Elucidating the Mystery of the Tripartite Symbiosis Plant – Mycorrhizal fungi – Dark Septate Endophytes

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Abstract
This study provides information on the tripartite symbiotic relationships formed by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in crops growing in the semiarid region of the Canadian Prairie. We found the symbiotic root systems of wheat, pea, chickpea and lentil to be morphologically distinct. The relationship between DSE and AMF abundance in roots ranged from negative in lentil to positive in wheat.

Introduction
Class II fungal endophytes, commonly called dark septate endophytes (DSE), and arbuscular mycorrhizal fungi (AMF) have a worldwide distribution. AMF are well known to increase plant growth, productivity and stress tolerance (Lozano *et al*., 2008; Tripathi *et al*., 2008) and improve P and N use efficiency (Fernández *et al*., 2011; Harman & Mastouri, 2010). However relatively few studies on DSE in the context of crop production have been conducted despite of the potential of these fungi to improve the productivity of different plants by reducing the impact of biotic and abiotic stresses (Yuan *et al.*, 2010). Possible interactions between DSE and arbuscular mycorrhizal fungi (AMF) colonizing the roots of crop plants still awaits research attention.

The goal of this study was to document and compare the symbioses formed between AMF and DSE in the roots of important crops grown in the semiarid region of the Canadian Prairie: wheat and three pulse, pea, chickpea and lentil.

Materials and methods
The field experiment was conducted in 2010 at the Agriculture and Agri-Food Canada Semiarid Prairie Agricultural Research Centre (SPARC), Swift Current, SK, Canada (latitude: 50°17'N; longitude: 107°41'W, elevation 825 m). The symbioses involving AMF and DSE were tested in four different crops using a randomized complete block design with 4 replicates per treatment. Plots were 4 x 8 m. Crop plants were: hard red spring wheat (cv. Lillian), kabuli chickpea (cv. CDC Frontier), yellow pea (cv. CDC Meadow), and red lentil (cv. CDC Maxim CL).

Root colonization was evaluated in root samples at mid-bloom stage after staining according to the protocol of Vierheilig *et al*. (1998). The percentage of root colonization by AMF and DSE structures was measured using the gridline intersect method (Giovannetti and Mosse, 1980) under the microscope, at 100 x magnifications.

Root density and root length colonized by the fungi were measured by staining the whole root system in 0.8% Toluidine Blue solution for 5 min just before scanning, as described in Costa *et al*. (2001). Images were saved and analyzed using the program WinRhizo PRO V 2003 for Windows (Régent Instrument Inc., Québec). Root length colonized (RLC) was calculated as: colonization values X root length measurements. Plant harvest index (HI) was calculated as the ratio of seed weight to the total weight of the harvested material.

Results were analyzed using one-way ANOVA and the significance between treatment means was assessed by protected LSD Student's t tests, in JMP version 8 (SAS Institute Inc., Cary, NC, USA). Pairwise correlations were also conducted using JMP version 8. Transformations were applied to the percentage data before analyses to comply with the requirement of the tests (Scherrer, 1984).

Results

In roots, AMF and DSE have different morphologies and form different structures that make possible their identification by microscopic examination of stained roots (Fig. 1). AMF colonized the roots more abundantly than DSE (Fig. 2). Melanized hyphae and microsclerotia were formed by DSE in the roots of the different crops roots (Fig. 1 D, E, F) often very close to AMF colonized spots (Fig. 1F). DSE hyphae were septate and usually thicker than those of AMF (Fig.1 B, E). Typically, AMF formed intraradical spores, vesicles and arbuscules, and their hyphae did not have septa (Fig. A, B, C). These structures were more or less abundant depending on the plant-fungal association.

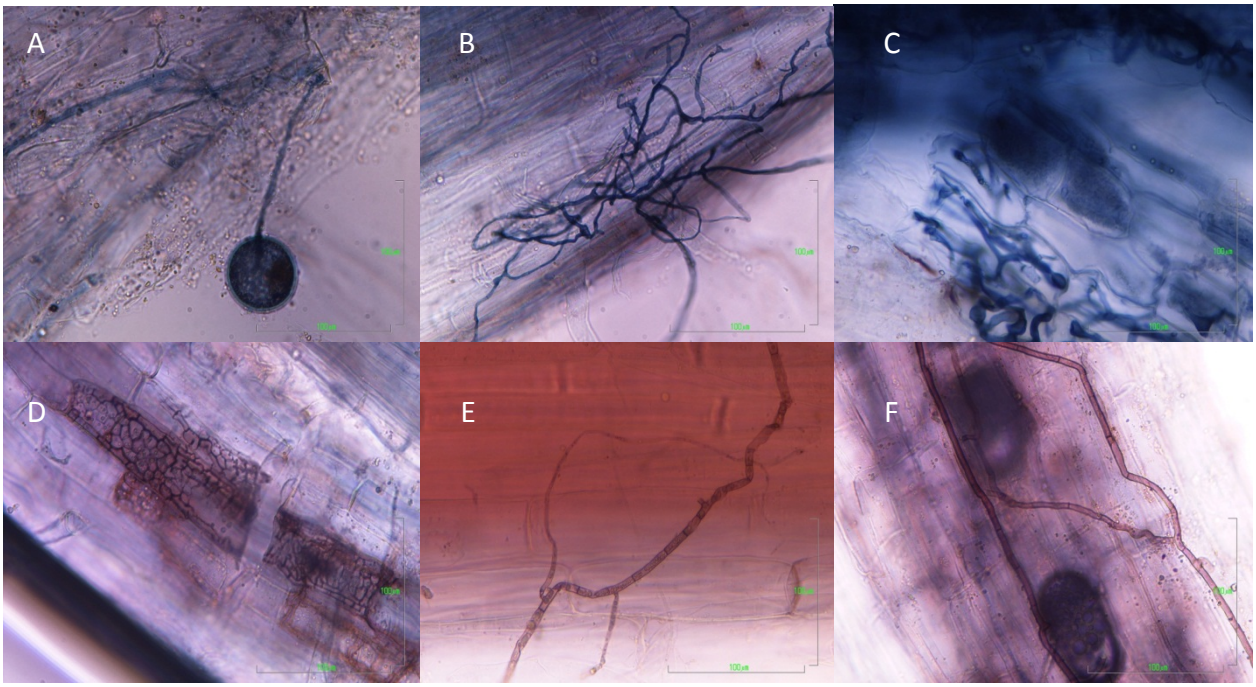

Figure 1. AMF and DSE structures observed in the roots of wheat. A: AMF hyphae with spore; B: AMF hyphae; C: AMF hyphae and intracellular arbuscules; D: DSE melanized microsclerotia; E: DSE melanized hyphae; F: DSE melanized hyphae overlapping AMF spores in the background.

Symbiotic development had different patterns in different crops. The percentage of colonization by AMF was higher in pulses than in wheat roots, and was most extensive in pea roots (Fig. 2). The percentages of root colonization by DSE were similar in all crop species, but the proportion of root occupation by DSE and AMF varied with crops. Wheat roots were colonized to the same extent by the two fungal groups, but the roots of pulses, especially pea, were mostly colonized by AMF (Fig. 2).

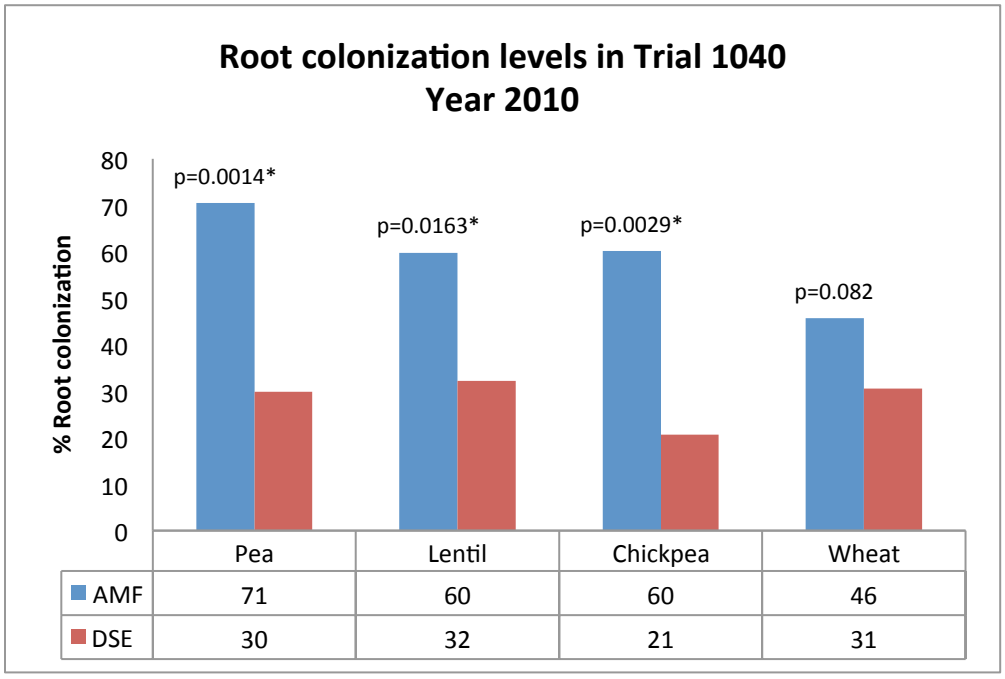

Figure 2. Percentage of root colonization by AMF and DSE in pea, lentil, chickpea and wheat crops in the years 2010. Data analyzed with ANOVA and LS Means Differences Student's t using JMP8 software (SAS Institute Inc., Cary, NC, USA). N=32, p values correspond to the comparison between AMF and DSE colonization within the crop.

The RLC by AMF or DSE in different crops reveals information about the nutrient uptake mechanisms of these crops, and in particular, on the role of AMF and DSE in the nutrition of these host crops. Different correlations were found between the percentage of root colonization by AMF or DSE and the RLC by these groups, suggesting that these fungi may have not only different patterns of colonization, but may also interact differently in different crops (Table 1). The higher the percentage of root colonization by AMF in lentil, the lower was the percentage of root colonization by DSE and RLC by DSE (Table 1), suggesting that the fungi have an antagonistic relationship in this crop. In wheat, by contrast, a positive correlation was found between RLC by AMF and RLC by DSE, suggesting the occurrence of a neutral or mutualistic relationship (Table 1).

Table 1. Pairwise correlation tables between AMF/DSE colonization, RLC by AMF/DSE and harvest index in pea, lentil, chickpea, and wheat in 2010. R square values are in bold when significant. * $p < 0.05$, ** $p < 0.01$

PEA	AMF	DSE	RLC AMF	RLC DSE	Harvest index
AMF	1.0000	-0.5064	**0.6155***	-0.0447	0.1887
DSE	-0.5064	1.0000	-0.2882	**0.6381***	-0.3310
RLC AMF	**0.6155***	-0.2882	1.0000	0.4930	0.2249
RLC DSE	-0.0447	**0.6381***	0.4930	1.0000	-0.0790
Harvest index	0.1887	-0.3310	0.2249	-0.0790	1.0000
LENTIL					
AMF	1.0000	**-0.8854****	0.2135	**-0.7228***	-0.2206
DSE	**-0.8854****	1.0000	-0.2015	**0.7356***	0.3781
RLC AMF	0.2135	-0.2015	1.0000	0.4576	-0.5575
RLC DSE	**-0.7228***	**0.7356***	0.4576	1.0000	-0.1072
Harvest index	-0.2206	0.3781	-0.5575	-0.1072	1.0000
CHICKPEA					
AMF	1.0000	0.1807	**0.8482****	0.0846	-0.0601
DSE	0.1807	1.0000	-0.0596	**0.7816***	**0.7453***
RLC AMF	**0.8482****	-0.0596	1.0000	0.1876	-0.4487
RLC DSE	0.0846	**0.7816***	0.1876	1.0000	0.3558
Harvest index	-0.0601	**0.7453***	-0.4487	0.3558	1.0000
WHEAT					
AMF	1.0000	0.2222	0.6527	0.5741	-0.6150
DSE	0.2222	1.0000	-0.5788	-0.5947	0.0075
RLC AMF	0.6527	-0.5788	1.0000	**0.9774****	-0.3940
RLC DSE	0.5741	-0.5947	**0.9774****	1.0000	-0.1924
Harvest index	-0.6150	0.0075	-0.3940	-0.1924	1.0000

Morphological differences in the symbioses of the different crops may be indicative of different strategies for nutrient uptake. Positive correlations have been found between the straw nitrogen or phosphorus content and the RLC by DSE in lentil and a similar relationship with straw nutrient contents occurred in chickpea, but with RLC by AMF rather than DSE (Data not shown). Besides, harvest index was positively correlated with DSE colonization in chickpea. No significant interactions were found between fungi-related variables and plant productivity (Table 1).

Highlights and Work Relevance

AMF are known to improve the performance and drought tolerance in crops plants (Marulanda *et al*., 2003; Porcel & Ruiz-Lozano, 2004), but little is known about the role of DSE in crop plants. Our study shows that DSE can colonize the roots of pea, lentil, chickpea and wheat crops, at least in a semiarid environment and that the colonization followed different patterns that appear to be specie specific.

Different environments and different plant genotypes may result in different endophytic fungal communities in plant roots. It was proposed that some DSE are better adapted than others to

stressful environmental conditions and then adapted plants "select" these symbionts as a way to escape from these extreme conditions (Yuan *et al.* 2010). In our study, the influence of plant genotype on symbiotic development is unclear, as it is counfounded by the influence of cropping practices such as pest management and fertilization, which are different for different crops, and create different soil environments.

Conclusions

This study provides new information about the symbioses in economically important crops growing in the semiarid region of the Canadian Prairie. Symbiotic relationships with AMF and DSE were crop specific, and the relationships between AMF and DSE in crop roots varied from mutualistic to antagonistic, or have no-effect, depending on the crop they colonized. Further studies are required to understand the role of crop genotype and crop-specific agronomic practices on the composition and function of the symbioses in these crops.

References

Costa, C., Dwyer, L.M., Hamel, C., Muamba, D.fF., Wang, X.L., Natais, L., Smith, D.L. 2001. Root contrast enhancement for measurement with optical scanner-based image analysis. Can. J. Bot. 79, 23-29.

Fernández, M.C., Gutiérrez, F.H., Rubio, G., 2011. Effect of indigenous mycorrhizal colonization on phosphorus-acquisition efficiency in soybean and sunflower. J. Plant Nutr. Soil Sci. 174, 673–677.

Giovanneti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist. 84, 489–500.

Lozano, J.M.R., Porcel, R., Aroca, R., 2008. Evaluation of the possible participation of drought-induced genes in the enhanced tolerance of arbuscular mycorrhizal plants to water deficit. Varma A (ed.) Mycorrhiza 3rd edition. Springer, pp. 185-208.

Marulanda, A., Azcón, R., Ruiz-Lozano, J.M., 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* L. Plants under drought stress. Physiol Plant 119: 526-533.

Porcel, R., Ruiz-Lozano, J.M., 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55: 1743-1750.

Scherrer, B., 1984. Biostatistique. Gaëtan Morin Editeur, Boucherville, Québec, 850p.

Tripathi, S., Kamal, S., Sheramati, I., Oelmuller, R., Varma, A., 2008. Mycorrhizal fungi and other root endophytes as biocontrol agents against root pathogens. Varma A (ed.) Mycorrhiza 3rd edition. Springer, pp. 281-306.

Vierheilig, H., Coughlan, A.P., Wyss, U., Piche, A.Y., 1998. Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. Applied and Environmental Microbiology. 64, 5004–5007.

Yuan, Z., Zhang, C., Lin, F., 2010. Role of diverse non-systemic fungal endophytes in plant performance and response to stress: progress and approaches. J Plant Growth Regul. 29, 116–126.