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Drought, but not salinity, determines the apparent effectiveness of halophytes colonized by arbuscular mycorrhizal fungi

Anna Füzy[a], **Borbála Biró**[a], **Tibor Tóth**[a], **Ulrich Hildebrandt**[b], **Hermann Bothe**[c,*]

[a]*Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Herman Ottó ut 15, P.O. Box 35, H-1525 Budapest, Hungary*
[b]*Universität Würzburg, Julius-von-Sachs-Institut für Biowissenschaften, Julius-von-Sachs-Platz 3, D-97082 Würzburg, Germany*
[c]*Universität Köln, Botanisches Institut, Gyrhofstr. 15, D-50923 Köln, Germany*

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Summary

The halophytes *Plantago maritima*, *Aster tripolium*, *Artemisia santonicum*, *Puccinellia limosa*, *Festuca pseudovina* and *Lepidium crassifolium* from two different saline soils of the Hungarian steppe were examined for colonization by arbuscular mycorrhizal fungi (AMF). The salt aster (*A. tripolium*) and the sea plantain (*P. maritima*) were examined more thoroughly by recording root colonization parameters, the salt content in the soil and monthly precipitations in 2001 and 2002. Mycorrhizal colonization was maximal in late spring to early summer and had a second peak later in the autumn. Arbuscule formation and overall mycorrhizal colonization appeared to be inversely correlated with the intensity of rainfall at the investigated sites. The results suggest that, in addition to seasonality, drought may play an important role in governing mycorrhizal activity in saline habitats. In greenhouse experiments, conditions in which AMF could overcome the inhibitory effects of sodium chloride on establishing plant–mycorrhizal symbiosis were not met.

Introduction

The literature on the effects of arbuscular mycorrhizal fungi (AMF) on salt-tolerant plants

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*Corresponding author. Tel.: +221 4702760; fax: +221 4705039.
E-mail address: Hermann.Bothe@uni-koeln.de (H. Bothe).

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(the halophytes) is controversial. Salt was reported to inhibit germination of AMF spores, colonization of the halophytes by the fungi and formation of new, fertile spores under laboratory conditions (Juniper and Abbott, 1993; Smith and Read, 1997). At field sites, however, several halophytes are heavily colonized by AMF. This was noted early (Mason, 1928; Boullard, 1959) for the salt aster (*Aster tripolium* L.). In our experience, this salt aster and other plants such as sea plantain (*Plantago maritima* L.), sea wormwood (*Artemisia santonicum* L.) and chamomile (*Matricaria chamomilla* L.) are among the most heavily colonized plants that we have examined over several years (Füzy et al., 2001; Landwehr et al., 2002). In addition, saline soils carry a high content of AMF spores, particularly of *Glomus geosporum* (Hildebrandt et al., 2001; Landwehr et al., 2002), indicating that saline soils are, indeed, sites where AMF thrive. In the field, other factors that allow extensive colonization of plants must override the inhibition by salt.

There is currently no clear relationship that links AM functioning with fungal colonization of roots (Smith and Read, 1997; Tonin et al., 2001; Vogel-Mikuš et al., 2005). Hence, a high level of colonization may not always indicate an extensive exchange of metabolites between the two symbiotic partners. A few active fungal structures in only a small number of roots may help plants to cope with all kinds of stress (drought, mineral deficiency, pollution by metals, salts or toxins, attack by pathogens, etc.). During the course of a year, the number of fungal structures within a plant may also vary significantly (Smith and Read, 1997; Füzy et al., 2001). This is particularly true of arbuscules, which have a short half-life of some 2 weeks. It is generally accepted that arbuscules are the sites of the most extensive transfer of metabolites. Glucose, primarily, is transferred from the plant to the fungus and, conversely, water and minerals from the fungus to the host (Smith and Read, 1997; Harrison, 2005).

The present study examined the level of AMF colonization of halophytes in two different saline habitats in the Hungarian steppe (Puszta) during the course of a year. Seasonal fluctuations of mycorrhiza (total colonization, arbuscule formation) were assessed during the course of the year. The study aimed at uncovering whether such fluctuations were dependent on the soil type or its salt composition, or more on other parameters such as rainfall and drought. If one accepts the notion that arbuscules are the sites of extensive metabolite transfer between the symbiotic partners, the arbuscule counts of the present study suggest that drought might be the major factor governing the activity of the symbioses between the AMF and halophytes in saline habitats rather than the soil type or its salt composition. Results of greenhouse experiments are also reported where AMF were tested in attempts to overcome the inhibitory effects of sodium chloride on the establishing of this symbiosis.

Materials and methods

Sites examined

Two saline sites were selected to examine the colonization of halophytes by mycorrhizal fungi:

(a) Puszta at Apaj (47°5.2′N; 19°5.8′E), an occasionally pastured grassland, with Solonetz soil type (for the term, see Horváth et al., 1974), with Na_2CO_3, Na_2SO_4 and NaCl as salts, pH between 8.5 and 9.6 (9.2±0.4), electric conductivity (1:2.5, soil:water ratio) of 0.38–0.82 (0.62±0.16) $\mathrm{dS\,m^{-1}}$, organic matter 0.86–1.78% (1.48±0.32%) and clay content 9–26% (18±6.5%). The site is classified as *Artemisia* saline puszta (15.A113) by Devillers and Devillers-Terschuren (1996).

(b) Border meadow of Lake Zabszék (46°50.6′N; 19°10.6′E), periodically flooded by Lake Solonchak (see Horváth et al., 1974), Na_2CO_3, and NaCl as main salts, pH 9.0–9.7 (9.4±0.3), electric conductivity of 0.70–1.17 (0.85±0.25) $\mathrm{dS\,m^{-1}}$, organic matter 0.71–1.14% (0.91±0.15%) and clay content 8–18% (14±4.5%). The plant community was classified as Pannonic *Puccinellia limosa* hollows (15.A131) (Devillers and Devillers-Terschuren, 1996). This site was some 50 km south of the first location.

Halophytes examined

Two typical halophytes, *A. tripolium* and *P. maritima*, were screened for the degree of mycorrhizal colonization and the formation of arbuscules in their roots at Apaj each month during 2001 and 2002. In 2002, *A. tripolium* was also examined at a similar site at Lake Zabszék.

Meteorological data, dates of sampling and determination of the degree of mycorrhizal colonization

The meteorological data recorded in the Kiskunság region (located at Kecskemét) are for 2001 in March, precipitation 64 mm, average temperature 8.7 °C sampling date 14.03 and the total precipitation in the 30-day period up to 10 days before the sampling date: 27 mm (given under Figures 2–5), April 37/10.2/04.04/35, May 17/17.1/10.05/20, June 94/17.9/20.06/29 July 127/21.5/18.07/89, August 21/22.1/27.08/8, September 98/14.8./25.09/77, October 14/13.9/27.10/7 and November

39/3.0/16.11/14. For 2002 the data were in March 22/7.8/14.03/14, April 36/11.0/26.04/18, May 41/19.0/23.05/28, June 57/21.3/25.06/65, July 34/23.4/30.07/33, August 75/21.3/30.08/74, September 73/15.1/30.09/6, October 30/10.6./27.10/23 and November 25/ 7.5/13.11/18.

Samples of lateral roots were taken from two parallels, in both cases consisting of a mixture of five randomly selected individuals of each halophyte species examined. The roots were mixed to yield approximately 1 g fresh weight, cleansed with tap water and stored in 70% alcohol prior to the examination of AMF colonization. After storage, root samples were cut into approximately 1 cm segments, boiled in 15% KOH for 40 min, stained with aniline blue and fixed in 40% lactic acid (Phillips and Hayman, 1970). Colonization was assessed by the five-class system of Trouvelot (Trouvelot et al., 1986) at 100 × magnification (or at 200 × or 400 × magnification when distinct AMF structures were counted). The abundance of hyphae, vesicles and arbuscules was determined in 30 root segments of each plant. The terminology on the intensity of mycorrhizal colonization (*M*% value) and on absolute arbuscule richness (*A*%) was described by Trouvelot et al. (1986).

Determination of the electric conductivity and of the water content in the soil samples

Roots were sampled at Apaj on July 18, 2001 to assess the degree of mycorrhizal colonization along the salt gradient (Figure 1). Electric conductivity was measured in the field at 0–20 cm depth with a four-electrode conductivity sensor SCT 12 apparatus (Martek Instruments Inc., Raleigh, NC, USA) and in the laboratory in a suspension of 1 g soil mixed with 2.5 mL water with a conductometer (Jeway 4020, Jeway Electric Co. Ltd., Fuzhou, PR of China). The water content in 10 g soil samples was determined by subtracting the weight of the soil dried overnight at 105 °C from its fresh weight.

Experimental details of the salt stress experiments in the greenhouse

The experimental procedures of these experiments (Figure 7) were as described previously (Scheloske et al., 2004). Seeds of either *A. tripolium* (collected at the German coast, island of Sylt, near List) or barley, *Hordeum vulgare* var. Carina (purchased from Schmitz and Laux, D-Hilden), were surface sterilized and germinated in sterilized garden soils ("Einheitserde"). Plantlets (3–4 weeks old) were transferred to 1–5 L pots filled with 80% quartz sand and 20% expanded clay as inert material, both sterilized. Roots were colonized by adding expanded clay containing a mixture of *G. geosporum* Br 1 and *Glomus intraradices* Sy167 produced as described (Hildebrandt et al., 1999). Plants were grown in the greenhouse at 25–30 °C, 70% relative air humidity, with 15 h photoperiods and a light intensity of 150–600 microEinstein for 6 months. Plants were watered every second day and once a week with Hoagland nutrient solution without phosphorus and once a month with complete Hoagland solution. The concentrations of NaCl in the abscissa of Figure 7 were carefully monitored by electric conductivity measurements every 3–4 days and adjusted to the correct value by a NaCl suspension whenever necessary.

Statistics

Statistical analysis of mycorrhizal colonization and arbuscule formation was conducted by one-way analysis using ANOVA. $LSD_{5\%}$ values are given in Figures 2–5. The correlation between mycorrhizal colonization and

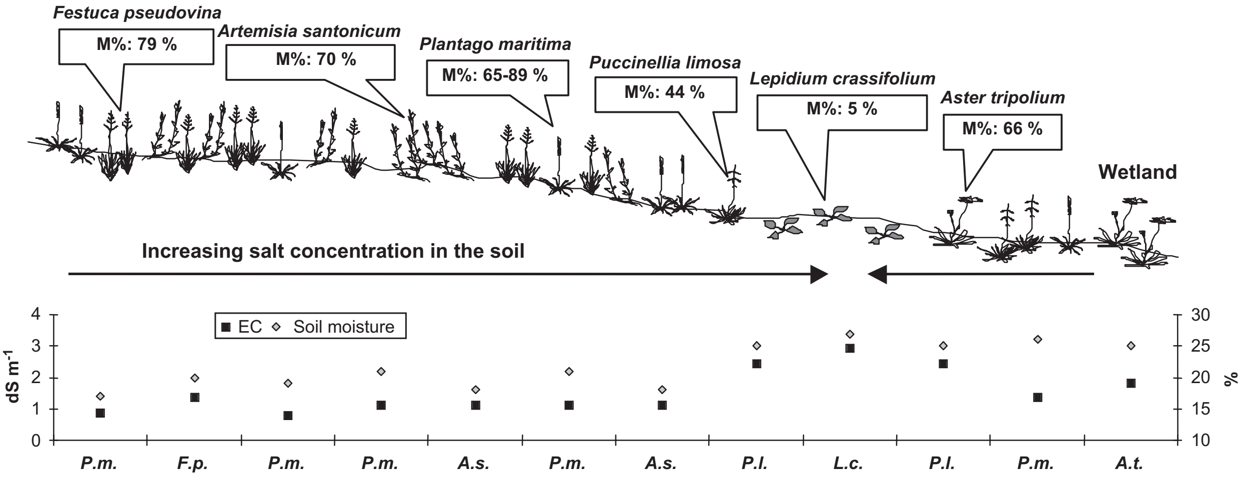

Figure 1. Schematic presentation of the relative location of halophytes along the salt gradient at Apaj. The haloseries (toposequence) of the plant species is based on Ellenberg and our own experience. *M*% values (determined as described by Trouvelot et al., 1986) indicate the percentage of mycorrhizal colonization of the halophytes in July 2001. ◇ = soil moisture, ■ = electric conductivity, determined directly at the site in 10–20 cm soil depth close to the roots of the plants indicated. P.m., *Plantago maritima*; F.p., *Festuca pseudovina*; A.s., *Artemisia santonicum*; P.c., *Puccinellia limosa*; L.c., *Lepidium crassifolium*; A.t., *Aster tripolium*.

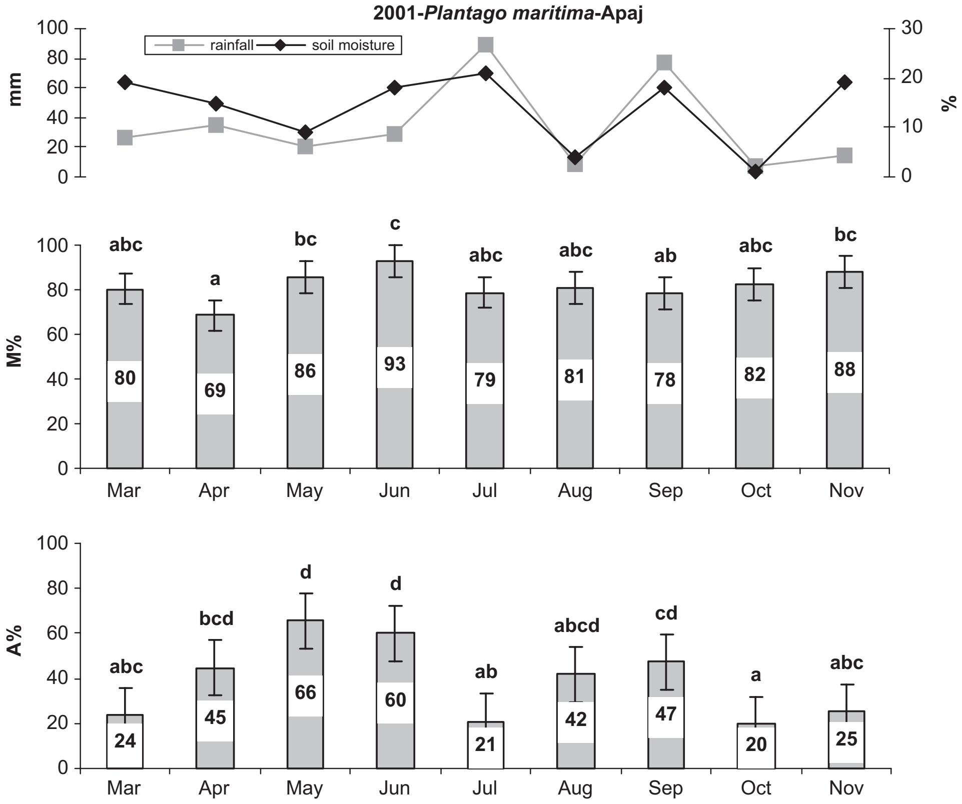

Figure 2. Mycorrhizal colonization and arbuscule frequency of *Plantago maritima* at Apaj in 2001 during the course of the year. M = total mycorrhizal colonization, A = arbuscule frequency (determined by the Trouvelot method). Different letters indicate significant differences as result of variance analyses ($p = 0.05$). Error bars represent the $\mathrm{LSD}_{5\%}$ values. ■ = rainfall, summed up for the 20-day interval ending 10 days prior to the sampling date, ◆ = moisture of a soil sample taken in 20 cm depth at the root of the plant. The significance level is $p = 0.162$ for $M\%$ values and $p = 0.004$ for $A\%$ values.

precipitation data was examined by linear regression at the $p = 0.05$ significance level.

Results

Colonization of different halophytes by AMF along a salt gradient

Plants in saline habitats colonize a marsh in strict dependence on the salt concentration in the soil (Ellenberg, 1988), resulting in a typical toposequence (haloseries) of the halophytes, often with distinct belt formations (Figure 1). Among the plants discussed in the present study, the sea plantain (*P. maritima* L.) occupies sites with lower salt concentration as inferred from the electric conductivity measurements (Figure 1, bottom). *Lepidium crassifolium* W. et K. occurs at the highest salt levels, whereas *A. tripolium* L. thrives mainly in wet areas but within a broad amplitude of salt and water content in the soil (Figure 1 bottom and unpublished observations). Several plants, such as the Asteraceae *A. tripolium* and *A. santonicum* L. = *A. maritima* auct. (Simon, 2000), as well as the sea plantain, *P. maritima*, or the grass *Festuca pseudovina* Hackel, all assessed at Apaj on July 18, 2001, showed a high degree of mycorrhizal formation with 2/3 or more of the roots being colonized. As *Festuca ovina/pseudovina* is not yet resolved taxonomically as an aggregate, this grass population at Apaj may represent a specifically salt-adapted ecotype. Along the toposequence, the degree of mycorrhizal colonization of plants seemed to decrease with increasing salinity in the soil (Figure 1), as also observed in other saline habitats (Hildebrandt et al., 2001; Landwehr et al., 2002). The grass *Puccinella limosa* (Schur) Holmb., like other species of this genus (Landwehr et al., 2002), exhibited a moderate degree of mycorrhizal colonization, which varied from one individual to the next. At the highest salt levels in the soils, colonization of the plants by AMF was low in most instances, as documented for *L. crassifolium* at Apaj (Figure 1). The low value of 5% colonization of *L. crassifolium* roots determined as described by

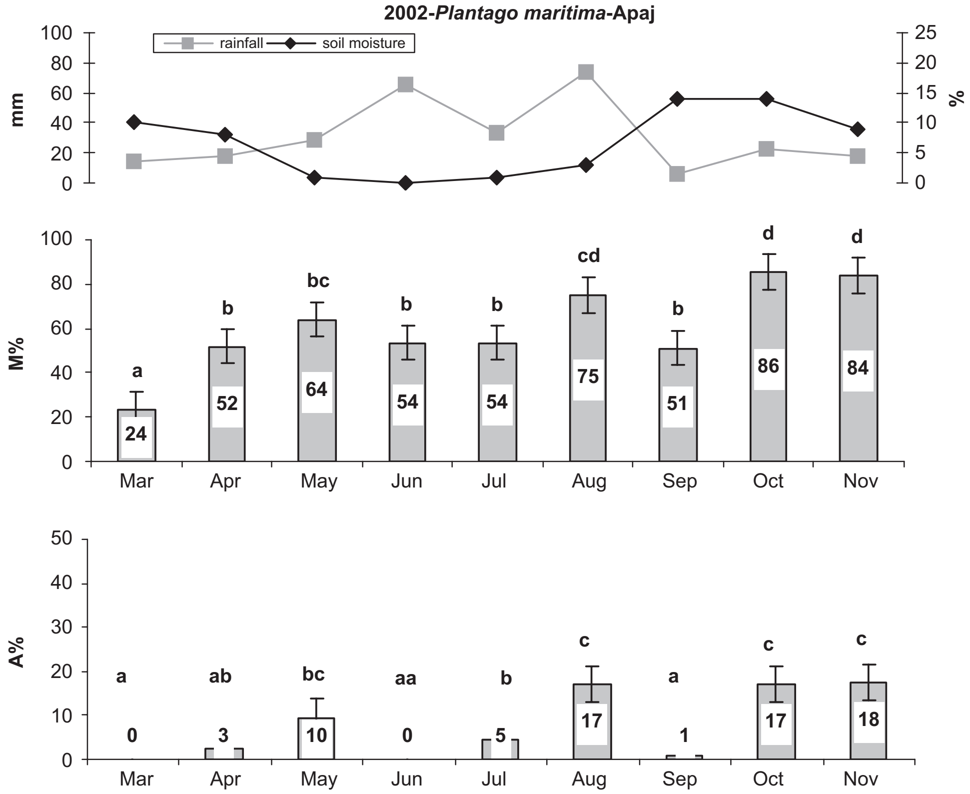

Figure 3. Mycorrhizal colonization and arbuscule frequency of *Plantago maritima* at Apaj in 2002 during the course of the year. For the abbreviations and symbols see legend to Figure 2. The significance level is $p = 0.000$ for *M*% values and $p = 0.002$ for *A*% values.

Trouvelot et al. (1986) might indicate an occasional spread of fungal hyphae within the roots of this Brassicaceae, a family generally considered to be non-mycorrhizal. Arbuscules were, however, not detected in the roots of this plant at the date examined.

Degree of colonization and arbuscule formation in halophytes during the course of the year

The amount of rainfall was low (much under the average) in the first half of 2001 (see Figures 2–5). During this period, the overall colonization rate of the roots of *P. maritima* and, more pronounced, the number of arbuscules steadily increased (Figure 2). Rainfall in July and September was high, which caused an apparent reduction in arbuscule numbers by more than 50% in July and October, respectively. August remained comparably dry, which coincided with an increase in arbuscule frequencies. Remarkably, the degree of colonization of *P. maritima* and the number of arbuscules on the roots of this perennial plant was still high in the late season (October/November). All these differences were statistically significant (Figure 2). The colonization intensity and arbuscule frequency were not well correlated with the water content in the upper 10 cm of the soil as with the rainfall as seen in Figure 2 and following figures.

In 2002, the overall degree of mycorrhizal colonization and arbuscule numbers of *P. maritima* (Figure 3) were not as high as in the previous year. However, a similar pattern of arbuscule formation was also observed in this year. Arbuscule formations were high in periods of low rainfall (April to May, July and late autumn), and decreased after periods of higher precipitation (June, August). The decrease in arbuscule numbers (e.g. in September) lagged slightly behind rainfall (Figure 3).

Essentially the same results were obtained with *A. tripolium* at Apaj in 2002 (Figure 4). Periods of higher rainfall in June and August were accompanied by low colonization and arbuscule frequency in June (in August not so distinct for arbuscules but for the degree of colonization). When precipitation was low at the beginning and end of the year as well as in July, both the degree of colonization and arbuscule numbers were higher than in the wetter months.

At the Zabszék site, some 50 km from Apaj, the mycorrhizal colonization of *A. tripolium* (Figure 5) showed the same dependence on rainfall as seen

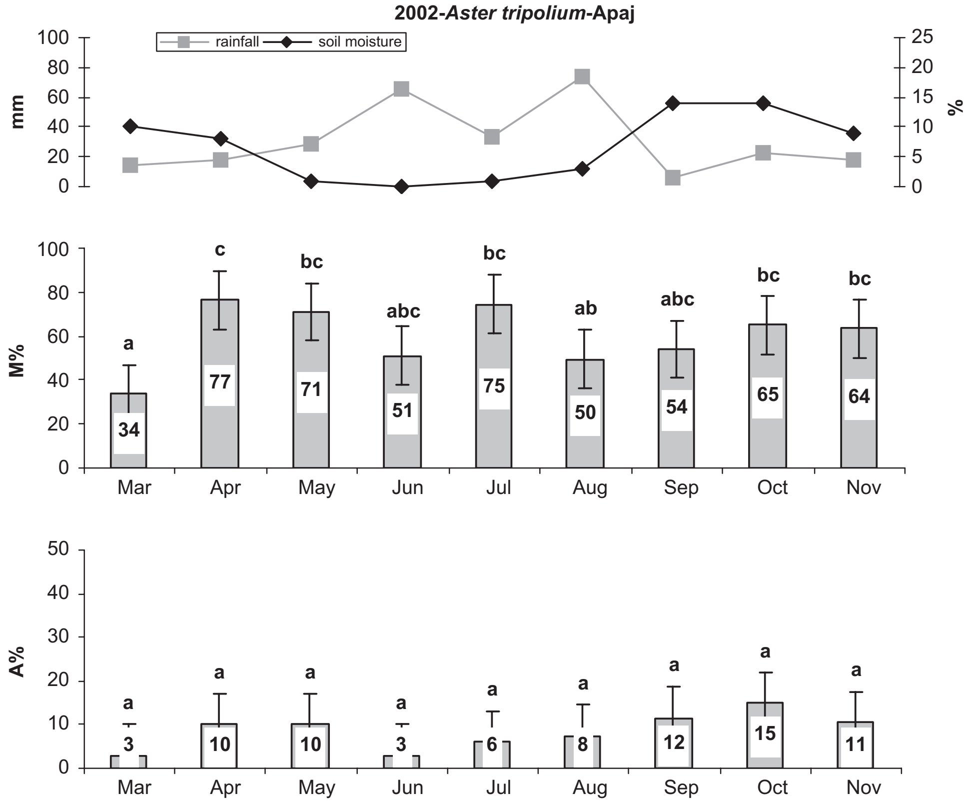

Figure 4. Mycorrhizal colonization and arbuscule frequency of *Aster tripolium* at Apaj in 2002 during the course of the year. For the abbreviations and symbols see legend to Figure 2. The significance level is $p = 0.068$ for M% values and $p = 0.592$ for A% values.

before. High levels of rainfall in June and August were accompanied by low arbuscule numbers, and during periods of drought at the beginning and end of the year and in July, arbuscule frequency was high. At Zabszék also, the mycorrhizal pattern did not correlate with the water content in the upper soil (Figure 5).

The data in Figures 2–5 were evaluated statistically (Figure 6a, Tables 1 and 2). The mycorrhizal intensity (M values) of the current and the previous month were subtracted from each other and correlated with the rainfall data. A linear regression model was found to predict the changes in the mycorrhizal colonization and rainfall for the months April until July at a significance level of $\alpha = 0.01$. A similar correlation was seen for the arbuscule values even for the months April until August (Figure 6b). Later in the year, however, such a correlation was not so distinct.

Failure to demonstrate salt resistance conferred by AMF in greenhouse experiments

A previous study (Landwehr et al., 2002) indicated that spores of one single species among AMF, *G. geosporum*, dominated in Central European saline soils, including the Hungarian salt marshes examined in the present study. In attempts to demonstrate the potential alleviation of salt stress by AMF, a mixture of *G. geosporum* (isolated from the salt marsh at Jerxheim in Northern Germany; Hildebrandt et al., 2001) and *G. intraradices* SY167 was used in greenhouse experiments (Figure 7). The salt content of the soils in the 1 L pots was carefully monitored twice a week by electric conductivity measurements and kept at the concentrations given in the abscissa of Figure 7 by adding different amounts of NaCl in the same volume of water. All attempts to find any positive effect of AMF on plant growth (biomass) failed in more than 20 different experiments between 1997 and 2003 at different seasons of the year. Even at lower concentrations (0.5%, 1%), NaCl suppressed mycorrhizal colonization of the salt-tolerant *A. tripolium*. No differences in root and shoot biomass were detected between AMF-inoculated plants and controls without the fungi. Similar results were obtained with maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* MILL.) and the sea plantain (*P. maritima*) in greenhouse experiments in the Cologne laboratory (not documented). The Budapest laboratory used

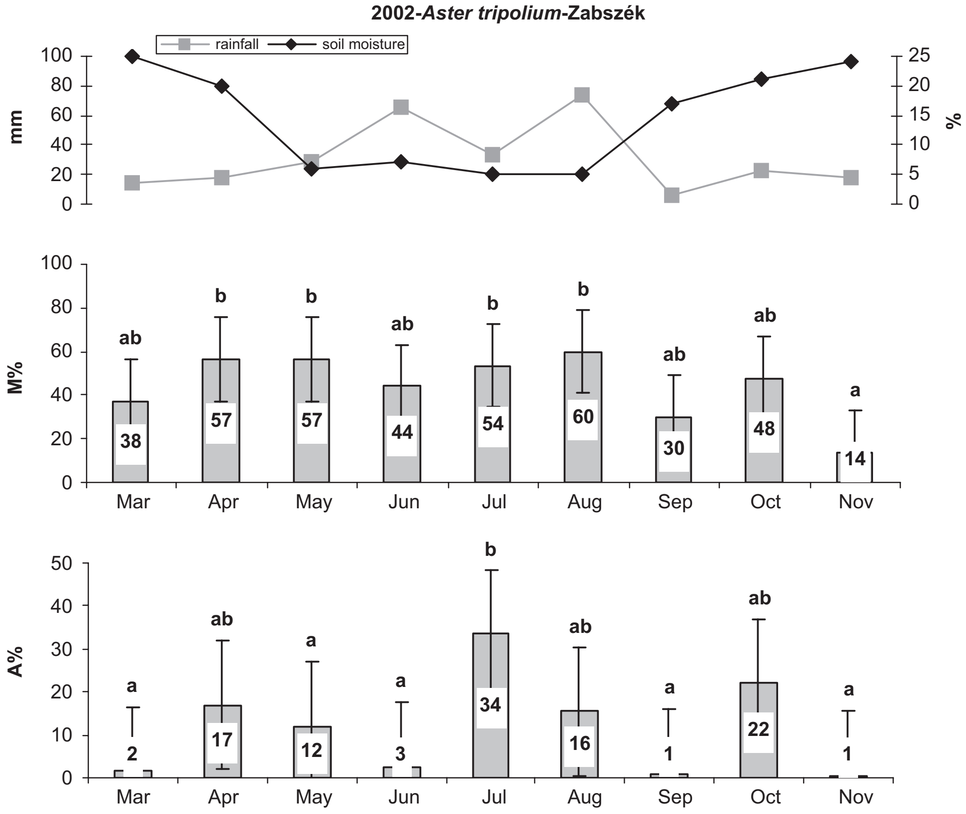

Figure 5. Mycorrhizal colonization and arbuscule frequency of *Aster tripolium* at Zabszék in 2002 during the course of the year. For the abbreviations and symbols see legend to Figure 2. The significance level is $p = 0.259$ for *M*% values and $p = 0.284$ for *A*% values.

white clover (*Trifolium repens* L.) and a split root system, where half of the roots were subjected to salt stress and the other half were not. Salt stress (1% NaCl) reduced AMF colonization from 65% (determined by the Trouvelot method) to 45% and arbuscule frequency from 63% to 34% (not reported).

Discussion

The two plant species selected for monthly counts of mycorrhizal colonization, *Plantago maritima* and *Aster tripolium*, have similar ecological demands in many respects (Ellenberg, 1988). Both are facultative halophytes, hemikryptophytes, helomorphs (= with large water-filled spaces, large intercellulars in the roots), show a tendency for leaf succulence, are evergreen and are strongly colonized by arbuscular mycorrhizal fungi (AMF) in nature. *P. maritima* prefers slightly drier stands and more basic soils than the salt aster and, in particular, has more shallow roots. In contrast, *A. tripolium* forms roots exploiting deeper soil layers, thus exhibiting a larger ecological spectrum with respect to soil moisture content. The present study showed that the degree of mycorrhizal colonization and, in particular, the number of arbuscules, increased continuously in spring, which is the generally observed seasonal onset of this symbiotic interaction (Escudero and Mendoza, 2005). Arbuscules were formed even late in the year on these perennial plants, though often in reduced numbers (cf. Figures 1–5). The surprising observation of the present study was that AMF colonization and arbuscule formation did not remain constant during the year nor did they increase specifically at distinct stages of plant growth, e.g. at flower or seed formation, but appeared to be inversely correlated with rainfall during the most productive season of the year, from late spring to early autumn. This was observed in all the counts in Figures 2–5, though sometimes the arbuscule response lagged behind the rainfall/drought cycle by 1 month. However, this variation could well reflect the number of days after rainfall when the samples were taken (soon or later after rainfall).

Early botanists noted that halophytes must cope not so much with the stress caused by Na^+ and Cl^- (or other) ions, but with the extremely negative water potential in salt marshes (Stocker, 1928).

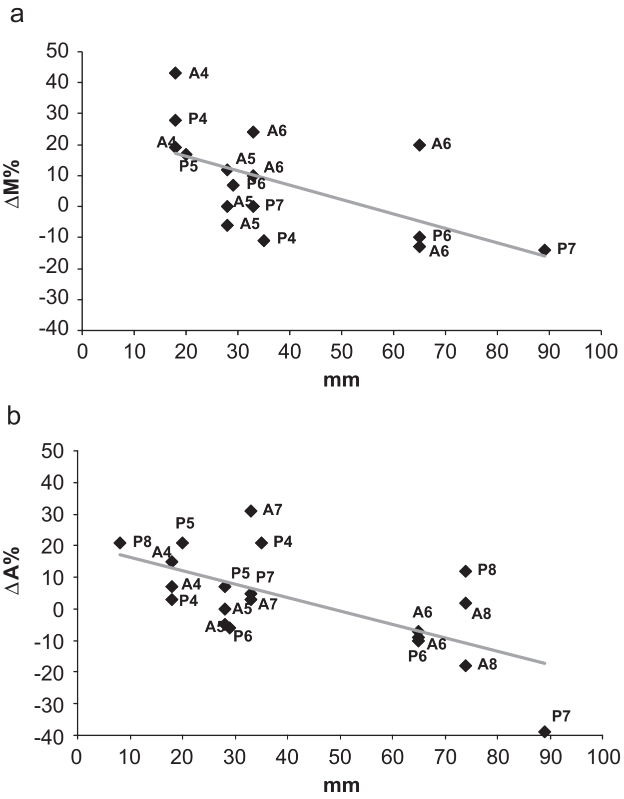

Figure 6. Linear regression between the precipitation data and the changes in the degree of mycorrhizal colonization of *Plantago maritima* and *Aster tripolium*. Data of Figures 2–5 have been taken for this analysis. Δ*M* (or Δ*A*) in % is the *M*(*A*) value at the sampling date minus the *M*(*A*) value at the previous month. Precipitation data were summed up for the period between the 30th and 10th day before the sampling day and were referred to the Δ*M* or Δ*A* values, respectively. (a) The correlation between mycorrhizal colonization (*M*%) and precipitation data was significant ($R = -0.75$, $p = 0.01$) for the data pairs from April until July, $y = -0.66x+29.8$. (b) The correlation between arbuscule richness (*A*%) and precipitation data was significant ($R = -0.65$, $p = 0.01$) for the data pairs from April until August, $y = -0.42x+20.3$. A, *Aster tripolium*; P, *Plantago maritima*; 4–8, months from April until August.

Table 1. Data pairs for Δ*M* and precipitation used and the corresponding numbers

		April	May	June	July	August	September	October	November
2001 – Apaj *P. maritima*	Precipitation	35	20	29	89	8	77	7	14
	Δ*M*%	−11	17	7	−14	2	−3	4	6
	Number in Figure 6a	1	2	3	4	5	6	7	8
2002 – Apaj *P. maritima*	Precipitation	18	28	65	33	74	6	23	18
	Δ*M*%	28	12	−10	0	21	−24	35	−2
	Number in Figure 6a	9	10	11	12	13	14	15	16
2002 – Apaj *A. tripolium*	Precipitation	18	28	65	33	74	6	23	18
	Δ*M*%	43	−6	20	24	−25	4	11	−1
	Number in Figure 6a	17	18	19	20	21	22	23	24
2002 – Zabszék *A. tripolium*	Precipitation	18	28	65	33	74	6	23	18
	Δ*M*%	19	0	−13	10	6	30	18	−34
	Number in Figure 6a	25	26	27	28	29	30	31	32

Both ions bind water that needs to be mobilized by the plants. Hence, plants of saline habitats have to cope with this "physiological drought", which is aggravated by prolonged periods without significant precipitation – a situation frequently encountered in saline habitats of the Hungarian steppe. Colonization by AMF may then help plants to acquire water from the soil, and the formation of additional

Table 2. Data pairs for ΔA and precipitation used and the corresponding numbers

		April	May	June	July	August	September	October	November
2001 – Apaj *P. maritima*	Precipitation	35	20	29	89	8	77	7	14
	ΔA%	21	21	−6	−39	21	5	−27	5
	Number in Figure 6b	1	2	3	4	5	6	7	8
2002 – Apaj *P. maritima*	Precipitation	18	28	65	33	74	6	23	18
	ΔA%	3	7	−10	5	12	−16	16	1
	Number in Figure 6b	9	10	11	12	13	14	15	16
2002 – Apaj *A. tripolium*	Precipitation	18	28	65	33	74	6	23	18
	ΔA%	7	0	−7	3	2	4	3	−4
	Number in Figure 6b	17	18	19	20	21	22	23	24
2002 – Zabszék *A. tripolium*	Precipitation	18	28	65	33	74	6	23	18
	ΔA%	15	−5	−9	31	−18	15	21	−21
	Number in Figure 6b	25	26	27	28	29	30	31	32

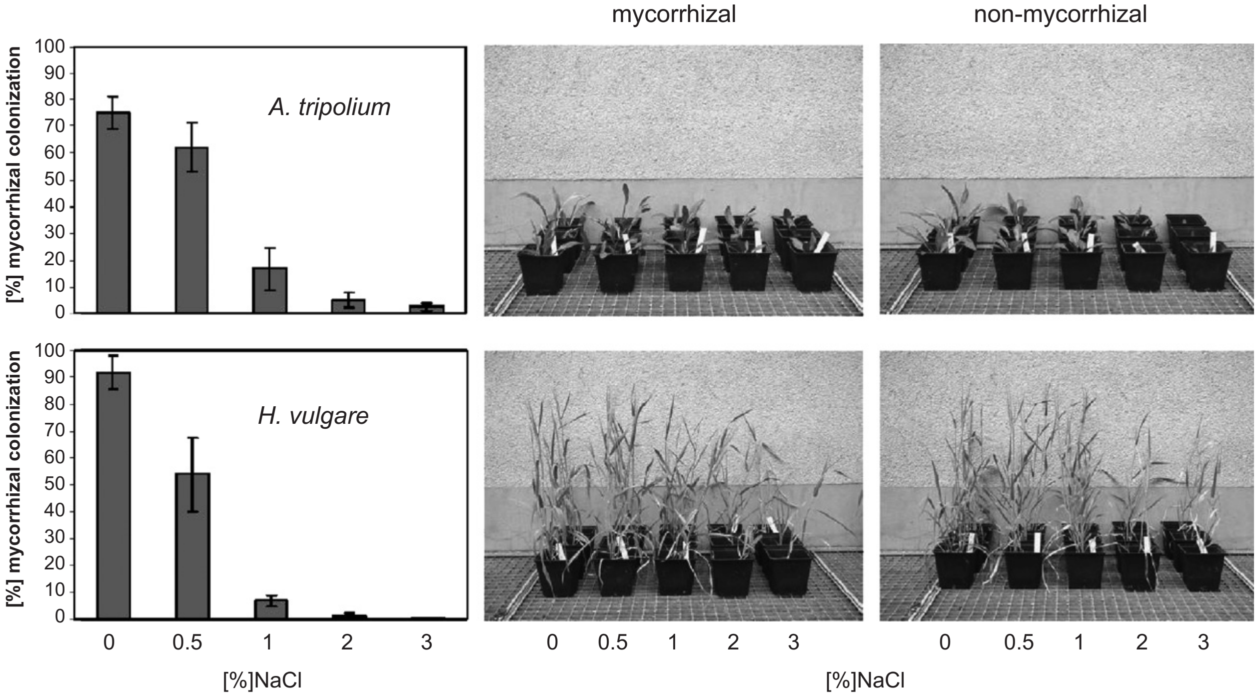

Figure 7. Inhibition of mycorrhizal colonization of the salt aster (*Aster tripolium*) or barley (*Hordeum vulgare*) by sodium chloride. A representative experiment from the many performed between 1997 and 2003 is shown. A mixture of *Glomus geosporum* and *G. intraradices* was used as inoculum. Bars represent standard deviations of the values from five plants for each concentration. For experimental details, see Materials and methods.

arbuscules may facilitate the transfer of water and nutrients to the plants. Consistent with this, a recent study (Ouziad et al., 2006) showed that the expression of both plasmalemma and tonoplast aquaporins was down-regulated in tomato roots colonized by AMF under NaCl stress. It is tempting to assume that the fungal structures (external hyphae) undertake the mobilization of water in drought-affected saline soils with the involvement of both fungal and plant aquaporins.

To our surprise, the degree of AMF colonization and arbuscule formation apparently did not correlate with the water content in the soil but negatively with rainfall over the whole vegetation period. This correlation is particularly distinct for mycorrhizal intensity between April and July and for arbuscule richness between April and August (Figures 6a and b). This response was similar in both halophyte species, although *A. tripolium* can tolerate more soil moisture and thus anoxic conditions than *P. maritima*. The aerial parts of the plants apparently recognize drought stress, and in order to avoid wilt, they may systemically use the AMF to form more arbuscules, apparently for water acquisition and drought avoidance, a response that may be regulated by absciscic acid. This phytohormone is synthesized by both the plants and the fungi (Danneberg et al., 1992).

There is good experimental evidence that AMF colonization protects plants against detrimental effects of water deficiency (Ouereieta et al., 2003; Ruiz-Lozano, 2003; Mena-Violante et al., 2006; Wu and Xia, 2006). AMF-induced increases in drought tolerance may involve avoidance and tolerance of dehydration (Cho et al., 2006). However, the AMF influence on plants in desiccating soils is unpredictable in the field (Cho et al., 2006). The literature on AMF and salt stress is even more controversial than that on drought. Whereas most summaries explicitly state that salt has negative effects on the colonization of plants by AMF (Juniper and Abbott, 1993, 2006; Smith and Read, 1997), several more recent studies report that AMF alleviate salt stress both in the laboratory (Feng et al., 2002; Sharifi et al., 2007) and in the field (Giri and Mukerji, 2004). Although mycorrhizal colonization is indeed reduced with increasing salt levels, the symbiosis between AMF and halophytes might be strengthened in saline environments once the partnership has been established (Tian and Feng, 2004; Neto et al., 2006). In saline soils, the species diversity of AMF, but not the spore abundance, is lower than in non-saline soils (Hildebrandt et al., 2001; Landwehr et al., 2002; Carvalho et al., 2004). This has been demonstrated for saline soils of both inland and coastal habitats. In the latter, plants are exposed to both salt stress and periodic anoxic conditions due to regular flooding. Both soil types in Western and Central Europe predominantly contain spores of one AMF species, *Glomus geosporum*, which can make up to 80% of all spores (Hildebrandt et al., 2001; Landwehr et al., 2002; Carvalho et al., 2004). It was therefore a straightforward approach to test whether isolates of *G. geosporum* confer salt tolerance to plants. Some positive effects were indeed reported with a *G. geosporum* isolate from a Portuguese flooded salt marsh (Carvalho et al., 2004). Unfortunately, in many greenhouse experiments between 1997 and 2003, we consistently failed to observe positive effects on drought tolerance with either *G. geosporum* (isolate from the salt marsh in Jerxheim) or a mixture of both the *G. geosporum* isolate and *Glomus intraradices* SY167, even with increasingly applied drought stress (data not shown). Consistent with these failures, no significant differences were detected in shoot biomass when comparing mycorrhizal and non-mycorrhizal *A. tripolium* subjected to conditions of tidal flooding with 50% diluted artificial seawater (Neto et al., 2006). As already noted, the effects of AMF on salt stress are unpredictable (Cho et al., 2006), and small experimental details may influence results in greenhouse experiments (Tian and Feng, 2004). Since NaCl is readily washed out from the experimental pots together with the drainage water, it is difficult to maintain a constant salt concentration in pots during experiments. A detailed laboratory study on the correlation between drought or salt stress and arbuscule frequency and their lifespan has not yet been reported to our knowledge. In nature, the occurrence of high numbers of *G. geosporum* spores in saline soils and the intensive AMF colonization of several halophytes such as *A. tripolium*, *A. santonicum* (in Western Europe *Artemisia maritima*), *P. maritima* or *Inula chrithmoides* might indicate that AMF play a beneficial role in plants of salt marshes. Due to drastic soil parameter changes within short distances and times ("microsite" formation), salt may, indeed, have detrimental effects on AMF colonization of halophytes at one site, but drought stress may override inhibition at another with lower NaCl concentrations, resulting in an overall effective *G. geosporum*/halophyte symbiosis in saline habitats. *G. geosporum* also occurs at non-saline sites, so the harsh conditions in salt marshes may cause this fungus to sporulate abundantly to help it to survive. Yet unpublished molecular data (Wilde, P., Manal, A., Stodden, M., Hildebrandt, U., Bothe, H., in preparation) indicate that roots of halophytes in Central European saline soils are preferentially colonized by still unidentified *Glomus* species. It therefore cannot be ruled out that AMF other than *G. geosporum* are the major players in plant tolerance of salt and drought stress in salt marshes.

It is necessary to comment on the colonization of the Brassicaceae *Lepidium crassifolium* in the Hungarian salt steppe. In comparison with the gridline intersect method, the Trouvelot protocol (Trouvelot et al., 1986) recommends counting of mycorrhizal structures on a fairly large root segment (1 cm). Thus, the occasional occurrence of an AMF structure (intraradical hypha) may be taken as faint indication of the colonization of plant roots of families such as Brassicaceae or Chenopodiaceae, which are generally considered to be non-mycorrhizal. On the other hand, as shown recently for the Brassicaceae *Biscutella laevigata* (Orłowska et al., 2002) and *Thlaspi* sp. (Regvar et al., 2003), all fungal structures (intraradical hyphae, vesicles, spores and even arbuscules), can be found, at low frequency, within the roots at certain growth stages of these plants, particularly during flowering. There are indications that such fungi, despite the low colonization rate, may relieve stress such as heavy metal toxicity (Biro et al., 2005; Vogel-Mikuš et al., 2005). In most saline habitats analyzed (Hildebrandt et al., 2001; Landwehr et al., 2002), plants thriving at the sites of highest salt concentration were members of plant families considered non-mycorrhizal (e.g. Brassicaceae and Chenopodiaceae). Any correlation between the frequency of fungal structures, their activity and their impact on plant growth is difficult to resolve. A low AMF colonization of plants such as *L. crassifolium* or *Puccinellia* sp. in salt marshes does not necessarily imply ineffectiveness of the plant–fungal interaction.

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References

Biró B, Posta K, Füzy A, Kádár I, Németh T. Mycorrhizal functioning as part of the survival mechanisms of barley (*Hordeum vulgare* L) at long-term heavy metal stress. Acta Biol Szegediensis 2005;49:65–8.

Boullard B. Relations entre la photopériode et l'abondance des mycorrhizes chez *Aster tripolium* L. Bull Soc Bot Fr 1959;106:131–4.

Carvalho LM, Correia PM, Martins-Loucao MA. Arbuscular mycorrhizal fungal propagules in a salt marsh. Mycorrhiza 2004;14:165–70.

Cho K, Toler H, Lee J, Ownley B, Stutz JC, Moore JL, Augé RM. Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. J Plant Physiol 2006;163:517–28.

Danneberg G, Latus C, Zimmer W, Hundeshagen B, Schneider-Poetsch HJ, Bothe H. Influence of vesicular arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.). J Plant Physiol 1992;141:33–9.

Devillers P, Devillers-Terschuren J. A classification of Palaearctic habitats. Nature and Environment 1996; 78: Council of Europe 〈http://www.botanika.hu/project/habhun/〉.

Ellenberg H. Vegetation ecology of Central Europe [translated by Strutt GK], 4th ed. Cambridge, New York: Cambridge University Press; 1988.

Escudero V, Mendoza R. Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. Mycorrhiza 2005;15:291–9.

Feng G, Zhang FS, Li XL, Tian CY, Tyang C, Rengel Z. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 2002;12: 185–90.

Füzy A, Vörös I, Takács T, Tóth T, Biró B. Colonisation of arbuscular mycorrhizal fungi on *Festuca pseudovina* and *Matricaria chamomilla* in two Hungarian salt-affected soils. Sci Bull North Univ Baia Mare Ser C 2001;14:109–17.

Giri B, Mukerji KG. Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 2004;14: 307–12.

Harrison MJ. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 2005;50:361–89.

Hildebrandt U, Kaldorf M, Bothe H. The zinc violet and its colonization by arbuscular mycorrhizal fungi. J Plant Physiol 1999;154:709–17.

Hildebrandt U, Janetta K, Ouziad F, Renne B, Nawrath K, Bothe H. Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. Mycorrhiza 2001;10:175–83.

Horváth I, Glavač V, Ellenberg H. Vegetation Südosteuropas. Stuttgart: Gustav Fischer; 1974.

Juniper S, Abbott LK. Vesicular–arbuscular mycorrhizas and soil salinity. Mycorrhiza 1993;4:45–7.

Juniper S, Abbott LK. Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. Mycorrhiza 2006;16:371–9.

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Tóth T, Biró B, et al. The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza 2002;12:199–211.

Mason E. Note on the presence of mycorrhizae in the roots of salt-marsh plants. New Phytol 1928;27:193–5.

Mena-Violante HG, Ocampo-Jimenez O, Dendooven L, Martinez-Soto G, Gonzalez-Castaneda J, Davies FT, et al. Arbuscular mycorrhizal fungi enhance fruit growth and quality of Chile ancho (*Capsicum annuum* L.). Mycorrhiza 2006;16:261–87.

Neto D, Carvalho LM, Cruz C, Martins-Louçao MA. How do mycorrhizas affect C and N relationships in flooded *Aster tripolium* plants? Plant Soil 2006;279:51–63.

Orłowska E, Zubek S, Jurkiewicz A, Szarek-Lukaszewska G, Turnau K. Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. Mycorrhiza 2002;12:1–17.

Quereieta JI, Barea JM, Allen MF, Caravaca F, Roldan A. Differential response of delta ^{13}C and water use efficiency to arbuscular mycorrhizal infection in two arid land woody plant species. Oecologia 2003;135: 510–5.

Ouziad F, Wilde P, Schmelzer E, Hildebrandt U, Bothe H. Analysis of aquaporins and Na^+/H^+ transporters in tomato colonized by arbuscular mycorrhizal fungi and affected by salt stress. Exp Environ Bot 2006;57: 177–86.

Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. Trans Br Mycol Soc 1970;55: 158–61.

Regvar M, Vogel K, Irgel N, Hildebrandt U, Wilde P, Bothe H. Colonization of pennycresses (*Thlaspi* spp.) of the Brassicaceae by arbuscular mycorrhizal fungi. J Plant Physiol 2003;160:615–26.

Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 2003;13:309–17.

Scheloske S, Maetz M, Schneider T, Hildebrandt U, Bothe H, Povh H. Element distribution in mycorrhizal and non-mycorrhizal roots of the halophyte *Aster tripolium* determined by proton induced X-ray emission. Protoplasma 2004;223:183–9.

Sharifi M, Ghorbanli M, Ebrahimzadeh H. Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. J Plant Physiol 2007; online available.

Simon T. Keys to the Hungarian vascular flora. Pteridophytes-flowering plants. Budapest: Tankönyvkiadó; 2000 (in Hungarian).

Smith SE, Read DJ. Mycorrhizal symbiosis, 2nd ed. San Diego, London: Academic Press; 1997.

Stocker O. Das Halophytenproblem. In: Frisch KV, Goldschmidt R, Ruhland W, Winterstein H, editors. Ergebnisse der Biologie. Berlin: Julius Springer; 1928. p. 266–353.

Tian CY, Feng G. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. Appl Soil Ecol 2004;26: 143–8.

Tonin C, Vandenkoornhuyse P, Joner E, Straczek J, Leyval C. Diversity of AM fungi in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. Mycorrhiza 2001;10: 161–8.

Trouvelot A, Kough JL, Gianinazzi V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de methodes d'estimation ayant une signification fonctionelle. In: Gianinazzi-Pearson V, Gianinazzi S, editors. Physiological and genetical aspects of mycorrhizae. Paris: INRA; 1986. p. 217–21.

Vogel-Mikuš K, Drobne D, Regvar M. Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. Brassicaceae from the vicinity of a lead mine and smelter in Slovenia. Environ Pollut 2005;133:232–42.

Wu QS, Xia RS. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of *Citrus* under well-watered and water stress conditions. J Plant Physiol 2006;163:417–25.