & Pruden 1989, Baxter et al. 1999) never observed in present survey (from 1997 to 2008) in the city of Oradea. Drought and oil pollution reported from Tinca influenced the mycorrhizal status of *Q. cerris* and *Q. frainetto,* reflected in low frequencies of mycorrhizal apices.

Modern investigations on the diversity of mycorrhiza, associated with a particular tree host, rely on DNA finger-printing. This diversity assessment takes into account all potential mycobionts associated with a tree and not those active at a particular moment. For active mycorrhiza at a time snapshot, the classical assessment based on morphotypes is a better approach on our opinion based on the presented results.

These results lead to the idea that artificial inoculation with site-adapted mycobionts would enhance plant growth and survival after outplanting, an opinion shared with other authors (e.g. Gerhardt et al. 2007). The mycorrhization is induced either under laboratory conditions on dual host-fungus systems, either during the seedlings growth by inoculation of the fungal inoculum in nursery containers, the inoculum being represented by spores, mycelia or simply, fragments of appropriate carpophores (Martinez-Amores et al. 1991). The micropropagated plantlets of *Quercus* species can be exposed to mycobionts in order to induce mycorrhization. In our opinion, a good candidate is *Cenococcum geophilum,* due to its large ecological and host range, and the capacity to induce frequent mycorrhizal tips, a hypothesis worth to test under experimental conditions. As a consequence, it is a good candidate for mycorrhization in dual systems (oak microcuttings or micropropagated plants + mycobiont) in Petri dishes, a similar approach being proposed by Herrmann et al. (1998).

Conclusion

The investigation of mycorrhizae using the classical approach of morphotype description yielded 30 types common for *Quercus robur, Q. cerris, Q. frainetto*. The original descriptions of the morphotypes provide a recognition

tool, to be used in further studies on *Quercus* spp. mycorrhizae, *Cenoccocum geophilum* being the exception of the already described morphotype by Agerer (1987-2002).

Although the number of described morphotypes is relatively high, at a specific time snapshot there are fewer, around 14 active morphotypes. Most frequently encountered and dominating mycobiont, under normal and stressful conditions, was *Cenococcum geophilum,* a good candidate for *in vitro* mycorrhization and further acclimatization of the plantlets. However, there is a geographical and host dependent pattern for the association of different morphotypes, as our study reveals. A general recommendation stresses the necessity, for nurseries, to provide seedlings with abundant mycorrhization, because the ectomycorrhizae can improve the success of acclimatization (Kropp & Langlois 1990).

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