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Journal:	<i>Canadian Journal of Microbiology</i>
Manuscript ID	cjm-2017-0637.R1
Manuscript Type:	Article
Date Submitted by the Author:	12-Feb-2018
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Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	durum wheat ( <i>Triticum turgidum</i> L. var. durum Desf.), arbuscular mycorrhiza, plant genetic improvement.

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**Abundance of the arbuscular mycorrhizal fungal taxa associated with the roots and rhizosphere soil of different durum wheat cultivars in the Canadian prairies**

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**Walid Ellouze** designed the experiments, acquired, analyzed, and interpreted the data, and wrote the manuscript with the help of Dr. Mishra and Dr. Hamel.

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**Asheesh Singh** contributed to the conceptualization and planning of the experiments, phenotyping, and finalizing of the manuscript. He piloted the grant submission as principal investigator.

**Vachaspati Mishra** helped in the literature search and in drafting the introduction and discussion.

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**Ron E. Knox** contributed to the conceptualization of the research, experimental design, interpretation of the results, and finalizing of the manuscript.

## Abstract

Understanding the variation in how wheat genotypes shape their arbuscular mycorrhizal (AM) fungal communities in a prairie environment is foundational to breeding for enhanced AM fungi–wheat interactions. The AM fungal communities associated with 32 durum wheat genotypes were described by pyrosequencing of amplicons. The experiment was set up at two locations in the Canadian prairies. The intensively managed site was highly dominated by *Funneliformis*. Genotype influenced the AM fungal community in the rhizosphere soil, but there was no evidence of a differential genotype effect on the AM fungal community of

durum wheat roots. The influence of durum wheat genotype on the AM fungal community of the soil was less important at the intensively managed site. Certain durum wheat genotypes, such as Strongfield, Plenty, and CDC Verona, were associated with high abundance of *Paraglomus*, and *Dominikia* was undetected in the rhizosphere of the recent cultivars Enterprise, Eurostar, Commander, and Brigade. Genetic variation in the association of durum wheat with AM fungi suggests the possibility of increasing the sustainability of cropping systems through the use of durum wheat genotypes that select highly effective AM fungal taxa residing in the agricultural soils of the Canadian prairies.

*Key words:* durum wheat (*Triticum turgidum* L. var. *durum* Desf.), arbuscular mycorrhiza, plant genetic improvement.

## Introduction

Arbuscular mycorrhizas are symbiotic associations between plant roots and fungi of the phylum Glomeromycota, a group containing some 250 described species (Redecker et al. 2013). Mycorrhizas form in more than 90% of all terrestrial plants, including wheat (Singh et al. 2012; Ellouze et al. 2016). The arbuscular mycorrhizal (AM) symbiosis plays an important role in the sustainability of agriculture. This symbiosis is a strategy used by many plant species for efficient extraction of soil nutrients (Yang et al. 2014), protection against abiotic stress (Hassan et al. 2013), and protection against root pathogens (Koide and Elliott 1989; Johnson and Graham 2013). Arbuscular mycorrhizas and other mutualists co-inhabiting roots could potentially be managed in agriculture in order to increase crop yields while reducing the dependence of crops on chemical fertilizers (Ellouze et al. 2008; Fraser et al. 2009). In spite of its value, the AM symbiosis is largely unmanaged in crop production (Ellouze et al. 2014). In the face of the increasing global demand for food and bioproducts and the disappearance of easily exploitable phosphate sources, it is important to increase the nutrient efficiency of crops. Integrating the management of the AM symbiosis into crop production systems would help reach this goal.

Genotypes of the same plant species can select different communities of root-colonizing microorganisms, including AM fungi (Bazghaleh et al. 2015). Genotypes of the same plant species release different cocktails of metabolites that either stimulate or repress AM fungi (Ellouze et al. 2012), thus creating selective influences on the root-associated AM fungi.

Durum wheat (*Triticum turgidum* L. var. *durum* Desf.) is an important cereal crop worldwide, providing some 36 million tonnes of durum grain yearly (<http://faostat.fao.org>). A large number of wheat cultivars with different agronomic traits have been developed. Glasshouse

studies showed that wheat cultivars vary in their responsiveness to AM symbioses (Graham and Abbott 2000; Ellouze et al. 2016). However, whether there are linkages between plant traits and the composition of the AM fungal community established in the roots of wheat in the field remains a matter of speculation. We tested the hypothesis that a wheat genotype shapes its associated AM fungal communities. If this hypothesis is true and if AM fungal taxa vary in their plant-growth-promotion ability, durum wheat genotypes that form AM symbioses with effective AM fungi in the agricultural soils of the Canadian prairies could be selected to improve the sustainability of intensive wheat-based cropping systems.

## Materials and methods

### *Experimental design and site description*

A field study comparing genotypes of durum wheat was conducted at two sites in Saskatchewan, in experimental plots belonging to the Government of Canada's Wheat Genetic Improvement Program. One site was near Swift Current, at Agriculture and Agri-Food Canada's Swift Current Research and Development Centre (50°15'N, 107°44'W), which is in the brown soil zone, and the other was near Regina, at Agriculture and Agri-Food Canada's Regina research farm (50°24.24'N, 104°33.64'W), which is in the black soil zone. The soil at the Swift Current experimental site was a Brown Chernozem (Aridic Boroll in the USA soil classification system) with a silt loam texture, and the soil at the Regina research farm was a Black Chernozem (Udic Boroll in the USA soil classification system) with a clay texture. The soil in Regina contained 7.5 and 27.5 mg of extractable (Olsen et al. 1954) NO<sub>3</sub>-N and PO<sub>4</sub>-P, respectively, per kilogram of soil and 25.4 g kg<sup>-1</sup> of organic C (Baccanti and Colombo 1992) after harvest, and the soil in Swift Current contained 9.6 mg kg<sup>-1</sup> of extractable NO<sub>3</sub>-N, 26.5 mg kg<sup>-1</sup> of extractable

PO<sub>4</sub>-P, and 18.9 g kg<sup>-1</sup> of organic C. The average (1981–2010) yearly temperature is 4.3 °C in Swift Current and 3.1 °C in Regina, and annual precipitation is 357.4 mm in Swift Current and 389.7 mm in Regina ([http://climate.weather.gc.ca/index\\_e.html](http://climate.weather.gc.ca/index_e.html)). The wheat plots were set up in soil that had been summer-fallowed the previous year to reduce the risk that soil moisture would limit grain yield. Summer fallow, a soil-moisture conservation practice that is used in dry areas such as the Canadian prairies, ensures a good wheat yield in the year following a fallow year. In Regina, a 2-year rotation was used, namely, fallow one year and wheat the next. In the fallow year, the soil was generally tilled twice and chemically cleaned once in spring. The site at Swift Current was managed as a 3-year rotation, namely, fallow–wheat–oats. In semiarid Swift Current, a chemical weed control treatment was also applied in spring, but generally a single tillage operation was sufficient to control the vegetation. Seeding operations were managed in a similar fashion with similar equipment at both sites.

The experiment was arranged in a randomized complete block design with three repetitions. The durum wheat cultivars used were a set of five landraces brought to Canada before 1920 (Knott 1995) and a set of 27 cultivars developed at different times in the modernization of crop fertilization practices, including six cultivars that were released recently (2004–2010) (Clarke et al. 2010). All the cultivars were developed in Canada except for Quilafen, which is from Chile, and for Ramsey and Lakota, which were developed at the North Dakota Agricultural Experimentation Station. The modern cultivar Coulter (developed in 1977) was not seeded at the Swift Current site because of a seed shortage; thus 31 cultivars were used there, and 32 were used at the Regina site. The durum wheat genotypes were grown in 3-m-long, single-row plots in Swift Current and in 3-m-long, four-row plots in Regina. The row spacing was 23 cm. The seeding rates targeted a plant density of 1000 plants m<sup>-2</sup>.

### ***Soil and root sampling and data collection***

The AM fungal community of the roots of each durum wheat genotype was sampled at heading in order to describe the community contributing to the development of yield. Two root samples were collected from the top 0-to-7.5-cm soil layer with a shovel. The samples were taken directly in the plant row following a systematic sampling plan (Fig. S1). The two root samples were then separated from shoots and pooled to obtain one sample per plot. The roots were washed thoroughly over a 2-mm sieve to minimize the loss of fine roots. The roots were then cut into 1-cm fragments that were dispersed and mixed in water. One root subsample from each plot was placed at  $-20^{\circ}\text{C}$  for DNA extraction, and another subsample was placed in labelled plastic biopsy cassettes (Unisette, Cat. No. U5260; Sigma-Aldrich, St. Louis, MO, USA), cleared, and stained using a Sheaffer black ink and vinegar solution (5:95 v:v), as described by Vierheilig et al. (1998). The percentages of durum wheat root colonization by AM fungi and by other fungal endophytes were then determined on these subsamples using the gridline intersect method (Giovannetti and Mosse 1980).

The AM fungal community of the rhizosphere soil was sampled at plant physiological maturity (hard dough stage) in order to describe the AM fungal legacy left by the durum wheat cultivars to the following crop in the rotation. Two soil samples were collected from the top 0-to-7.5-cm soil layer of each plot using a hand sampler ( $r = 2.5\text{ cm}$ ). The soil samples were taken directly in the plant row where root density was very high. The samples were pooled to obtain one composite sample per plot and were placed at  $-20^{\circ}\text{C}$  for DNA extraction.

### ***DNA extraction from soil and roots and PCR conditions***

Total DNA was extracted from 0.27 g of soil using a PowerSoil DNA Isolation Kit (Cat. No. 12888-100; Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's



instructions. The 0.27-g subsamples of rhizosphere soil were obtained after sieving the samples through a 2-mm sieve and mixing the soil by agitation in a 15-mL tube.

About 3 g of fine roots were taken from each field plot. The samples were crushed using a Retsch MM301 ball mill machine (Haan, Germany) and Lysing Matrix A tubes (MP Biomedicals, Santa Ana, CA, USA) at maximum speed, eight times for 1 min each time. Total DNA was extracted from a 100-mg (fresh weight) subsample of each root sample using an UltraClean Microbial DNA Isolation Kit (Cat. No. 12224-250; Mo Bio Laboratories, Inc.) according to the manufacturer's instructions for maximum yield.

A nested-PCR approach was used to amplify AM fungal DNA. The first PCR round used the universal fungal primer pair NS1 and NS4 (White et al. 1990) to amplify a 1.5-kb fragment of the 18S rRNA gene. The PCR assay was conducted with a Bio-Rad C1000 Touch Thermal Cycler (Hercules, CA, USA) in a 30- $\mu$ L volume made of 23  $\mu$ L of Platinum PCR SuperMix High Fidelity (Cat. No. 12532-016; Invitrogen, Carlsbad, CA, USA), 3  $\mu$ L (5  $\mu$ M) of NS1, 3  $\mu$ L (5  $\mu$ M) of NS4, and 1  $\mu$ L of extracted DNA (diluted 1:10). The PCR conditions were as follows: 94 °C for 3 min; 35  $\times$  (94 °C for 45 s; 51 °C for 45 s; and 72 °C for 1 min); and 72 °C for 7 min. The PCR products were analyzed by agarose gel electrophoresis (1.0% w:v agarose), stained with SYBR Safe DNA Gel Stain (Invitrogen), and visualized using a gel imaging system (MultiImage Light Cabinet; Alpha Innotech, Thermo Fisher Scientific, Hampton, NH, USA).

The product of the first PCR round with a visible band was diluted 1:10 and used as template in the subsequent PCR round. The primers for this second stage were AMV4.5NF and AMDGR, which were previously used successfully in other studies (Sato et al. 2005; Lumini et al. 2010; Dai et al. 2013). These primers amplify sequences from environmental samples of all four AM fungal orders, namely, the Diversisporales, Glomerales, Archaeosporales, and

Paraglomerales, as well as other fungi of the Ascomycota, Basidiomycota, and Chytridiomycota. All amplicons from each sample were barcoded with one of 31 Roche multiplex identifiers (MIDs) (Roche Diagnostics, Laval, QC, Canada) (Rothberg and Leamon 2008), thus allowing the sequencing of pools of 31 samples.

The PCR products from the soil and root samples were purified using the Agencourt AMPure XP PCR purification system (Beckman Coulter, Brea, CA, USA). The concentration of purified amplicons was measured using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and pyrosequencing was performed on the equimolar mixtures of differently tagged samples in  $6 \times 1/8$  of the reaction at the Vancouver Prostate Centre (Vancouver, BC, Canada) for the soil samples and in  $6 \times 1/8$  of the reaction at the McGill University and Génome Québec Innovation Centre (Montreal, QC, Canada) for the root samples, using Roche 454 FLX Titanium technology.

### ***Bioinformatics***

The sequences were filtered for quality and trimmed to remove MIDs and primers using mothur v.1.30 (Schloss et al. 2009). Only sequences with quality control values greater than 30, no ambiguous bases, fewer than nine homopolymers, and a minimum length of 200 bp were included in further analysis (Huse et al. 2010). Chimeric sequences were detected and removed using UCHIME (Edgar et al. 2011) in mothur. Sequences belonging to groups other than Glomeromycota were identified by comparison with the Silva eukaryotic reference for 18S rDNA sequences (<http://www.arb-silva.de/>) and AM fungal reference sequences obtained from GenBank. All unique sequences were filtered for reduced computational complexity using mothur. The average length of the cleaned sequences was 262 bp. After sequence cleaning, we retained 299,201 of the 1,420,433 reads obtained from the sequencing of the 378 samples

[(31 genotypes in Swift Current + 32 genotypes in Regina)  $\times$  3 repetitions = 189 soil samples + 189 root samples]. The primer sets NS1/NS4 and AMV4.5NF/AMDGR are not specific to AM fungi, and 78.7% of the reads belonging to non-Glomeromycota phyla were discarded, resulting in a 63,776-read AM fungal dataset.

The clean AM fungal sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity using the CD-HIT-EST program (Huang et al. 2010). Taxonomic assignment was performed by comparing a representative sequence of each OTU to the GenBank non-redundant nucleotide database (Zhang et al. 2000).

### ***Statistical analysis***

The effects of genotype and site on multiple variables and on single variables were tested using multivariate and univariate statistical tests, as described below. Because site properties and communities were very different, the AM fungal communities of each site were considered separately.

The significance of durum wheat genotype effects on the structure of AM fungal communities was assessed by PerMANOVA (Anderson 2001), in PC-ORD v.6.1 (Peck 2010), using Jaccard as distance in a model considering blocks. Non-metric multidimensional scaling ordination was constructed, using PC-ORD v.6.1, to visualize the significant effect of genotype on the abundance of AM fungal genera in rhizosphere soil.

The significance of durum wheat genotype effects on the relative abundance of *Paraglomus*, *Archaeospora*, *Claroideoglomus*, *Funneliformis*, *Rhizophagus*, and *Dominikia* reads was assessed by ANOVA using JMP v.6 (SAS Institute Inc., Cary, NC, USA). The *Archaeospora* and *Dominikia* abundance data were subjected to log-normal ( $\ln [n+1]$ ) transformation before analysis. The *Funneliformis* and *Rhizophagus* abundance data were

subjected to square-root transformation before analysis. The significance of genotype effects on the relative abundance of *Diversispora* and *Septoglomus* reads was tested by Kruskal–Wallis tests, as these data could not be transformed to meet the normality requirement of ANOVA.

The effect of genotype on the relative abundance of AM fungal reads measured in roots and in soil was compared by subjecting the data to a pairwise Kruskal–Wallis test in JMP v.6.

The effect of genotype on the relative abundance of AM fungal reads in these niches (roots and soil) in Regina and Swift Current was compared in different tests.

## Results

A total of 190 AM fungal OTUs were identified in the roots and soil of the two field sites, based on clustering at 97% sequence similarity. The majority of the OTUs and reads belonged to the genus *Funneliformis* (58 OTUs and 50.3% of the reads). The remaining OTUs belonged to the genera *Claroideoglossum* (35 OTUs and 10.7% of the reads), *Dominikia* (22 OTUs and 2.9% of the reads), *Archaeospora* (20 OTUs and 5.8% of the reads), *Paraglossum* (18 OTUs and 23.8% of the reads), *Rhizophagus* (16 OTUs and 4.1% of the reads), *Diversispora* (16 OTUs and 2.0% of the reads), and *Septoglomus* (5 OTUs and 0.4% of the reads).

### *Communities of AM fungi inhabiting soil and roots of durum wheat*

Of the 190 OTUs detected in total, 71 and 137 were detected in the root and rhizosphere soil samples, respectively, in Swift Current; in Regina, where *Funneliformis* was extremely dominant, 33 and 136 of these OTUs were detected in roots and rhizosphere soil, respectively (Fig. 1). At both sites, 104 OTUs were detected in rhizosphere soil, and 12 were detected in roots. In both niches at both sites, 9 OTUs were detected: one *Paraglossum* and eight *Funneliformis*.

*Funneliformis* was extremely abundant in Regina, accounting for 81.1% of the AM fungal reads obtained from soil (Fig. 2). This genus also dominated the AM fungal community hosted by roots, accounting for 99.3% of the AM fungal reads (Fig. 2). Only four root samples contained another AM fungal genus in addition to *Funneliformis* in Regina. In Swift Current, the AM fungal communities were more diverse both in roots and in soil. *Paraglomus* was the most abundant genus in soil (48.6% of the reads) and in roots (34.9% of the reads), whereas *Archaeospora*, which accounted for only 1.7% of the AM fungal reads obtained from soil, was abundant in roots (25.1% of the reads). In contrast, *Claroideoglomus*, which accounted for only 2.6% of the AM fungal reads obtained from roots, was the second most abundant genus in soil (22.4% of the reads). *Funneliformis* was slightly less abundant in the soil (16.6% of the reads) than in the roots of durum wheat (25.6% of the reads) in Swift Current (Fig. 2). Overall, read frequency revealed a high ratio of intraradical growth to extraradical growth in *Archaeospora* and, to a lesser extent, in *Funneliformis*, whereas *Claroideoglomus* and, to a lesser extent, *Rhizophagus*, *Dominikia*, and *Paraglomus* grew relatively more extraradically than intraradically. *Septoglomus* was detected only in soil. *Diversispora* reads were much more frequent in roots than in soil in Swift Current but were detected only in soil in Regina, where *Funneliformis* dominated the root niche, with a relative read abundance of 99.3% (Fig. 2).

#### ***Genotype effect on AM fungal communities of roots and soil***

The data show that plant genotype can influence the structure of the AM fungal community, at least in rhizosphere soil. This effect of genotype on community structure was detected at both sites but was weaker in Regina (Table 1). As a result, the effect of genotype on the relative abundance of genera was detected in Swift Current only. Durum wheat genotype influenced the relative abundance of *Paraglomus* in rhizosphere soil in Swift Current, which

266 ranged from 11.1% in Ramsay to 93.7% in Strongfield (Table 2; Fig. 3). *Dominikia* was detected  
267 in the rhizosphere of 80% of landrace genotypes and in 60% of modern genotypes but in only  
268 two of the six (33%) recently released durum wheat genotypes, namely, Strongfield and  
269 Transcend.

270 No effect of genotype on the AM fungal community was detected in roots (Table 1). The  
271 AM fungi were heterogeneously distributed in roots, and this heterogeneity and the small size of  
272 the root samples used may explain the presence of multiple zeros in the data file, which  
273 negatively impacted the quality of the dataset.

## 275 Discussion

276 The different assemblages of AM fungal communities observed at the Regina and Swift  
277 Current sites indicate that environmental factors such as climate, soil properties, and field  
278 management influence the composition of AM fungal communities. Our results are consistent  
279 with other research showing a large impact of the soil environment on the distribution of AM  
280 fungi (Castro et al. 2010; Andrew et al. 2012; Yu et al. 2012; Ellouze et al. 2013; Liu et al. 2015;  
281 Bainard et al. 2016; Oehl et al. 2017).

282 Increasing the sustainability of cropping systems through the use of selected plant  
283 genotypes that form symbioses with highly effective AM fungal taxa in agricultural soils is  
284 possible. Our results and those of other authors show that genotypes can influence associated  
285 AM fungal communities (Ellouze et al. 2012, 2013; Bazghaleh et al. 2015). A negative effect of  
286 plant domestication on the AM symbiosis was previously reported (Xing et al. 2012). However,  
287 the evidence that plant selection reduces the ability of crop plants to respond to the AM  
288 symbiosis is rather thin (Lehmann et al. 2012 and references therein). Differences in plant

susceptibility to the AM symbiosis was found in many crop plants, such as tomato (Steinkellner et al. 2012), cowpea (Oruru et al. 2018), and durum wheat (Singh et al. 2012), but this variation did not support the hypothesis of a negative effect of plant breeding on the AM symbiosis.

The historical set of durum wheat genotypes that we used includes 32 genotypes introduced to North America or produced through genetic selection at different times in the development of modern fertilization practices initiated after World War II. Similarly to previous studies (Lehmann et al. 2012; Singh et al. 2012; Steinkellner et al. 2012; Oruru et al. 2018), our study provides no evidence of a negative impact of durum wheat genetic selection on the symbiosis of this plant. On the contrary, four of the six newest high-yielding durum wheat genotypes (Commander, Enterprise, Eurostar, and Brigade) seemed to favour the proliferation of *Diversispora* and *Claroideoglossum* and exclude *Dominikia*. The recent durum wheat genotypes might trigger a positive feedback reaction from the soil fungal community. In a survey of wheat fields in the Canadian prairies, the abundance of *Diversispora* and *Claroideoglossum* in soil was reported to be positively related to wheat productivity and to N and P uptake efficiency, whereas the abundance of *Dominikia* in soil was negatively related to wheat productivity and to N and P uptake efficiency (Dai et al. 2014). Correlations are not necessarily the expression of causal relationships, and at this time, the relative growth-promoting value of AM fungal genera remains uncertain. However, it makes sense that genetic selection for the most productive plant genotypes would also select for genotypes that form AM symbiotic associations benefiting plant production.

*Dominikia*, the genus deemed detrimental to wheat based on a large correlation study (Dai et al. 2014), had a higher relative abundance in soil than in roots, and *Diversispora*, a genus deemed beneficial to N and P uptake in wheat (Dai et al. 2014), had a higher relative abundance

in roots than in soil. Extraradical development is essential for effective harvesting of soil resources, and intuitively, the growth form of the AM fungal associate of a plant should be a determinant of the growth-promoting value of the AM fungus. However, the relationship between the partitioning of AM fungal growth and plant-growth promotion is not linear. The development of photosynthetic tissues and mycorrhizas create competing C sinks in mycorrhizal plants (Johnson 2010). The AM fungal species that form large extraradical networks should have more capacity to extract soil nutrients than species with reduced extraradical mycelia but should represent a larger C drain that may or may not be offset by plant photosynthetic plasticity. The efficiency of the symbiotic interface in roots may also be a determinant of AM symbiotic efficiency. Understanding the variation in the formation of mycorrhizas by crop plants could lead to the identification of traits that are related to symbiotic effectiveness in the AM fungi and that would therefore be useful in plant breeding programs. Our observations suggest that the ratio of extraradical development to intraradical development is not such a trait.

Now that selecting durum wheat genotypes for their influence on associated AM fungal community is a possibility, it is urgent to identify effectiveness traits in AM fungi or in AM symbiotic associations. One way for mycorrhizal plants to optimize their efficiency of soil nutrient extraction is through the selection of the most effective AM fungal partners. It was shown using a root-culture model system that plants preferentially invest C in the AM fungal species that brings them more nutrients in comparison with other competing species (Kiers et al. 2011). However, a high ability to provide soil nutrients may be related to extensive growth forms in AM fungal partners that are large C sinks, and the regulation of AM symbiotic development in crops may involve other concurrent mechanisms. Variation in the strength of these concurrent AM regulation mechanisms among the genotypes of a plant species may explain the variation in



the composition of the root-associated fungal communities selected by different genotypes of chickpea (Ellouze et al. 2013; Bazghaleh et al. 2015) and other crop plants (Dunfield and Germida 2001; Berg and Smalla 2009) growing in the same soil.

Very different root- and soil-inhabiting AM fungal community structures were found at the Regina and Swift Current sites. In Regina, the almost absolute domination of *Funneliformis* was a striking characteristic of the AM fungal communities, particularly in roots. The AM fungal communities in Swift Current differed from those in Regina mainly by the relative abundance of the genera *Archaeospora* and *Paraglomus*. Soil available N and P levels at the study sites were similar, but the level of soil organic C and site management differed. Soil organic matter is known to influence the growth of AM fungi (Shrestha Vaidya et al. 2008) and the composition of their communities (Montiel-Rozas et al. 2017). The AM fungi are obligate biotrophs, and the use of summer fallow every other year in Regina appears to be a likely factor shaping AM fungal communities at this site. No matter the cause of variation in the AM fungal community from site to site, it appears that site characteristics had a stronger influence than plant genotype did.

In general, the soil-based AM fungi represent a pool of taxa from which plants recruit their partners and obtain support for their growth (Johnson et al. 2003; Davison et al. 2011). The over-dominance of *Funneliformis* may be a reason for the almost exclusive occupation of roots by this taxon in Regina. *Funneliformis* is considered a generalist AM fungal taxon and is predicted to be a pioneer species with a high dispersal capacity (Schwartz et al. 2006). A known cosmopolitan species (Avio et al. 2009), *Funneliformis* is very successful in croplands throughout the world (Rosendahl et al. 2009), including in Canadian wheat fields (Dai et al. 2013). Our results also concur with the abundance of *Funneliformis* in wheat breeders' fields.

## Conclusion

Our observations support the notion that durum wheat genotype can contribute to shaping AM fungal diversity in cultivated soils. Thus, selected durum wheat genotypes could be a tool for managing the communities of AM fungi that naturally reside in cultivated fields and for maintaining a soil biological state that is conducive to high crop yield. Furthermore, the extreme dominance of *Funneliformis* found in Regina suggests that plant genetic selection for a highly effective AM symbiosis might be better achieved using AM fungal inoculants.

## Acknowledgements

The authors gratefully acknowledge the financial support of the Western Grains Research Foundation and Agriculture and Agri-Food Canada (AAFC). Technical assistance provided by members of the wheat breeding and soil microbiology research group at the Swift Current Research and Development Centre is sincerely appreciated.

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542 **Table 1.** Significance of the effects of block and genotype on the structure of the community of  
543 arbuscular mycorrhizal (AM) fungi associated with durum wheat, according to perMANOVA  
544 ( $n = 3$ ).

Site	Source of variation	Roots	Rhizosphere soil
Regina	Block	<b>0.0194*</b>	<b>0.0002</b>
	Genotype	0.8331	<b>0.0010</b>
Swift Current	Block	<b>0.0002</b>	<b>0.0002</b>
	Genotype	0.6848	<b>0.0002</b>

545 \* $P$ -values in bold are significant at  $\alpha = 0.05$ .

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**Table 2.** Percentage  $\pm$  standard error of arbuscular mycorrhizal fungal reads belonging to taxa that were found in soil planted with durum wheat genotypes in Swift Current, SK, according to Kruskal–Wallis tests for *Diversispora* and *Septoglomus* and to ANOVA for other genera.

Family	Paraglomaceae	Archaeosporaceae	Diversisporaceae	Claroideoglomeraceae	Glomeraceae			
Genus	<i>Paraglomus</i>	<i>Archaeospora</i>	<i>Diversispora</i>	<i>Claroideoglomus</i>	<i>Funneliformis</i>	<i>Septoglomus</i>	<i>Rhizophagus</i>	<i>Dominikia</i>
<b>Most recent</b>								
Brigade*	50.79 $\pm$ 20.60	0.75 $\pm$ 0.75	0.38 $\pm$ 0.38	24.72 $\pm$ 5.34	5.24 $\pm$ 5.24	1.00 $\pm$ 1.00	2.99 $\pm$ 2.04	0.00 $\pm$ 0.00
Commander	45.91 $\pm$ 15.29	9.97 $\pm$ 9.97	1.39 $\pm$ 1.39	28.68 $\pm$ 4.43	3.96 $\pm$ 1.00	0.00 $\pm$ 0.00	9.67 $\pm$ 2.53	0.00 $\pm$ 0.00
Enterprise	57.49 $\pm$ 14.46	2.08 $\pm$ 2.08	0.69 $\pm$ 0.69	29.63 $\pm$ 16.31	1.00 $\pm$ 0.71	0.00 $\pm$ 0.00	4.54 $\pm$ 1.39	0.00 $\pm$ 0.00
Eurostar	50.18 $\pm$ 19.56	1.49 $\pm$ 1.58	1.05 $\pm$ 1.05	29.04 $\pm$ 13.42	6.35 $\pm$ 2.02	0.00 $\pm$ 0.00	6.81 $\pm$ 0.69	0.00 $\pm$ 0.00
Strongfield	93.77 $\pm$ 3.53	2.89 $\pm$ 2.89	0.00 $\pm$ 0.00	1.98 $\pm$ 1.26	0.48 $\pm$ 0.48	0.00 $\pm$ 0.00	0.08 $\pm$ 0.08	4.85 $\pm$ 4.85
Transcend	41.86 $\pm$ 21.23	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	7.74 $\pm$ 4.07	18.06 $\pm$ 8.88	0.00 $\pm$ 0.00	3.61 $\pm$ 0.92	13.20 $\pm$ 1.28
<b>Modern</b>								
AC Avonlea	21.22 $\pm$ 12.31	0.24 $\pm$ 0.24	0.04 $\pm$ 0.04	8.46 $\pm$ 7.46	33.18 $\pm$ 8.53	5.56 $\pm$ 5.56	7.51 $\pm$ 3.39	0.49 $\pm$ 0.49
AC Melita	45.86 $\pm$ 19.41	0.00 $\pm$ 0.00	0.75 $\pm$ 0.75	14.68 $\pm$ 1.43	24.11 $\pm$ 2.76	0.00 $\pm$ 0.00	2.96 $\pm$ 1.42	4.90 $\pm$ 1.03
AC Morse	54.38 $\pm$ 21.81	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	41.30 $\pm$ 23.96	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	2.89 $\pm$ 0.72	0.00 $\pm$ 0.00
AC Napoleon	58.52 $\pm$ 2.34	6.17 $\pm$ 1.46	0.00 $\pm$ 0.00	13.38 $\pm$ 2.84	13.84 $\pm$ 1.10	0.00 $\pm$ 0.00	2.56 $\pm$ 1.14	0.00 $\pm$ 0.00
AC Navigator	42.67 $\pm$ 13.73	9.12 $\pm$ 1.12	0.48 $\pm$ 0.48	36.35 $\pm$ 8.78	0.36 $\pm$ 0.36	0.00 $\pm$ 0.00	10.63 $\pm$ 1.35	0.00 $\pm$ 0.00
AC Pathfinder	59.31 $\pm$ 16.30	5.75 $\pm$ 1.40	0.00 $\pm$ 0.00	16.03 $\pm$ 12.95	7.56 $\pm$ 0.18	0.00 $\pm$ 0.00	7.67 $\pm$ 0.28	2.75 $\pm$ 1.51
Arcola	61.59 $\pm$ 3.15	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	29.75 $\pm$ 1.69	1.02 $\pm$ 0.25	0.00 $\pm$ 0.00	3.72 $\pm$ 0.25	3.97 $\pm$ 1.57
CDC Verona	74.62 $\pm$ 12.13	7.24 $\pm$ 7.24	0.00 $\pm$ 0.00	9.01 $\pm$ 5.18	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	3.84 $\pm$ 3.84	7.24 $\pm$ 7.24
Hercules	32.98 $\pm$ 11.68	3.90 $\pm$ 1.97	0.37 $\pm$ 0.37	41.51 $\pm$ 10.59	12.89 $\pm$ 3.72	0.00 $\pm$ 0.00	1.04 $\pm$ 0.26	0.00 $\pm$ 0.00
Kyle	42.71 $\pm$ 3.24	1.75 $\pm$ 1.75	0.00 $\pm$ 0.00	29.54 $\pm$ 4.30	19.89 $\pm$ 0.24	0.00 $\pm$ 0.00	1.04 $\pm$ 0.27	7.10 $\pm$ 1.58
Lakota	32.57 $\pm$ 12.62	5.05 $\pm$ 1.58	2.08 $\pm$ 2.08	30.56 $\pm$ 16.90	12.32 $\pm$ 0.41	0.00 $\pm$ 0.00	7.62 $\pm$ 1.93	12.55 $\pm$ 12.55
Macoun	46.73 $\pm$ 17.03	0.00 $\pm$ 0.00	2.32 $\pm$ 1.64	15.13 $\pm$ 9.58	1.00 $\pm$ 7.45	0.00 $\pm$ 0.00	9.55 $\pm$ 1.64	2.08 $\pm$ 2.08
Medora	36.52 $\pm$ 3.49	0.78 $\pm$ 0.78	0.84 $\pm$ 0.84	42.71 $\pm$ 2.01	4.45 $\pm$ 1.49	4.27 $\pm$ 4.27	7.18 $\pm$ 0.41	0.00 $\pm$ 0.00
Plenty	87.68 $\pm$ 7.48	3.39 $\pm$ 3.39	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.23 $\pm$ 1.23	0.00 $\pm$ 0.00	2.50 $\pm$ 2.50	0.00 $\pm$ 0.00
Quilafen	36.18 $\pm$ 10.68	3.25 $\pm$ 1.75	1.26 $\pm$ 0.63	21.88 $\pm$ 2.44	28.84 $\pm$ 0.72	0.00 $\pm$ 0.00	7.84 $\pm$ 0.02	0.00 $\pm$ 0.00
Ramsey	11.11 $\pm$ 11.11	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.67 $\pm$ 1.67	28.84 $\pm$ 8.47	0.00 $\pm$ 0.00	0.66 $\pm$ 0.66	18.36 $\pm$ 18.36
Sceptre	60.94 $\pm$ 20.33	3.53 $\pm$ 3.53	0.00 $\pm$ 0.00	24.03 $\pm$ 15.42	2.22 $\pm$ 0.62	0.00 $\pm$ 0.00	5.95 $\pm$ 1.51	2.05 $\pm$ 1.17
Stewart 63	35.66 $\pm$ 5.84	5.75 $\pm$ 5.75	0.50 $\pm$ 0.50	24.09 $\pm$ 12.09	17.39 $\pm$ 0.34	0.00 $\pm$ 0.00	0.94 $\pm$ 0.94	11.94 $\pm$ 4.01
Wakooma	57.33 $\pm$ 17.64	2.41 $\pm$ 1.49	14.43 $\pm$ 7.89	13.02 $\pm$ 9.97	2.00 $\pm$ 0.05	0.00 $\pm$ 0.00	8.94 $\pm$ 0.06	0.00 $\pm$ 0.00
Wascana	30.01 $\pm$ 16.86	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	25.98 $\pm$ 14.17	0.55 $\pm$ 0.55	0.00 $\pm$ 0.00	1.08 $\pm$ 1.08	7.85 $\pm$ 1.60
<b>Landrace</b>								
Arnautka	19.78 $\pm$ 17.98	4.53 $\pm$ 4.53	1.26 $\pm$ 0.75	12.56 $\pm$ 8.51	52.71 $\pm$ 4.12	0.04 $\pm$ 0.04	1.80 $\pm$ 0.46	1.73 $\pm$ 1.12
Golden Ball	43.15 $\pm$ 23.04	0.00 $\pm$ 0.00	0.43 $\pm$ 0.43	25.83 $\pm$ 13.15	8.35 $\pm$ 8.35	0.00 $\pm$ 0.00	3.06 $\pm$ 0.77	2.94 $\pm$ 2.94
Kubanka	59.96 $\pm$ 7.49	5.42 $\pm$ 5.42	0.00 $\pm$ 0.00	17.78 $\pm$ 12.29	2.43 $\pm$ 2.43	0.00 $\pm$ 0.00	9.18 $\pm$ 0.44	4.57 $\pm$ 1.19

Mindum	51.66 ± 2.78	1.95 ± 2.01	0.00 ± 0.00	29.57 ± 2.33	9.86 ± 0.48	0.00 ± 0.00	4.20 ± 1.06	0.00 ± 0.00
Pelissier	32.48 ± 3.02	0.74 ± 1.90	0.87 ± 0.46	33.06 ± 4.67	2.00 ± 0.34	1.38 ± 0.79	1.49 ± 0.07	0.93 ± 2.51
<i>P</i> -values <sup>†</sup>	<b>0.029</b>	0.16	0.42	0.094	0.079	0.18	0.93	0.28

\*References to the genotypes can be found in Knott (1995), Clark et al. (2009*a*, 2009*b*, 2010), and Singh et al. (2010).

<sup>†</sup>*P*-value in bold is significant at  $\alpha = 0.05$ ,  $n = 3$ .

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### Figure legends

**Fig. 1.** Venn diagram of the distribution among niches and sites of the 190 OTUs found in roots and rhizosphere soil in Regina and Swift Current.

**Fig. 2.** Abundance of reads of the genera of arbuscular mycorrhizal (AM) fungi representing the families Paraglomaceae, Archaeosporaceae, Diversisporaceae, Claroideoglomeraceae, and Glomeraceae among the niches (soil and roots) of durum wheat growing at the Regina and Swift Current sites. Abundance is expressed as a percentage of total AM fungi reads. The differences between root- and soil-inhabiting AM fungal communities were significant at both sites ( $P < 0.0001$ ;  $n = 96$  for Regina and  $n = 93$  for Swift Current, according to a multiresponse permutation procedure).

**Fig. 3.** Non-metric multidimensional scaling ordination plots of durum wheat genotypes according to the arbuscular mycorrhizal (AM) fungal communities established in their rhizosphere soil, in Swift Current. Final stress = 0.098 and final instability = 0.00000, after 76 iterations. The AM fungal community of rhizosphere soil was significantly influenced by genotype ( $P = 0.0002$ ,  $n = 3$ ) according to perMANOVA. Cyan markers indicate the most recent genotypes (2004–2010), grey markers indicate the modern genotypes (1957–2001), and violet markers indicate the landraces introduced into Canada (1916–1920).

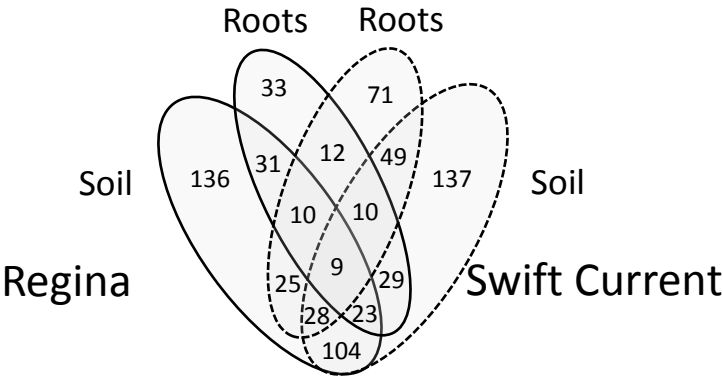


Fig. 1.

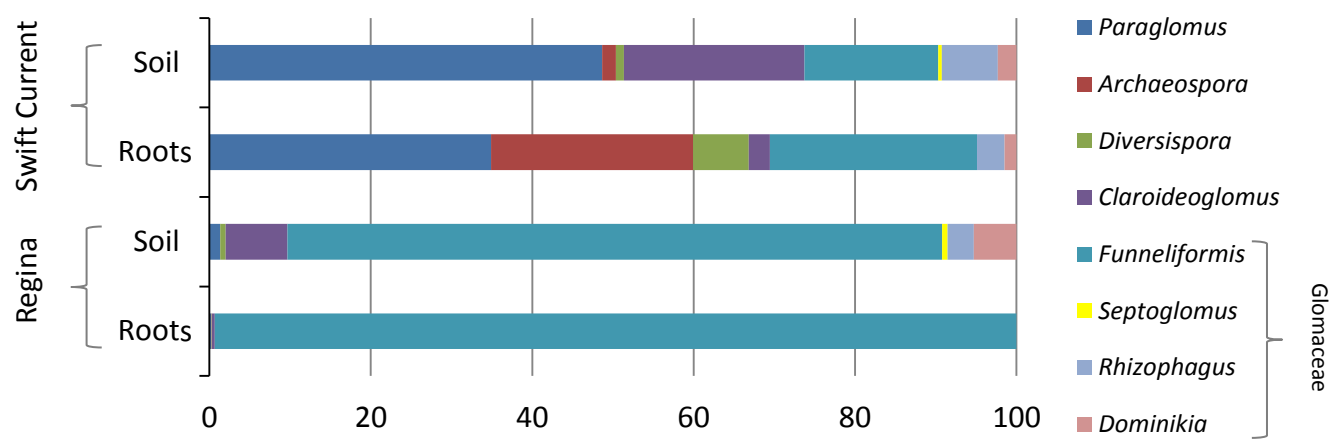


Fig. 2.



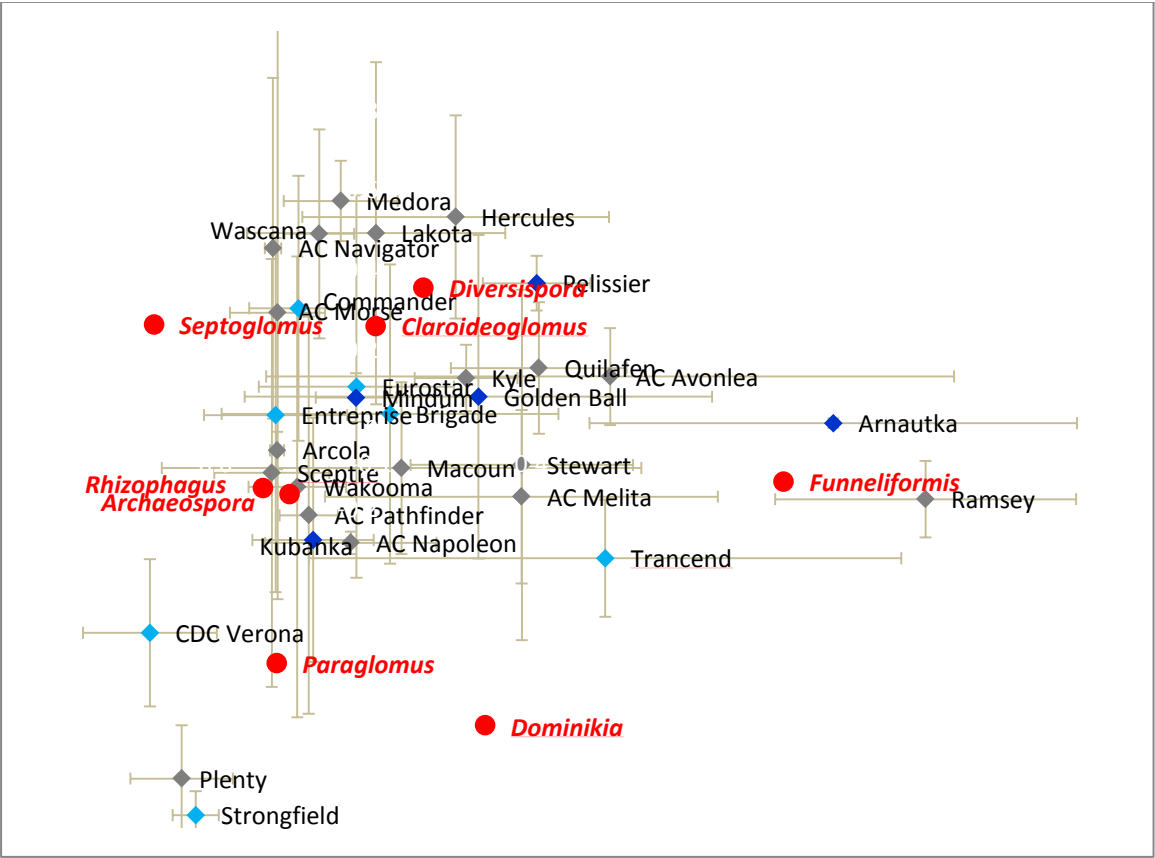


Fig. 3.