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Arbuscular mycorrhizal and dark septate fungal associations in shallot (*Allium cepa* L. var. *aggregatum*) under conventional agriculture

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Abstract – We examined roots of the shallot (*Allium cepa* L. var. *aggregatum*), one of the most popular cultivated crops of the family Aliaceae, cultivated under conventional agriculture for arbuscular mycorrhizal fungal (AMF) and dark septate fungal endophyte (DSE) associations. All the plants had dual colonization of both AMF and DSE associations. The intermediate-type AMF morphology in the shallot is the first report of this AMF type for the family Aliaceae. The extents of total AMF and DSE colonization ranged from 20.7 to 67.3% and 3.6 to 35.3% respectively and varied significantly among fields. Though no significant relationship existed between total AMF and DSE variables, they were correlated to the soil variables. Significant correlations existed between soil P and microsclerotia and also between soils N and K and AMF spore numbers. A total of six AMF spore morphotype belonging to *Glomus* and *Scutellospora* were identified. *Scutellospora calospora* was the most dominant morphotype in the studied fields.

Keywords: *Allium*, arbuscular, endophyte, mycorrhiza, *Glomus*, *Scutellospora*

Abbreviations: AMF – arbuscular mycorrhizal fungus, **DSE –** dark-septate endophyte

Introduction

The herbaceous biennial *Allium cepa* L. (onion) is the most widely cultivated taxon in the family Alliaceae. The onion *A. cepa* var. *aggregatum* is native to South West Asia, but cultivated worldwide. Crops benefit from arbuscular mycorrhizal fungus (AMF) through enhanced uptake of nutrients with low mobility, especially phosphorus. The non-nutritional benefits of AMF include alleviation of plant stresses caused by biotic and abiotic fac-

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tors, and the stabilizing of soil aggregates (SMITH and READ 2008, BOLANDNAZAR et al. 2007, JAIME et al. 2008, GIANINAZZI et al. 2010).

The onion has an inefficient, mostly unbranched, shallow root system with sparse root hairs, and cannot maintain adequate uptake of nutrients such as phosphorus (P) that diffuse slowly through the soil solution, which has a negative effect on yields (MENGEL and KIRKBY 2001). Onions are highly mycorrhizal-dependent (DERESSA and SCHENK 2008). Previous studies do indicate that the onion is highly responsive to mycorrhization, resulting in improved plant growth and yield under normal as well as stressed conditions (JAIME et al. 2008, BOLANDNAZAR et al. 2007, GOUSSOUS and MOHAMMAD 2009, GALVÁN et al. 2011). A significant correlation between natural AMF colonization and onion yields in conventionally managed onion farmlands has recently been reported (GALVÁN et al. 2009). In conventional agriculture, large amounts of fertilizers are used to increase the yield of onion (BOSCH-SERRA and CURRAH 2002). Additionally large amounts of biocides are used in shallot cultivation as this plant is very susceptible to pests and diseases (ANONYMOUS 1986, SUHARDI 1996). Large scale use of fertilizers, biocides and tillage affect both arbuscular mycorrhizal formation and function (see PLENCHETTE et al. 2005).

Diversity of AMF species seems to be essential for sustainable functioning of the ecosystem in the event of sudden changes in environmental conditions (WANG et al. 1985, ABBOTT and GAZEY 1994). Studies indicate the existence of variation in life histories, reproduction abilities and morphology among AMF species (DICKSON 2004, HART et al. 2001, HART and READER 2002). Agricultural practices may induce selection pressure in such a way that a certain AMF group could adapt to changes, establish and proliferate better than others (SINGH et al. 2008). For example, SINGH et al. (2008) indicated that *Camellia sinensis* growing under natural conditions harboured a greater diversity of AMF species than those under cultivation. Further the composition of AMF community could be strongly influenced by the individual plant species through differential effects on mycorrhizal establishment and sporulation (SANDERS and FITTER 1992, BEVER et al. 1996). It is becoming increasingly important to gain a better understanding of AMF diversities under field conditions, as suggested by HUSBAND et al. (2002). Limited studies have examined AMF diversities associated with specific plant species like *Vitis vinifera* (BALESTRINI et al. 2010), *Solanum tuberosum* (DAS and KAYANG 2010b), *Michelia champaca* (DAS and KAYANG 2010a), *Phaseolus aureus* (VALSALAKUMAR et al. 2007) and *Camellia sinensis* (SINGH et al. 2008). Recently, GALVÁN et al. (2009) reported AMF polytypes in onion roots under organic and conventional farming systems using rDNA sequencing.

Crop roots are also colonized by a diverse group of melanaceous, septate fungi known as dark septate fungal endophytes (DSE). The function of DSE in plant roots is unclear and may range from pathogenic or saprophytic to mutualistic (JUMPPONEN and TRAPPE 1998, JUMPPONEN 2001). The occurrence of DSE on several temperate and tropical crop species has been reported (JUMPPONEN and TRAPPE 1998, MUTHUKUMAR and TAMILSELVI 2010). ADDY et al. (2005) speculated that the nature of DSE association with roots may be broader than nutrient transfer as there is no evidence of any specific fungal requirements from the host. An analysis of the role of DSE in ecosystems (MANDYAM and JUMPPONEN 2005) indicated facilitation of nutrient uptake of the host plant, alterations in host water uptake, stress tolerance and utilization of wider nutrient pools by the host through DSE. MANDYAM and

JUMPPONEN (2008) also suggested that a knowledge of DSE abundance in relation to root-associated fungi is essential to understand this significance.

In this study, we examined root colonization by AMF and DSE in shallots under conventional agriculture. The three main questions addressed in this study were (i) Does AMF and DSE colonization vary within and between cultivated fields? (ii) Is there any relationship between AMF and DSE within roots? (iii) Do soil factors influence AMF and DSE colonization or structures within roots? In addition we also assessed the AMF spore numbers to see if they were related to colonization levels and assessed their diversity with the shallot.

Materials and methods

Sampling

Onion root and soil samples were collected during December 2009 from 20 different onion fields (hereafter referred to as F1 to F20) from Sathyamangalam (11°28'N and 77°59'E, 540 m a.s.l), Tamil Nadu, Southern India. The average annual rainfall of this area is 360–600 mm. All the fields selected had uniform cultivation practices and cultivation periods (November to January). The fields were canal-irrigated. Fertilization began with the application of diammonium phosphate (DAP, 16–18% N, 40–48% P) during tillage. Fertilizers were further sequentially applied at 25 days (urea-46%N and DAP), 50 days (ammonium sulphate-21% N, 24% S) and 60 days (muriate of potash) after planting. The crop duration was 70–75 days.

Five randomly selected plants were sampled from each field resulting in a total of 100 plants. Root samples were collected by uprooting the plants. The roots were gently washed free of soil with water, fixed in formalin-acetic acid-70% alcohol (5:5:90; v:v:v) and brought to the laboratory for further processing. Soil shaken from roots and next to plants was collected and shade dried. The dried soil was divided into two halves. One half of the air-dried soil collected from individual plants was packed separately in polythene bags and stored at 4°C to enumerate and extract AMF spores. The second half of the soil of all the individuals from a field was mixed together to form a composite soil sample. These composite soil samples were used to assess the soil chemistry.

Determination of soil characters

Soil pH was determined in 1:1 soil: water (v:v) using a digital pH meter soon after the soil samples arrived in the laboratory. Soil samples were analyzed for sand, silt and clay fractions by the hydrometer method (BOWELS 1992). The total nitrogen (N) and available P were determined according to JACKSON (1971) and exchangeable potassium (K) was determined after extraction with ammonium acetate (JACKSON 1971). The iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were determined according to DTPA method (LINDSAY and NORVELL 1978).

Preparation of roots for AMF and DSE assessment

Fixed roots were washed thoroughly in tap water. The roots were cut into 1-cm bits, cleared in 2.5% KOH at 90 °C (KOSKE and GEMMA 1989) for 60 min, acidified with 5N HCl

and stained with tryphan blue (0.05% in lactoglycerol) overnight. The stained roots were examined with an Olympus BX 51 compound microscope (400×) for AMF and DSE structures. Aseptate inter or intracellular, linear or coiled fungal hyphae accompanied with arbuscules or arbusculate coils were used to designate AMF colonization. Melanized, regularly septate fungal hyphae with microsclerotia or moniliform cells characterized DSE colonization. The percentage of root length colonized by AMF and DSE was estimated according to the magnified intersection method (MCGONIGLE et al. 1990). Classification of AMF morphology of the shallot was as per DICKSON (2004).

Enumeration and isolation of AMF spores

Spores were extracted from 100 g of soil by modifications of the wet-sieving and decanting techniques. After wet-sieving and decanting, the residues on the sieves (710 and 37 μm) were washed into beakers. The beaker contents were collected over a filter paper, spread on Petri dishes and scanned under a dissection microscope at 40× magnification. All intact spores (non-collapsed with cytoplasmic content) free from parasitic attack were counted. Sporocarps and spore clusters were considered as one unit. For identification, spores with similar morphology were mounted on slides with polyvinyl alcohol-lactic acid-glycerol (PVLG) or PVLG Melzer's reagent for identification (KOSKE and TESSIER 1983). Spores were identified based on spore morphology and sub-cellular characters (SCHENCK and PEREZ 1990) using descriptions available on Schüsslers web site (http://www.lrz.de/~schuessler/amphylo/amphylogeny.html), and INVAM (http://www.invam.caf.wvu.edu).

Frequency of occurrence (%) for each AMF spore morphotype was calculated as the percentage of the number of the field soil possessing spores of that morphotype. AMF species richness represents the number of taxa occurring in a field.

Statistical analysis

Data on AMF and DSE colonization and AMF spore numbers were subjected to analysis of variance (ANOVA) to assess the significance of variance among the plants and fields. Pearson's correlation was used to assess the relationship between the root colonization, spore numbers and the soil parameters (SPSS, Windows version 9). Spore numbers were log transformed and the percentage data on root colonization was arcsine transformed prior to analysis.

Results

Soil characteristics

Seventy percent of the fields studied had sandy loam soil with 30% of the remaining fields having sandy clay loam (Tab. 1). Soil chemistry varied between fields. The soil was moderately basic with the pH ranging from 7.9 (F4-6, 9) to 8.5 (F19). The EC ranged between 0.18 (F16) and 0.52 (F9) dS m^{-1}. The total N, available P and exchangeable K range observed were 67 (F7, 18) to 118 (F19) mg kg^{-1}, 5 (F7) to 8 (F17) mg kg^{-1} and 200 (F16) to 300 (F19) mg kg^{-1} respectively. Similarly, soils had micronutrient concentration ranges of 3.82 (F1, 11) to 7.94 (F19) mg kg^{-1} Fe, 2.08 (F1, 11) to 2.99 (F18) mg kg^{-1} Mn, 1.33 (F1, 17) to 1.98 (F13) mg kg^{-1} Zn and 0.69 (F16) to 1.66 (F3) mg kg^{-1} Cu.

Tab 1. Soil characteristics of shallot onion cultivated fields in Tamil Nadu.

Field	Sand	Silt	Clay	Soil type	pH	Ec	Total N	Available P	Exchangeable K	Fe	Mn	Zn	Cu
						(d Sm^{-1})	(mg kg^{-1})						
F1	58	13	29	Sandy clay loam	8.3	0.26	73	5.4	225	3.82	2.08	1.33	1.41
F2	40	16	44	Sandy loam	8.0	0.30	76	6.4	215	4.22	2.16	1.43	1.32
F3	35	30	35	Sandy loam	8.1	0.23	78	5.2	220	4.30	2.18	1.63	1.66
F4	62	19	19	Sandy loam	7.9	0.29	70	5.6	235	4.72	2.19	1.52	1.23
F5	55	23	23	Sandy loam	7.9	0.31	76	6.8	215	3.96	2.57	1.72	1.42
F6	60	16	24	Sandy clay loam	7.9	0.50	80	7.6	205	3.90	2.52	1.59	1.37
F7	45	32	23	Sandy loam	8.2	0.26	67	5.0	205	5.12	2.47	1.63	1.40
F8	45	23	32	Sandy loam	8.1	0.27	72	5.8	220	5.69	2.52	1.78	1.52
F9	57	10	33	Sandy loam	7.9	0.52	80	6.2	225	4.42	2.62	1.72	1.52
F10	57	24	19	Sandy loam	8.0	0.34	77	5.8	220	3.85	2.67	1.66	1.12
F11	25	33	42	Sandy loam	8.1	0.43	71	7.2	230	3.82	2.08	1.53	1.63
F12	42	29	29	Sandy loam	8.2	0.26	76	6.4	215	4.22	2.36	1.46	1.52
F13	43	29	29	Sandy loam	8.3	0.22	74	5.8	220	4.17	2.64	1.98	1.43
F14	55	20	25	Sandy loam	8.1	0.23	71	7.2	235	4.20	2.64	1.55	1.27
F15	52	9	39	Sandy clay loam	8.2	0.33	78	6.2	210	3.85	2.60	1.92	1.36
F16	36	44	20	Sandy loam	8.1	0.18	81	7.5	200	4.72	2.84	1.54	0.69
F17	46	19	35	Sandy clay loam	8.4	0.27	84	8.0	220	5.92	2.55	1.33	0.93
F18	76	12	12	Sandy loam	8.2	0.38	67	7.0	230	6.88	2.99	1.42	0.98
F19	53	20	27	Sandy clay loam	8.5	0.35	118	6.0	300	7.94	2.50	1.53	0.84
F20	52	19	30	Sandy clay loam	8.2	0.32	84	7.0	235	6.16	2.74	1.40	0.79

Determination of AMF and DSE associations

Roots of all the onion plants examined from different fields had dual colonization of both AMF and DSE. AMF colonization was characterized by the formation of an appressorium on the root surface (Fig. 1a). A colonization peg arising from the appressorium spread within the roots to form intraradical colonization. Colonization is characterized by intracellular hyphae with arbuscules (Fig. 1b), intracellular hyphal coils or arbusculate coils (Fig. 1c), intercellular hyphae and vesicles (Fig. 1d). Arbuscules were present in all the root samples examined. Septate hyphae (Fig. 1e) along with moniliform cells and/or microsclerotia (Fig. 1f) characterized DSE colonization. Microsclerotia was absent in onion roots collected from three fields (F11, F12, F14), whereas moniliform cells were not observed in shallot roots collected from five fields (F1-3, F15, F17) (Tab. 2).

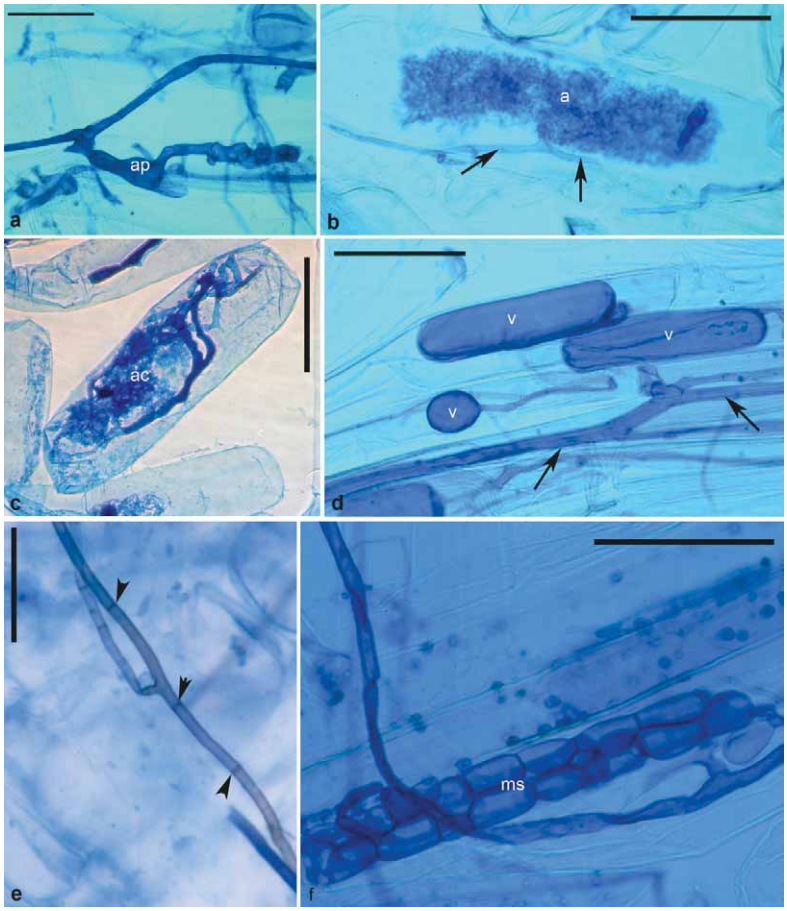

Fig. 1. Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal colonization in the shallot. **a** – Appressorium (ap) of AMF on the root surface; **b** – Arbuscule (a) and intracellular hyphae (arrows) in root cell; **c –** Arbusculate coil (ac) within a root cell; **d –** Vesicles (v) and intercellular hyphae; **e** – Septation (arrow heads) in DSE hyphae; **f** – Microsclerotia (ms) of DSE in cortical cell. Scale bars = 50μm.

Tab. 2. Extent of arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal association in rhizosphere soils of shallot onion.

Fields	AM						DSE			
	Colonization (%)				Spore number ($100g^{-1}$)	AM fungal species	Colonization (%)			
	RLH/RLHC	RLA/RLAC	RLV	RLTC			RLDH	RLMO	RLMI	RLDTC
F1	31.8 ± 6.2	5.7 ± 2.2	11.0 ± 4.3	48.5 ± 11.7	11.2 ± 2.5	GE, GS1, SC	13.5 ± 3.0	–	0.7 ± 0.5	14.2 ± 3.3
F2	20.5 ± 4.8	2.4 ± 0.2	2.7 ± 1.6	25.6 ± 6.1	14.3 ± 0.9	GS, GA, SC	33.8 ± 4.7	–	1.5 ± 0.8	35.3 ± 5.0
F3	37.0 ± 5.2	10.1 ± 2.2	0.2 ± 0.1	47.3 ± 6.6	8.9 ± 2.6	GA, GS1	14.1 ± 4.9	–	0.2 ± 0.1	14.3 ± 5.0
F4	37.1 ± 2.5	7.4 ± 2.7	1.0 ± 0.8	45.5 ± 4.6	10.3 ± 1.6	GE, GA	12.4 ± 3.4	0.4 ± 0.5	0.7 ± 0.4	13.5 ± 4.0
F5	15.7 ± 3.2	3.9 ± 0.7	1.1 ± 0.7	20.7 ± 3.7	8.6 ± 1.4	GE, GA, SC	3.8 ± 1.4	1.4 ± 1.0	0.2 ± 0.1	5.4 ± 2.4
F6	31.2 ± 4.2	15.2 ± 3.6	0.4 ± 0.3	46.8 ± 6.3	16.7 ± 1.6	GS, GV, SC	4.3 ± 0.9	0.1 ± 0.1	0.1 ± 0.1	4.5 ± 0.9
F7	34.8 ± 3.0	9.5 ± 3.3	1.7 ± 1.2	46.0 ± 4.9	15.3 ± 2.2	GV, GE, SC	14.4 ± 3.2	0.8 ± 0.6	1.3 ± 0.5	16.5 ± 3.1
F8	34.5 ± 4.5	18.4 ± 3.7	0.2 ± 0.1	53.1 ± 4.9	17.1 ± 2.8	GA, SC	4.5 ± 0.7	0.3 ± 0.2	0.9 ± 0.3	5.7 ± 1.3
F9	35.1 ± 6.5	16.7 ± 5.2	0.3 ± 0.1	52.1 ± 10.8	15.1 ± 0.8	GS1, SC	3.1 ± 1.0	1.7 ± 1.2	0.3 ± 0.1	5.1 ± 1.9
F10	38.1 ± 4.3	12.9 ± 4.0	0.1 ± 0.1	51.1 ± 6.6	13.9 ± 2.2	GE, SC	6.2 ± 2.9	0.9 ± 0.5	0.2 ± 0.1	7.3 ± 3.1
F11	35.9 ± 4.6	16.3 ± 5.0	–	52.2 ± 8.2	8.9 ± 2.3	GE, GS1	4.7 ± 0.6	1.0 ± 0.9	–	5.7 ± 1.2
F12	33.5 ± 3.4	18.1 ± 4.5	0.5 ± 0.4	52.1 ± 5.5	11.9 ± 2.8	GA, GS, SC	4.4 ± 1.4	0.4 ± 0.5	–	4.8 ± 1.6
F13	38.7 ± 2.8	19.1 ± 3.0	0.1 ± 0.1	57.9 ± 5.6	8.8 ± 2.1	GS1	3.5 ± 1.3	1.9 ± 1.0	0.4 ± 0.6	5.8 ± 1.6
F14	30.6 ± 5.7	14.9 ± 3.2	0.6 ± 0.3	46.1 ± 8.5	7.4 ± 1.5	GE, GS1	3.8 ± 1.2	0.4 ± 0.4	–	4.2 ± 1.6
F15	30.3 ± 1.8	15.1 ± 2.3	0.1 ± 0.1	45.5 ± 3.5	8.1 ± 1.3	GS1	3.5 ± 1.2	–	0.1 ± 0.1	3.6 ± 1.2
F16	28.0 ± 3.5	21.6 ± 4.2	0.2 ± 0.1	49.8 ± 7.8	12.6 ± 2.7	GA, GE, GS, GS1, SC	21.7 ± 1.8	0.6 ± 0.4	0.2 ± 0.1	22.5 ± 1.8
F17	41.1 ± 2.0	22.8 ± 3.7	2.9 ± 1.4	66.8 ± 3.4	12.8 ± 3.2	GE, GS, GV,SC	18.1 ± 1.7	–	0.4 ± 0.3	18.5 ± 1.8
F18	38.8 ± 1.9	24.5 ± 1.9	1.4 ± 1.0	64.7 ± 2.1	13.3 ± 3.3	GA, GS, SC	19.7 ± 1.1	0.2 ± 0.2	0.5 ± 0.4	20.4 ± 0.7
F19	42.1 ± 1.1	23.9 ± 3.8	1.3 ± 0.7	67.3 ± 4.2	3.5 ± 0.7	GE, SC	19.5 ± 3.1	0.8 ± 0.6	0.2 ± 0.1	20.5 ± 3.8
F20	35.1 ± 3.6	8.5 ± 1.7	–	43.6 ± 5.2	3.7 ± 1.0	GE, GS, SC	13.0 ± 2.1	1.1 ± 0.7	0.3 ± 0.2	14.4 ± 2.3
Plants($P_{4,200}$)	1.6 **	2.4**	5.9**	1.8**	<1 ns		<1 ns	3.7**	2.6*	<1 ns
Fields($F_{19,200}$)	12.0**	17.5**	20.9**	17.0**	3.71**		47.8**	3.0**	1.7*	34.4**
P×F ($F_{99,200}$)	2.81**	2.7**	3.0**	13.7**	<1 ns		2.5**	1.6**	<1 ns	2.2**

RLH/RLHC – root length with hyphae or hyphal coils, RLA/RLAC – root length with arbuscles or arbusculate coils, RLV – root length with vesicles, RLTC – total colonization, RLDH – root length with dark septate hyphae, RLMO – root length with moniliform hyphae, RLMI – root length with microsclerotia, RLDTC – dark septate endophyte total colonization, GS1 – *Glomus* species 1, GA – *Glomus aggregatum*, GE – *Glomus etunicatum*, GS – *Glomus sinuosum*, GV – *Glomus viscosum*, SC – *Scutellospora calospora*, Numbers represent Mean ± SE

**Significant at 1% level, *Significant at 5% level, ns-non significant.

Extent of AMF colonization

The extent of AMF colonization and the root length with different AMF structures varied significantly among roots collected from various fields (Tab. 2). The percentage of total root length with AMF colonization (%RLTC) ranged from 20.7 (F5) to 67.3 (F19). Total AMF colonization and the percentage of root length with AMF structures significantly varied among plants collected from a field and from different fields. The ranges of percentage root length with AMF hyphae/ hyphal coils (%RLH/ RLHC), arbuscules/ arbusculate coils (%RLA/ RLAC) and vesicles (%RLV) were 15.7 (F5) to 42.1 (F19), 2.4 (F2) to 24.5 (F18) and 0.1 (F10, F13, F15) to 11.0 (F1) respectively. Compared with average %RLH/ RLHC (33.5) and %RLA/ RLAC (14.3), %RLV was lower (1.3) and vesicles were not observed in onion roots collected from two fields (F11 and F20). Correlation analysis indicated %RLH/ RLHC, %RLA/ RLAC and %RLTC to be significantly and positively correlated to soil pH and Fe. Similarly, a significant positive correlation also existed between %RLA/ RLAC and soil Mn. In contrast %RLV was significantly and negatively correlated to soil Zn content.

Extent of DSE colonization

Significant variation in percentage of root length with DSE colonization (%RLDTC) was evident among onion roots collected from different fields and ranged between 3.6 (F15) and 35.3 (F2). The percentage of root length with dark septate hyphae (%RLDH) ranged from 3.1 (F9) to 33.8 (F2) and varied significantly among fields. Likewise, the percentage of root length with moniliform hyphae (%RLMO) and microsclerotia (%RLMI) varied significantly both within and among fields ranged from 0.1 (F6) to 1.9 (F13) and 0.1 (F6, F15)to 1.5 (F2) respectively.

The %RLMI was significantly and negatively correlated with soil P. Similarly %RLDH and %RLDTC was also significantly and negatively correlated to soil Zn and Cu. In contrast, %RLMO was significantly and positively correlated to Zn. Neither %RLDTC nor root length with DSE structures were correlated to %RLTC or AMF structures (Tab. 3).

Distribution of AMF spores

Total spore counts significantly varied among the fields and ranged from 4 (F19, 20) to 17 (F18) spores per 100g soil. Correlation analysis indicated the lack of correlation between %RLTC and spore numbers ($r = -0.022$; $p > 0.05$). However, spore numbers were significantly and positively correlated to soil K and negatively to soil N (Tab. 3).

A total of six AMF morphotypes of two genera were distinguished on the basis of spore morphology; they included *Glomus* species 1, *Glomus aggregatum* N. C. Schenck et G. S. Sm., *Glomus etunicatum* W. N. Becker et Gerd., *Glomus sinuosum* (Gerd. et B.K. Bakshi) Almeida et Schenck, *Glomus viscosum* T. H. Nicolson and *Scutellospora calospora* (T. H. Nicolson et Gerd.) C. Walker et F.E. Sanders, one of which could not be identified at species level (Fig. 2). *Scutellospora calospora* (70%) was the most frequent AMF morphotype associated with onion (Figs. 3, 4).

Tab. 3. Pearson's correlation coefficients between various arbuscular mycorrhizal fungi (AMF), dark septate fungal endophyte (DSE) and soil variables (SV).

SV	AMF					DSE			
	RLH/ RLHC	RLA/ RLAC	RLV	RLTC	SP	RLDH	RLMO	RLMI	RLDTC
pH	0.519*	0.477*	0.306	0.638**	–0.414	0.252	–0.118	–0.041	0.241
EC	0.085	0.095	–0.166	0.068	0.204	–0.256	0.185	–0.212	–0.252
N	0.246	0.328	–0.057	0.318	–0.466*	0.230	0.087	–0.283	0.224
P	–0.161	0.374	–0.203	0.083	–0.023	0.050	–0.082	–0.446*	0.026
K	0.442	0.258	0.041	0.410	–0.608**	0.146	0.129	–0.173	0.148
Fe	0.488*	0.498*	–0.064	0.553*	–0.242	0.426	–0.048	0.091	0.425
Mn	0.099	0.525*	–0.403	0.275	0.014	–0.085	0.264	–0.277	–0.080
Zn	–0.074	0.072	–0.487*	–0.105	0.029	–0.577**	0.459*	–0.118	–0.551*
Cu	–0.184	–0.341	0.016	–0.299	0.267	–0.507*	0.055	0.079	–0.500*

Significance at 1% level (**) and 5% level (*)
RLH/ RLHC - root length with hyphae or hyphal coils
RLA/RLAC - root length with arbuscles or arbusculate coils
RLV - root length with vesicles
RLTC - total colonization
SP - spore number
RLDH - root length with dark septate hyphae
RLMO - root length with moniliform hyphae
RLMI - root length with microsclerotia
RLDTC - dark septate endophyte total colonization

Discussion

Taxa in *Allium* are known to form typical *Arum*-type morphologies (Smith and Smith 1997, Dickson et al. 2007) and this is the first report of the intermediate-type AMF in the genus *Allium* and family Aliaceae. The intermediate-type AMF morphology of the shallot is intermediate to the I3 and I4 subtypes described by Dickson (2004). Although the exact factors influencing AMF morphology are not known, it has been suggested that both host root structure and AMF fungal genotypes influence AMF morphology (Dickson et al. 2007). Shallot roots in conventional agricultural fields had AMF colonization levels within the ranges of those reported in other studies (Muthukumar and Tamilselvi 2010, Aliasgharzad et al. 2009, Goussous and Mohammad 2009, Jaime et al. 2008). However, the average AMF colonization (49.12%) is lower than those reported for onions under conventional cultivation in Fevoland (91%) and Zeeland (72%) of the Netherlands (Galván et al. 2009). Similarly, the %RLA/RLAC in the present study was three- to five-fold lower than reported for conventional onion cultivation in the Netherlands (Galván et al. 2009). Generally, low levels of AMF colonization in conventionally managed soils were explained by high soil P concentrations. This was not clearly the case of the onion fields in the present study as we found those AMF colonization parameters were not correlated to the concentrations of P in the soil. Our observations on the lack of correlation between soil P and AMF variables suggests that the soil P concentrations in the field soils were well below the threshold levels that could influence AMF. The average soil P in the present study (6.4 mg kg^{-1}) was almost seven fold lower than P concentrations reported for Dutch soils used for con-

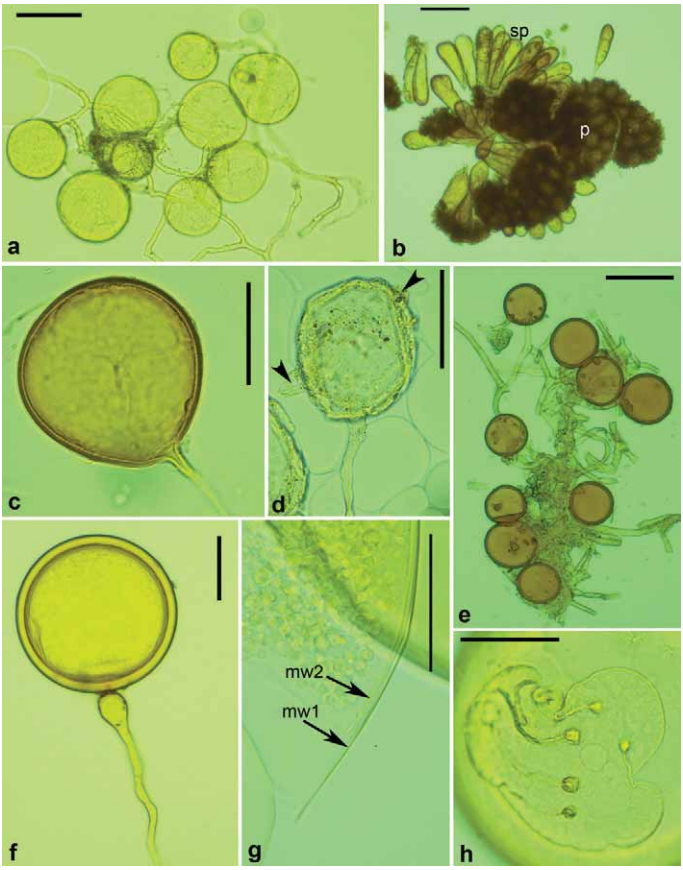

Fig. 2. Arbuscular mycorrhizal spores isolated from the soils of shallot onion. **a** – *Glomus aggregatum*; **b** – Fractured sporocarp of *Glomus sinuosum*; p, peridium; sp, spores; **c** – *Glomus etunicatum*; **d** – *Glomus viscosum* with attached soil particles (arrowheads); **e** – *Glomus* sp.1; **f** – *Scutellospora calospora*; **g** – Membranous walls (mw1, mw2) of *S. calospora*; **h** – Germination shield of *S. calospora*. Scale bars: a, b, e, f = 100 µm; c, d, g, h = 50µm.

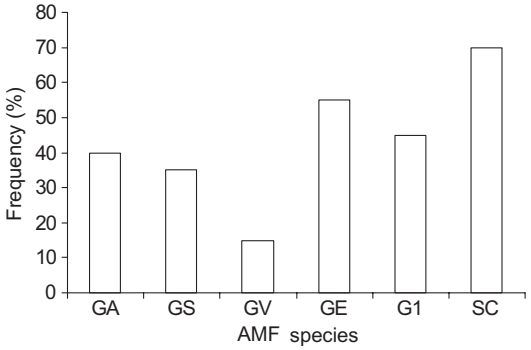

Fig. 3. Arbuscular mycorrhizal fungal (AMF) frequency in the soils used for shallot cultivation. For AMF species abbreviations see Tab. 2.

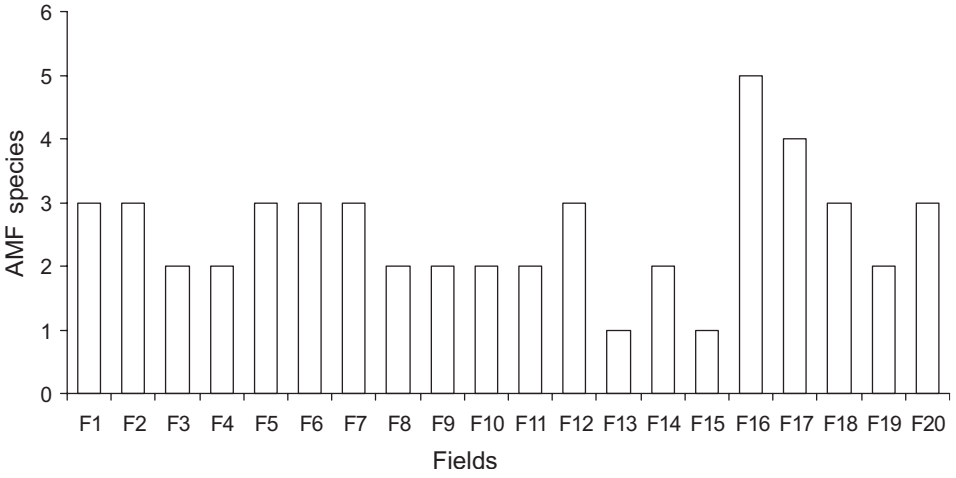

Fig. 4. Arbuscular mycorrhizal fungal (AMF) diversity in shallot soils.

ventional cultivation of onions (GALVÁN et al. 2009). The lack of correlation between soil P and mycorrhizal parameters agreed with the observation of GALVÁN et al. (2009), but contrasted with studies where an inverse correlation had been reported between these variables (ANANTHAKRISHNAN et al. 2004, LINGFEI et al. 2005, KHANAM et al. 2006, VALSALAKUMAR et al. 2007, Das and KAYANG 2010b).

Soil pH has a great relevance for plant growth as it influences nutrient mobilization as well as availability (MARSCHNER 1995). Many studies reporting the influence of soil factors on AMF colonization have failed to find any relationship between soil pH and colonization (ZAHKA et al. 1995, KHADE and RODRIGUES 2008, VALSALAKUMAR et al. 2007, OLIVEIRA and OLIVEIRA 2010, KHANAM et al. 2006). In the present study pH of the soil was positively correlated to all AMF colonization parameters except %RLV. LINGFEI et al. (2005) found results similar to ours, where soil pH was found to be correlated with the extent of hyphal and arbuscular colonization in grasses. Colonization and extraradial mycelium formation by AMF are known to be altered by soil pH (VAN AARLE et al. 2002). COUGHLAN et al. (2000) inferred that the tendency for colonization levels to increase with pH was due to the stimulation of the additional AMF taxa and/or a greater ability of the taxa present to colonize host roots. However, the positive influence of pH on AMF colonization contradicts the observations of WANG et al. (2008) and FORTIN et al. (2002) where increasing soil pH had detrimental effects on AMF spore germination and mycorrhization.

Though less studied than those of other soil nutrients, Fe deficiencies at high soil pH are known to suppress colonization by AMF (WANG et al. 2008). The positive correlation between soil Fe and AMF variables indicate that Fe could stimulate colonization by AMF. A similar correlation has been reported in *Paullinia cupana* during the rainy season by OLIVEIRA and OLIVEIRA (2010). MICHELINI et al. (1993) indicated that Fe interacts with other soil nutrients like Ca, Mn or P to influence AMF colonization and can greatly be influenced by soil pH. In the present study, soil Fe was also significantly and positively correlated to soil pH ($r = 0.565$; $p<0.01$; $n = 20$). Contrarily, to the observations of AUDET and CHAREST (2006), Zn and %RLV had an inverse correlation in the present study. The nega-

tive effect of soil Zn can be due to the directly suppressive effect of Zn on AMF propagules or the indirect effect on host roots.

The average AMF spore number (11 spores per 100g soil) recorded in the present study is lower than the range of 54–3920 spores per 100g soil reported for tropical soils (VALSALAKUMAR et al. 2007, KHANAM et al. 2006, KHADE and RODRIGUES 2008, OLIVEIRA and OLIVEIRA 2010, ANANTHAKRISHNAN et al. 2004, DAS and KAYANG 2010b). Major portions of AMF spores occurring in field soils are either dead or spore cases (MUTHUKUMAR and UDAIYAN 1999) and the spore numbers presented in this study are only for intact spores. The lack of correlation between %RLTC and spore numbers agrees with the observations of VALSALAKUMAR et al. (2007) in *Phaseolus aureus*, ZAHKA et al. (1995) in *Acer saccharum*, KHALIL and LOYNACHAN (1994) in *Glycine max* and SASAI (1992) in cultivated plants of Miyagi prefecture, Japan. Such a lack of correlation between AMF colonization and spore numbers indicate that the factors influencing these variables are totally different. In this study AMF spore numbers were negatively correlated to soil N and K. This contradicts the suggestions that soil K could stimulate the production of AMF spores (OLIVEIRA and OLIVEIRA 2010). This influence of soil N and K on AMF spore numbers can be attributed to their influence on soil pH as soil N ($r = 0.463$) and soil K ($r = 0.464$) were correlated ($p<0.05$) to soil pH.

A total of one to five AMF taxa were detected in conventionally cultivated onion field soil. This is lower than the 8 to 20 species that are usually reported for arable lands (LAND and SCHÖNBECK 1991, DOUDS and MILLNER 1999, FITTER, 2001, JANSA et al. 2002). However, the AMF diversity in the present study is within ranges observed for a site by ANANTHAKRISHNAN et al. (2004) and SJÖBERG et al. (2004). VALSALAKUMAR et al. (2007) also reported very low AMF diversities of one to three taxa for the 21 sampling locations under *Phaseolus aureus* cultivation in Tamil Nadu and Karnataka of South India. The presence of low AMF diversity could be due to the selection pressure imposed by cultivation practices resulting in the dominance of fast growing species (OEHL et al. 2004) and species that are able to tolerate stresses like tillage, fertilizer and biocide applications (GOSLING et al. 2006, GALVÁN et al. 2009).

DSE colonization had been reported in roots of several crop species and in roots also colonized by AMF (JUMPPONEN and TRAPPE 1998, MUTHUKUMAR and TAMILSELVI 2010). In this study, the root systems of all shallot onions examined were commonly colonized by both DSE and AMF. DSE colonization has been reported in onions from both temperate and tropical agroecosystems (JUMPPONEN and TRAPPE 1998, MUTHUKUMAR and TAMILSELVI 2010). It is interesting to note that the extent of %RLDTC was always lower (except F2) than %RLTC. Further, the lack of correlation between root lengths colonized by AMF and DSE clearly suggest that these two fungal types do not compete for resources within roots. However, the results of a recent study by SCERVINO et al. (2009) show that DSE could modify mycorrhizal status of plants.

The correlation analysis showed that soil Zn and Cu influenced DSE colonization and structures, which is in line with the observation of CHRISTIE and KILPATRICK (1992). In contrast, LINGFEI et al. (2005) found no significant correlations between DSE colonization and soil factors. The response of DSE to soil nutrients appears to vary with soil conditions. For example, CHRISTIE and KILPATRICK (1992) found an inverse correlation between Cu and Zn to DSE colonization in soil amended cow slurry but not in soil amended with pig slurry. At present, the ecology of DSE association in crop plants has not been documented. As grow-

ing evidence suggests that DSE may influence plant growth in a way similar to AMF (Wu et al. 2010, Wu and Guo 2008, Fumiaki and Kazuhiko 2007), an understanding of the factors influencing their associations and function would enable their possible exploitation in agriculture.

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