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Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes

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Abstract Halophytes from both coastal and inland Central European salt marshes were examined for colonization by arbuscular mycorrhizal (AM) fungi. Plants from different families were strongly colonized but the degree of colonization varied with the individual plant and apparently during the vegetation period, too. Members of the typical non-mycorrhizal families like *Armeria maritima* of the Plumbaginaceae and *Salicornia europaea* of the Chenopodiaceae were found to be colonized, particularly in the drier salt marshes. High numbers of *Glomus* spores were found in the saline soils, especially those of the inland locations examined. Approximately 80% of these spores were from *Glomus geosporum* as shown by a typical restriction fragment length polymorphism (RFLP) pattern of the amplified internal transcribed spacer regions. The present study demonstrates that RFLP analysis is useful when screening habitats for the occurrence of mycorrhizal fungi which can be identified only with difficulty by morphological criteria.

Keywords Salt marsh mycorrhizae · Mycorrhizal halophytes · *Glomus geosporum* · Restriction fragment length polymorphism · Salt tolerance

Introduction

Approximately 7% of the global land surface is covered with saline habitats (Ruiz-Lozano et al. 1996) which have remarkably similar plant communities and zonal distributions of species in dependence on the salt concentration (Chapman 1960; Walter 1968). In such soils, the degree of salinity apparently governs the competitiveness of plants, whereas other parameters like soil texture, structure or pH are of minor importance for plant life. Literature on the mycorrhizal colonization of plants in such habitats is controversial. Besides grasses, plant species of salt marshes belong to the families Chenopodiaceae, Plumbaginaceae, Juncaceae, Juncaginaceae, Brassicaceae, and others which are believed to be non-mycorrhizal (Hirrel et al. 1978; Brundrett 1991; Smith and Read 1997). An extensive review (Juniper and Abbott 1993) summarized the evidence that germination of mycorrhizal spores, their subsequent hyphal growth and their effectiveness in colonizing plant roots are reduced by increasing concentrations of salts. Salt marshes are rich in water and are often waterlogged. High salt and water contents in soils are unfavourable for the growth of mycorrhizal fungi. Thus it is believed that plants of such locations are generally not colonized by arbuscular mycorrhizal (AM) fungi (Peat and Fitter 1993). On the other hand, data are scattered amongst the literature showing that AM plants can occur in salt marshes world-wide, and that the content of AM fungal spores in saline soils can be high. Colonization of halophytes by AM fungi had already been described a long time ago (Mason 1928), and also more recently (Kahn 1974; Hoefnagels et al. 1993; Brown and Bledsoe 1996). The degree of mycorrhizal colonization of the roots of the salt aster (*Aster tripolium*) is rather high and depends on the carbohydrate supply (Boullard 1959). Even species of the Chenopodiaceae, the most salt-tolerant *Salicornia* sp. and *Suaeda maritima* can be colonized; this has been described for samples from Utah (Kim and Weber 1985), The Netherlands (Rozema et al. 1986; Van Duin et al. 1989) and west Bengal (Sengupta and Chaudhari 1990).

This somewhat controversial literature encouraged us to screen plants of several saline habitats in Central Europe for their colonization by mycorrhizal fungi.

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One coastal site (Schiermonnikoog island, The Netherlands) was selected on the North Sea, and two others (Hiddensee, and Fehmarn islands) on the shores of the Baltic Sea. At inland locations, ground water occasionally transports NaCl from soil depths to the soil surface, and such places carry a fascinating vegetation with remarkably similar zonal distributions to those at coastal marshes. Such sites turned out to be particularly rewarding in the present study because they were found to contain extraordinarily high numbers of AM spores. The predominant fungal species at such locations was identified as *Glomus geosporum*. Its identity at the different locations was ascertained by restriction fragment length polymorphism (RFLP) analysis using the polymerase chain reaction (PCR) products of the internal transcribed spacer (ITS) region of DNA from single spores.

Materials and methods

The details of the sites investigated are listed in Table 1. Plants investigated or mentioned were from the Apiaceae: *Oenanthe lachenalii* C.C. Gmelin (parsley, water dropwort); Asteraceae: *Artemisia maritima* L. (sea wormwood), *Artemisia rupestris* L. (continental wormwood), *Aster tripolium* L. (sea aster); Caryophyllaceae: *Sagina maritima* G. Don. (sea pearlwort), *Spergularia salina* J. et C. Presl. [*Spergularia marina* (L.) Griseb.], lesser sand-spurrey, *Minuartia verna* (L.) Hiern. (spring sandwort); Chenopodiaceae: *Salicornia europaea* L. (glasswort, marsh sampire); Juncaceae: *Juncus gerardii* Loisel. (salt mud rush); Juncaginaceae: *Triglochin maritimum* L. (sea arrow grass); Plantaginaceae: *Plantago coronopus* L. (buck's horn plantain), *Plantago maritima* L. (sea plantain); Plumbaginaceae: *Armeria maritima* (Miller) Willd. (thrift, sea pink), *Limonium vulgare* Miller (common sea lavender); Poaceae: *Agropyron junceum* (L.) P.B. (sand couch), *Ammophila arenaria* (L.) Link (marram grass), *Festuca rubra* L. f. *litoralis* (strand fescue), *Puccinellia distans* (Jacq.) Parl. (reflexed salt marsh grass), *Puccinellia maritima* (Huds.) Parl. (common salt marsh grass), *Spartina anglica* C. E. Hubbard (common cord grass), *Zea mays* L. (maize); Primulaceae: *Glaux maritima* L. (sea milkwort); Corylaceae: *Carpinus betulus* L. (hornbeam); Tiliaceae: *Tilia cordata* Miller (small-leaved lime); Violaceae: *Viola calaminaria* (DC) Lej. (zinc violet); Brassicaceae: *Thlaspi calaminare* (Lej.) Lej. et Court. (T. *caerulescens* J. et C. Presl) (zinc pennycress); Fagaceae: *Quercus robur* L. (pedunculate oak). The English names are as in Blamey and Grey-Wilson (1989). The fungi were *Glomus geosporum* (Nicolson and Gerdemann) Walker, and *Glomus intraradices* (Schenck and Smith).

To determine the degree of mycorrhizal colonization, plants were collected at the sites, roots were cleansed with tap water, transferred to FAA [mixture of 13 ml formaldehyde solution (min 37%; Merck), 5 ml of 96% acetic acid, 100 ml of 96% ethanol and 100 ml H_2O], stained with lactophenol blue and counted by a slightly modified version of the gridline intersect method (Giovannetti and Mosse 1980), described in detail by Schmitz et al. (1991). Counting of 200–300 root segments for each plant was sufficient to determine the degree of mycorrhizal colonization accurately. The percentages of intraradical hyphae, extraradical hyphae, vesicles or arbuscules are the mean percentages of root segments among all counted which distinctly showed at least one intraradical hypha, extraradical hypha, vesicle or arbuscule, respectively, per root segment. For assessing the amount of extraradical hyphae, hyphae were counted which still adhered to the roots and were thus distinctly stained after taking the plants out of the soils and rinsing them with tap water.

For the isolation of the AM fungal spores, 2- to 5-g soil sample were suspended in about 2 l tap water by extensive stirring, and poured onto sieves with different pore sizes (first 1-mm, then 0.5-mm, 0.08-mm and finally 0.045-mm pore diameter). The spores which were retained on the sieves with 0.045- and 0.08-mm pore widths were transferred to a Petri dish containing water. The spores were stirred by gentle shaking, removed from the upper layer of the suspension with a pipette and transferred into approximately 60 ml water and then into centrifuge tubes containing the same volume of 70% sucrose (household quality). After centrifuging (10 min, 1,400 *g*), the spores in the sucrose layer were removed with a pipette and thoroughly washed on the 0.045-mm sieve to remove the residual sucrose. The spore fraction was transferred into a Petri dish, and any remaining root and debris were removed with a pipette. The fraction obtained was pure enough to be counted.

Conductivity in the soil was determined by a WTW LF 537 microprocessor conductivity meter. For this, 2 g soil was suspended in 10 ml double distilled water and gently stirred to obtain a homogeneous suspension.

For the characterization of the DNA by PCR, the spores were mechanically crushed on a microscope slide and transferred to 0.5-ml microtubes. The method of White et al. (1990) was used to amplify the DNA region between the end of the 18S rRNA, ITSI, 5.8S rRNA, ITSII and the beginning of the 28S rRNA using the primers ITS1 and ITS4. The PCR products were separated by electrophoresis in an 1% (v/v) agarose gel (GibcoBRL ultrapure) followed by staining the DNA with ethidium bromide and photographing it. Another aliquot of the PCR product was restricted by *Alu*I, *Hin*fI, *Hpa*II, *Hae*III, *Bsu*RI and *Taq*I (all from MBI Fermentas). Digests were separated in 2% agarose gels and the DNA was stained with ethidium bromide for photographing.

Results

The degree of mycorrhizal colonization of halophytes

Since the degree of mycorrhizal colonization can vary from plant to plant, and even in individual plants during the vegetation period, within one species (Smith and Read 1997), data for the AM colonization of each plant are given individually, and the total values can be compared from one salt marsh to the next (Table 2). The composites *Aster tripolium, Artemisia maritima* and *Artemisia rupestris* were distinctly colonized at all sampling locations, and the degree of total colonization varied between 6% and 90%. High amounts of extraradical and intraradical hyphae and vesicles were also scored to a similar extent in all composites examined. Arbuscules were detected in plants sampled in August and September, but not in material collected in November. Among plants from the other families, *Oenanthe lachenalii* showed the highest degree of mycorrhizal colonization of all. Plantains are often used as model plants for studying mycorrhiza, so, not unexpectedly, *Plantago maritima* and *Plantago coronopus* and also the Primulaceae, *Glaux maritima*, were colonized at all places examined. Members of typical non-mycorrhizal families, like the Chenopodiaceae: *Salicornia europaea* and the Plumbaginaceae: *Armeria maritima*, did show AM colonization, whereas *Limonium vulgare* of the latter family was not colonized (values $<3\%$ were regarded as not meaningful). Individual plants of the Caryophyllaceae, *Spergularia salina* and *Sagina maritima*,

Table 1 Characteristics of the sites where the plants were sampled and special features of the plant community

Site	Location	Description of the place	Soil type	Special features of the plant community
Saline soils				
Schiermonnikoog I, North Sea (The Netherlands)	6°12′55″E, 53°29′40″N	Near Kobbediunen, typical fertile coastal salt marsh	Silt, Mollisol	Dominated by *Limonium vulgare*, *Aster tripolium*, *Puccinellia maritima*
Schiermonnikoog II	6°15′0″E, 53°29′58″N	Sand bank along an estuarine-type watercourse, directed outwards to the North Sea, near Oosterstand	Sandy loam, Inceptisol	Dominated by *Armeria maritima*, *Festuca rubra*
Schiermonnikoog III	6°9′10″E, 53°28′30″N	Wet marsh (inwards) at Westerplas, between Westerstrand and the old harbour	Rich silt, Histosol	Wet marsh with *Spartina anglica*, *Triglochin maritimum*, *Salicornia europaea*
Fehmarn, Baltic (Burg)	11°1′20″E, 54°26′40″N	Wet marsh near the village of Flügge at the entrance to the Krumm Steert	Silt, Mollisol	Drier salt marsh with lower salt content, *Artemisia maritima*, *Spergularia salina*, *Plantago maritima*
Hiddensee I, Baltic sea (Kloster)	13°6′40″E, 54°35′0″N	Wet marsh between Kloster and Vitte, so-called Langer Ort	Silt, Mollisol	Wetter than Hiddensee II, with *Salicornia europaea*
Hiddensee II	13°6′40″E, 54°35′0″N	Wet marsh between Kloster and Vitte, so-called Langer Ort, distance from no. I ~100 m	Silt, Mollisol	Large variability in H_2O content within short distance, with *Oenanthe lachenalii* preferring waterlogged stands and *Plantago maritima*, and *Armeria maritima* drier spots
Barnstorf (Uehrde)	10°48′30″E, 52°5′50″N	Continental salt marsh, ground H_2O transports NaCl from a stock to the soil surface	Silt loam, Histosol	Zonal distribution of the halophytes depending on the NaCl concentration, as at the coast, *Salicornia europaea* in the centre with, *Juncus gerardii* at the edge, fairly disturbed by cattle grazing
Seckertrift (Jerxheim)	10°54′58″E, 52°4′30″N	Similar to Barnstorf, also NaCl transported upwards from a salt stock in the earth	Silt loam, Histosol	Not disturbed by cattle grazing, also clear zonal distribution of plants depending on salinity
Salt spring (Artern)	11°17′10″E, 51°22′30″N	Watercourse, rich in NaCl	Clay loam, Vertisol	Dominated by *Artemisia rupestris* (or *A. maritima*?) and *Aster tripolium*, a municipal employee cleaned the water course of plants at the sampling date in April 1998
Control soils				
Garden of the Botanical Institute (Köln)	6°55′20″E, 51°00′30″N	Weakly fertilized garden	Sandy loam, Vertisol	Mixed planted vegetation
Forest soil (Chorbusch, Köln-Worringen)	6°48′40″E, 51°02′50″N	Soil of richer forest, typical *Carpino-Quercetum*, soil N content high, inorganic P content low	Clay loam, Alfisol	Oak-hornbeam forest, with typical trees like *Carpinus betulus*, *Quercus robur*, *Tilia cordata*
Heavy metal soil (Schlangenberg, Stolberg)	6°15′30″E, 50°44′20″N	Heavy metal heap, particularly rich in Zn and Fe, near the village of Breinigerberg	Sandy loam, Spodosol	Covered with typical heavy metal-tolerant plants like *Viola calaminaria*, *Armeria maritima*, *Thlaspi calaminare*, *Minuartia verna*
Heavy metal soil (Bottendorfer Höhe, Bottendorf)	11°17′20″E, 51°21′40″E	Heavy metal heap, particularly rich in Fe and Zn	Sandy loam, Spodosol	Covered with heavy metal-tolerant plants like *Armeria maritima*, *Minuartia verna*

showed distinct colonization. Arbuscules could, however, often not be detected, probably due to the fact that the plants were collected late in the year and thus at the end of their life (Table 2). Members of the typical non-mycorrhizal families Juncaceae (*Juncus gerardii*) and Juncaginaceae (*Triglochin maritimum*) did not, indeed, show any indication of AM colonization. Among the typical grasses of the salt marshes, *Festuca rubra* was distinctly colonized in Schiermonnikoog, whereas *Puccinellia maritima* (from the coastal marshes) and *Puccinellia distans* (abundantly in the inland salt habitats) showed a variable pattern of colonization. *Spartina anglica,* a typical constituent of the wet marshes, was not colonized in contrast to *Ammophila arenaria* growing on sand dune patches within the marshes.

Spore numbers in saline and non-saline soils

Similar to the mycorrhizal colonization of the plants, the spore content of the soil adjacent to the roots was patchy. Therefore, the variations were large and com-

Table 2 Mycorrhizal colonization of the roots of halophytes in diverse habitats. When more than one plant has been counted, the numbers for mycorrhizal colonization for each of them is given. *First numbers* are always the counts for the first plant, *second number* for the second plant, etc. The percentage values for intraradical hypha, extraradical hyphae, vesicles and arbuscules are the mean percentages of roots segments among all those counted which distinctly showed at least one intraradical hypha, extraradical hypha, vesicle, or arbuscule, respectively, per root segment. Nature conservation (and in some cases also logistic problems) did not permit us to examine more than one plant at some dates and sites. *Reprod.* Reproductive

Plant	Place where collected	Date	Plant phenophase	Degree of colonization (%)	Extraradical hyphae (%)	Intraradical hyphae (%)	Vesicles (%)	Arbuscules (%)
Asteraceae								
Aster tripolium	Schiermonnikoog I	October 1998	Seeds formed	68	10	62	51	0
A. tripolium	Schiermonnikoog III	October 1998	Seeds formed	15/24/34	11/13/24	10/20/21	3/4/8	0
A. tripolium	Fehmarn	August 1996	Flowering	34	8	29	24	11
A. tripolium	Barnstorf	September 1996	Late flowering stage	82	19	73	59	47
A. tripolium	Barnstorf	August 1999	Flowering	48/6/26	6/1/8	47/6/24	22/2//9	2/0/6
A. tripolium	Barnstorf	November 1998	Seeds formed	75/96	33/74	66/96	19/68	0/3
A. tripolium	Jerxheim	August 1999	Flowering	86/17	27/25	81/13	22/16	25/0
A. tripolium	Jerxheim	November 1998	Seeds formed	85/90/79	39/47/13	67/84/75	22/20/17	2/8/24
A. tripolium	Hiddensee I	August 1999	Flowering	77	25	75	41	21
A. tripolium	Hiddensee II	August 1999	Flowering	88/75/37	21/18/8	82/73/37	67/46/11	30/29/6
A. tripolium	Artern	April 1998	Four–five leaves formed	33	9	30	10	8
Artemisia rupestris	Artern	April 1998	First leaves formed	36	10	35	12	5
Artemisia maritima	Fehmarn	August 1996	Late flowering stage	13	4	8	7	4
A. maritima	Schiermonnikoog I	October 1998	Seeds formed	28	7	23	17	0
A. maritima	Schiermonnikoog III	October 1998	Seeds formed	30/19	28/16	9/21	6/6	0/0
Poaceae								
Puccinellia maritima	Schiermonnikoog II	October 1998	After reprod. phase	35	13	29	16	0.3
P. maritima	Schiermonnikoog III	October 1998	After reprod. phase	5	1	5	0	0
P. maritima	Fehmarn	August 1996	Seeds formed	4	1	2	0	0
P. maritima	Hiddensee I	August 1999	Seeds formed	9/6	2/0	9/6	2/3	1/0
Puccinellia distans	Barnstorf	August 1999	Seeds formed	0/0/8	0/0/0	0/0/8	0/0/3	0/0/2
P. distans	Jerxheim	August 1999	Seeds formed	18/2/3/15	0/1/2/7	13/2/3/15	8/0/0/9	4/0/0/2
P. distans	Barnstorf	November 1998	After reprod. phase	49/6/3	18/1/0	42/6/3	17/0/0	0/0/0
P. distans	Jerxheim	November 1998	After reprod. phase	11/7/1	8/2/0	3/6/1	1/3/0	0/0/0
P. distans	Barnstorf	September 1996	After reprod. phase	0	0	0	0	0
Festuca rubra f. litoralis	Schiermonnikoog I	October 1998	After reprod. phase	33/13/30	23/5/18	20/9/20	20/8/11	0/0/0.2
F. rubra f. litoralis	Schiermonnikoog III	October 1998	After reprod. phase	20/16	5/9	14/12	13/5	0/0
Ammophila arenaria	Schiermonnikoog I	October 1998	After reprod. phase	62	18	55	44	0
Agropyron junceum	Schiermonnikoog III	October 1998	After reprod. phase	8/17	7/15	4/8	1/3	0/0
Spartina anglica	Schiermonnikoog III	October 1998	After reprod. phase	4/1	2/1	3/0	1/0	0/0
Zea mays, field grown[a]	Barnstorf/Jerxheim	August 1998	Seeds formed	2/6	0/5	2/6	0/1	1/0
Plantaginaceae								
Plantago maritima	Schiermonnikoog I	October 1998	After reprod. phase	43/50	26/22	42/44	23/23	0/1.6
P. maritima	Schiermonnikoog III	October 1998	After reprod. phase	30/21	15/11	26/15	14/9	0/0
P. maritima	Fehmarn	August 1996	Seeds formed	20	4	12	3	12
Plantago coronopus	Schiermonnikoog II	October 1998	After reprod. phase	46	31	28	20	0.2
P. coronopus	Schiermonnikoog III	October 1998	After reprod. phase	44	10	38	24	0
Plumbaginaceae								
Armeria maritima	Schiermonnikoog I	October 1998	After reprod. phase	36	11	34	24	0.3
A. maritima	Schiermonnikoog III	October 1998	After reprod. phase	43	30	28	16	0
A. maritima	Hiddensee I	August 1999	Flowering	60/62/64	27/19/27	57/57/59	19/23/21	8/11/12
Limonium vulgare	Schiermonnikoog III	October 1998	Seeds formed	1	0.4	0.4	0	0

Table 2 (continued)

Plant	Place where collected	Date	Plant phenophase	Degree of colonization (%)	Extraradical hyphae (%)	Intraradical hyphae (%)	Vesicles (%)	Arbuscules (%)
Chenopodiaceae								
Salicornia europaea	Barnstorf	August 1999	Flowering	23/20/17	9/12/6	23/17/17	3/2/12	0/2/0
S. europaea	Jerxheim	August 1999	Flowering	14/15/0/24	9//7/0/13	4/15/0/24	3/9/0/5	0/2/0/3
S. europaea	Barnstorf	November 1998	After reprod. phase	64/45/41/5/24	33/32/33/1/19	45/31/24/5/10	17/17/2/0/0	0/0/0/0/0
S. europaea	Jerxheim	November 1998	After reprod. Phase	11/50	6/21	7/37	1/14	0/0
S. europaea	Fehmarn	August 1996	Flowering	3	0	1	1	2
Primulaceae								
Glaux maritima	Schiermonnikoog II	October 1998	After reprod. Phase	42	10	32	23	0
G. maritima	Schiermonnikoog III	October 1998	After reprod. Phase	10	9	7	3	0
G. maritima	Fehmarn	August 1996	Seeds formed	20	4	12	3	12
Juncaginaceae								
Triglochin maritimum	Schiermonnikoog III	October 1998	After reprod. phase	1	1	1	0.3	0
T. maritimum	Hiddensee I	October 1998	After reprod. phase	0/0	0/0	0/0	0/0	0/0
Caryophyllaceae								
Spergularia salina	Hiddensee I	August 1999	Seeds formed	0/14	0/4	0/13	2	3
S. salina	Barnstorf	November 1998	After reprod. phase	12	6	7	2	0
S. salina	Jerxheim	November 1998	After reprod. phase	19	9	10	6	0
S. salina	Fehmarn	August 1996	Seeds formed	2	0	0	0	0
Sagina maritima	Schiermonnikoog II	October 1998	After reprod. phase	43	37	29	7	0
Juncaceae								
Juncus gerardii	Barnstorf	November 1998	After reprod. phase	0/6	0/2	0/3	0/0	0/0
J. gerardii	Jerxheim	November 1998	After reprod. phase	1	0	0	0	0
Apiaceae								
Oenanthe lachenalii	Hiddensee II	August 1999	Flowering	91/93/92	68/76/51	91/90/92	61/56/87	27/16/41

[a] In the direct vicinity of the inland salt marsh, non-saline Histosol (Chernozem-type) soil

parisons can only be made between the spore numbers among the different salt marshes (Tables 3, 4). Soils from the plant roots of the continental salt marshes at Jerxheim as well as at the Baltic island of Hiddensee had high amounts of spores compared to the situation in the Atlantic environment, at Schiermonnikoog. It happened that no spore was detected in one sample from Barnstorf. However, extremely high spore numbers were scored in other samples from Barnstorf, and also from Jerxheim and Hiddensee. It was not feasible to analyse samples larger than 2–5 g for practical reasons, and this would also have meant disturbing the plant life in these areas significantly. Taking all the data together, the average spore numbers were particularly high at the continental salt marshes of Jerxheim, Barnstorf and also of the Baltic island, Hiddensee (Tables 3, 4). The spore numbers in the soils around the roots were apparently not correlated with the degree of mycorrhizal colonization. Plants with a high degree of mycorrhizal colonization like *Artemisia maritima* or *Aster tripolium* did not show high spore numbers in the soil adjacent to their roots (Table 3). The soil around the roots of *Artemisia rupestris* and *Aster tripolium* (taken out of the creek originating from the salt spring) at Artern had an extremely high salt content (as indicated by the conductivity measurement) and also contained AM spores.

A comparison between spore numbers of these soils and of non-saline habitats (Table 4) indicated that the spore content was very high in the continental salt marshes at Jerxheim and Barnstorf. These saline habitats are located within one of the most fertile areas in Germany. The non-saline, Histosol (Chernozem type) soil in the immediate neighbourhood and even at a ~1 km distance had fairly high numbers of mycorrhizal spores. Since saline and heavy metal sites share features, e.g. *Armeria maritima* occurs in both soil types and only there, spore content was also determined in two heavy metal soils of different areas in Germany for comparison. Both also contained spores, though in drastically lower amounts than the salt marshes. Surprisingly, the phosphorus-limited soil of an oak/hornbeam forest in the vicinity of Köln had a very low number of spores (Table 4), and the herbs in this soil were not colonized by AM fungi (details not documented). In the non-saline soils, the fluctuations in the spore

Table 3 Spore content of soil samples from the roots of plants of salt marshes. Soil samples were taken from the rhizosphere of the different plants. The spore content was determined as number of spores isolated/g soil wet weight. Determinations of two different soil samples from one plant are separated by /. The letters a, b, c indicate individual soil samples from different plants at the same location. For the closely related salt marsh grasses, *P. maritima* is the predominant coastal grass, whereas *P. distans* settles inland saline habitats. Means, SDs and the number of determinations (in parentheses) are given. Conductivity of the soil was determined in 2 g soil suspended in 10 ml H_2O in each case. * indicates the data given for the second species of the same genus.

Plant		Barnstorf	Jerxheim	Hidden-see I	Hidden-see II	Schier-monni-koog I	Schier-monni-koog II	Schier-monni-koog III	Artern
Salicornia europaea	a	44/26	147/227	73/19	132				
	b	19/19	74/185	23/20	21				
	c		23/269						
Puccinellia distans/maritima*	a	0/2	92/52	27/30*			2/3		
	b		63/121	15/6*					
	c		141/178						
Aster tripolium	a	2/0	205/81	20/35	407	2/34		2/8	6/9
	b	50/57	105/65		34				
	c		105/88						
Spergularia salina	a	8/11		9/10					
	b			27/40					
Armeria maritima	a			32/53		11/33		1/1	
	b			541/258					
Plantago maritima	a				99				
	b				29				
	c				68				
Oenanthe lachenalii	a				68				
	b				108				
	c				198				
*Plantago maritima/coronopus**						1/17	1/3	1/27*	
*Artemisia maritima/rupestris**						1/2		1/1	8*
Average spore content in the soil		20 ± 20 (12)	123 ± 67 (18)	69 ± 130 (18)	116 ± 115 (10)	13 ± 14 (8)	2 ± 1 (4)	5 ± 9 (8)	8 ± 2 (3)
Conductivity of the soil (dS/m)		*11.2*	*21.9*	*11.7*	*5.1*	*1.9*	*0.1*	*4.6*	*25.8*
Zea mays from the surroundings; *conductivity*		16/17; *0.3*	20/10; *0.8*						

Table 4 Amount of arbuscular mycorrhizal fungal spores isolated from different soils; mean ± SD, *n* in parentheses

Soil	Mean number of spores/g soil wet weight ± SD (*n*)
Salt marshes (inland)	
Jerxheim, undisturbed	108 ± 64 (25)
Barnstorf, partly disturbed	56 ± 75 (16)
Artern, in the water course	8 ± 1.5 (3)
Salt marshes (coastal)	
Hiddensee, Baltic sea I (wet)	69 ± 131 (18)
Hiddensee, Baltic sea II (drier)	116 ± 115 (10)
Schiermonnikoog I, North Sea, wet marsh	13 ± 15 (8)
Schiermonnikoog II, North Sea, dryer marsh	2.3 ± 1.0 (4)
Schiermonnikoog III, North Sea, wetter marsh	5.3 ± 9.1 (8)
Heavy metal soils	
Breinigerberg/near Aachen	3.5 ± 0.2 (3)
Bottendorfer Höhe, Sachsen-Anhalt	2.6 ± 0.15 (3)
Control (non-polluted) soils	
Cologne (oak/hornbeam forest), inorganic P limited	0.7 ± 0.1 (6)
Cologne, garden of the Botanical Institute	4.7 ± 0.15 (10)
Jerxheim, directly neighbouring the saline area	16 ± 4 (6)
Barnstorf, directly neighbouring the saline area	18 ± 10 (2)
Barnstorf, ~1 km away from the saline area	12 ± 5.5 (2)

numbers were not so extreme as at Jerxheim, Barnstorf and Hiddensee (Table 4).

Identification of the main fungus in the salt marshes as *Glomus geosporum* using RFLP analysis

Spores from the different salt marshes were used for PCR amplification of the ITS regions between the 18S and 28S rRNA and for subsequent restriction enzyme analysis. DNA from 10–30% of visibly intact spores was successfully amplified by PCR under the conditions employed (without using nested PCR, spores from Schiermonnikoog gave fewer PCR products than those from Jerxheim for unknown reasons). About 80% of the spores from the salt marshes provided one distinct RFLP pattern (Fig. 1a, Table 5). Prof. Janusz Blaszkowski (Botanical Institute of the University Szczecin, Poland) identified these spores as *Glomus geosporum* (Nicolson and Gerdemann) Walker by morphological criteria. A culture of *G. geosporum* from the Banque Européenne des Glomales (BEG11, provided by Dr V. Gianinazzi-Pearson, INRA, Dijon, France) gave a restriction pattern identical to that from the spores of the salt marshes, with the exception of two minor additional bands in the case of BEG11 DNA restricted with *Bsu*RI (Fig. 1b). The *G. geosporum* RFLP pattern was not detected in spores from the four non-saline habitats

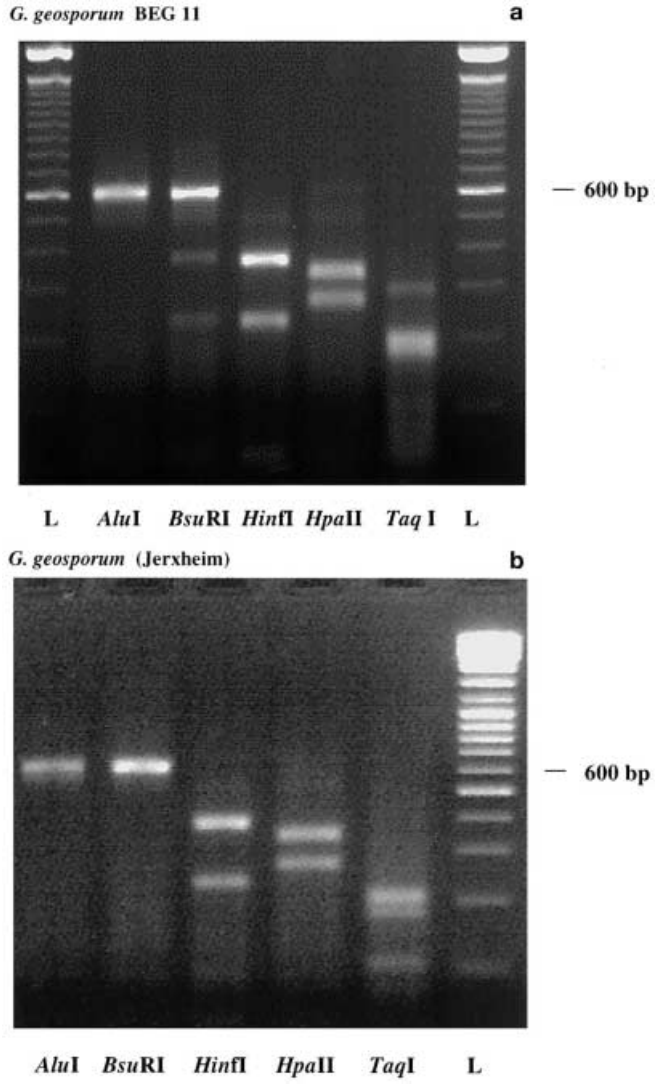

Fig. 1a,b The restriction fragment length polymorphism pattern of the polymerase chain reaction products obtained from spores of *Glomus geosporum* BEG11 (**a**) and of *Glomus geosporum* isolated from the salt marsh at Jerxheim (**b**). The lengths (bp) of the fragments obtained after restriction with: *Alu*I 610, *Bsu*RI 610 (and *Bsu*RI 390, *Bsu*RI 240 in the case of *G. geosporum* BEG11), *Hin*fI 390, 240; *Hpa*II 340, 290; *Taq*I (300), 210 190, 100, (80). *S* 100-bp ladder (Gibco)

examined far away from the salt marshes (Table 5). It was, however, detected several times in spores from soil samples collected ~1 km from the salt marshes at Barnstorf and Jerxheim (not documented). The *G. geosporum* PCR product of 610 bp was cloned and sequenced. The sequence was deposited into the EMBL databank (accession no. GenBank AF231469) and showed 93% identity to the deposited sequence for BEG11 in the same databank (accession no. AJ239122).

Table 5 The frequency of the *Glomus geosporum* restriction pattern from spores of different soils. Spores were isolated from the different locations and their DNA was used for amplification by polymerase chain reaction and restriction enzyme analysis. The major pattern (lane 2) was from *Glomus geosporum* (see text). Samples from the non-saline, Histosol (Chernozem-type) soil were taken from the close vicinity (~1 km distant) of the salt springs at Barnstorf and Jerxheim

Location	*G. geosporum* pattern	Other patterns		% *G. geosporum* pattern
		Sum	Numbers of different patterns	
Saline soils				
Barnstorf, saline	14	1	1	93.3
Non-saline, vicinity	6	3	3	66.7
Jerxheim, saline	15	2	1	88.2
Non-saline, vicinity	7	1	1	87.5
Hiddensee I	7	4	3	63.6
Hiddensee II	4	6	1	40.0
Schiermonnikoog, sum	13	2	2	87.0
Schiermonnikoog I	9	1	1	90.0
Schiermonnikoog II	2	0	0	100
Schiermonnikoog III	2	1	1	67.7
Artern	6	4	1	60
Non-saline soils				
Botanical garden Köln	0	8	5	0
Heavy metal soils				
Breinigerberg	0	22	8	0
Bottendorfer Höhe	0	10	2	0
Hornburg, near Bottendorf	0	11	1	0

Discussion

In the present study, many individuals of different plant species in the salt marshes were >3% mycorrhizal, and typical intraradical and extraradical hyphae as well as vesicles and – in summer – arbuscules were detected. Spore numbers in the saline soils were particularly high, especially in the inland and Hiddensee salt marshes. All these data indicate that mycorrhiza might play a role in such wet habitats, and this is in line with other published results (Read et al. 1976; Kim and Weber 1985; Rozema et al. 1986; Ho 1987; Van Duin et al. 1989; Sengupta and Chaudhari 1990; Cooke and Lefor 1990). However, a statistical evaluation of this type of data is difficult to achieve because the degree of mycorrhizal colonization is not constant during a plant's life cycle, and because spores can be patchily distributed in soils. Strings of spores bundled to hyphae often occur in soils, which can occasionally lead to extremely high counts for spore numbers in a single soil sample. It would have been interesting to analyse a transect in a salt marsh at any place bordering the Atlantic Ocean, from a *Salicornia* stand approaching the low tidal zone across the marsh till drier, sand dune-like areas. This could reveal whether AM fungal species richness influences plant biodiversity, as has been

shown for other ecosystems (Van der Heijden et al. 1998).

The study of the inland salt marshes provided the most interesting data. Extremely high spore numbers were found in the saline soils of Barnstorf and Jerxheim, with approximately 80% of these spores belonging to *Glomus geosporum*. These salt marshes are located within one of the most fertile soils (Histosol, Chernozem type) in Germany, and the neighbouring, non-saline areas had a much lower, though still surprisingly high, spore content. Mineral nutrition in these nutrient-rich soils is unlikely to be the major factor determining the colonization of the plants by AM fungi. The fungal structures may bind or exclude NaCl and may thereby confer salt tolerance to the plants in these habitats, as has recently been described for heavy metals and a *Glomus intraradices* isolate from the zinc violet, *Viola calaminaria* (Hildebrandt et al. 1999; Kaldorf et al. 1999). Another explanation is even more likely. The salt marshes at Barnstorf and Jerxheim are at the leeside of the Harz mountains and belong to the drier areas in Germany with an annual precipitation of <500 mm. These inland areas regularly suffer from drought over longer periods, particularly in summer, and the water potential in such saline soils can be highly negative, as indicated by the conductivity parameters. Thus halophytes might have to endure "physiological drought" (Schimper 1898) which may partly be relieved by the effective water and nutrient exploitation of the soils achieved by the mycorrhizal hyphae. The salt marshes investigated in the Baltic Sea are also drier than the Atlantic ones in Schiermonnikoog, which may explain the higher spore content in the soil and the larger degree of mycorrhizal colonization of the plants at Hiddensee. The plants in the wet salt marsh at Schiermonnikoog (site III) were apparently less colonized than those at the drier site II which had a very low conductivity value.

The present study provided the somewhat unexpected information that plants of typical non-mycorrhizal families can be colonized by AM fungi, which is, however, partly in agreement with the literature (Kim and Weber 1985; Van Duin et al. 1989; Sengupta and Chaudhari 1990; Bhardwaj et al. 1997). The Plumbaginaceae species *Armeria maritima* was also found to be colonized by AM fungi in heavy metal soils (as ssp. *halleri*), though to a highly variable extent (Hildebrandt et al. 1999). *Salicornia europaea* (Chenopodiaceae) can be colonized by AM fungi (Kim and Weber 1985; Rozema et al. 1986; Van Duin et al. 1989), but has never been found to show such a high degree of mycorrhizal colonization as in the present study. This annual plant colonizes sites with the highest salt content where no other species can survive. Thus any contribution by any other mycorrhizal plant associated with *Salicornia* (Hirrel et al. 1978) can be excluded at the "monoculture" stands at Jerxheim or Barnstorf. It remains to be shown whether *Glomus geosporum* and *Salicornia* establish a partnership with mutual benefits. The very high spore content in such soils may force the AM fungi to colonize plants which they avoid in other habitats. On the Atlantic Ocean, *Salicornia europaea* (as the ssp. *dolichostachya*) can settle the wettest stands, and extends even to the low tide zone (Ellenberg 1996). Preliminary data (unpublished) indicate that *Salicornia* and also the other member of the Chenopodiaceae found there, *Suaeda maritima* Forsk., are not colonized at such stands. The genus *Salicornia* is currently subdivided into several species and subspecies by not totally resolved and definitive criteria, and an imperfectly known distribution (Rothmaler 1988; Blamey and Grey-Wilson 1989). It remains to be shown whether the prevailing subspecies of the inland marshes, *Salicornia europaea* ssp. *europaea*, is highly colonized by AM fungi while other subspecies of the genus are not. Whereas most halophytes grow better in non-saline soils, only few plants like *Lepidium crassifolium* W. and K. (Weissenböck 1969) and *Salicornia europaea* (own unpublished data; Bothe 1976) need NaCl for growth from germination on. It is possible that mycorrhizal fungi may be particularly helpful in the germination of *Salicornia*, as seen in orchids where their germination was described as being dependent on their specific fungi (Burgeff 1934).

The present study showed that RFLP analysis of the ITS region can be successfully applied to screen for the distribution of a species like *Glomus geosporum* at different locations. The RFLP pattern for *G. geosporum* was not detected with spores from the three other locations (two heavy metal soils and soil from a garden) which, however, does not mean that *G. geosporum* is restricted to salt marshes. The BEG11 isolate used as a reference was isolated from pasture grassland according to information provided by the BEG (see http://www.bio.ukc.ac.uk/beg/). Spores with the same RFLP pattern were also isolated from the Chernozem soils in the vicinity of the salt marshes. These spores had a different appearance under the light microscope and would not have been recognized by us as belonging to *G. geosporum* without using PCR. DNA from spores of one species can occasionally provide more than one PCR product (Sanders et al. 1995) which was confirmed in our own work (Hildebrandt et al. 1999). On the other hand, the ITS region is highly variable and should provide PCR product(s) and restriction pattern(s) at the subspecies or even ecotype level. In the present study, only one PCR product was obtained with *G. geosporum* both from the salt marshes and from the isolate BEG11. The DNA restriction pattern of the PCR product was uniform for the *G. geosporum* spores from the different salt marshes, which was slightly different (in the restriction by *Bsu*RI) from that obtained for the isolate BEG11. However, the close similarities in the RFLP patterns and the high DNA sequence identity of 93% strongly indicate that the isolate from the salt marsh belongs to the species *Glomus geosporum*. The nature of these additional weaker bands after restriction with *Bsu*RI is uncertain in the case of the *G.*

geosporum BEG11 PCR product. They could indicate two ITS populations in the spores from this *G. geosporum* isolate. The slight differences in the RFLP patterns could also mean that a *G. geosporum* ecotype exists which is specifically adapted to the adverse conditions of salt marshes. Recently, a *Glomus intraradices* isolate was obtained from the roots of the zinc violet which allows several plants to grow in diverse heavy metal soils provided the nutrient supply is optimized (Hildebrandt et al. 1999). In the case of lettuce, colonization of the roots by AM fungi has been described to partly alleviate salt stress (Ruiz-Lozano et al. 1996). Future work has to show whether the *G. geosporum* from salt marshes confers salt tolerance to plants, either by relieving water stress or by directly binding or excluding salt.

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