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Effect of Inoculation with Arbuscular Mycorrhizal Fungi on Selected Spring Wheat Lines

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Abstract

An experiment was performed in a completely randomized split-plot design using five lines of spring wheat (*Triticum aestivum* L.) (AW-774, AC Carberry, HY-162, Major and AAC Scotia) and two arbuscular mycorrhizal fungi (AMF) strains (*Rhizoglomus irregulare* and *Glomus cubense*). Two different inoculant forms (solid and liquid) for the *G. cubense* strain were evaluated. The main plot was AMF, and the subplot was spring wheat lines. Data on heading date, plant height, fresh, and dry biomass, yield, grain quality (chemical composition of the seeds, gluten, and sugar), root structure, and colonization by AMF were collected. The results show a positive effect of inoculation in comparison with the control treatment. The liquid and solid *G. cubense* inoculants provided better results than inoculation with *R. irregulare*. Fungus indicators were in agreement with root morphological parameters because of the effect induced by AMF activity. Yield increased significantly in the mycorrhizal treatments.

Keywords: fungus, colonization, yields, cereal

1. Introduction

Wheat (*Triticum aestivum* L.) is the world's third produced cereal after maize and rice. It is estimated that by 2015, the production of this cereal will increase by 70% (Tilman et al., 2011) because of the growing needs of the human population for food, feed, fibre, and fuel (Fedoroff et al., 2010). To meet this demand, and because of the limited availability of uncultivated land (Garnett et al., 2013), agriculture faces a problem related to the transformation of many ecosystems to intensive grain production (Mueller et al., 2012).

Strategies such as the use of more environmentally friendly alternatives, natural processes, and environmental conservation play a vital role in current agricultural production. A large number of microorganisms exert positive effects on the growth and development of plants in the rhizosphere region, and are involved in various activities, including dynamic resources availability to plants and preservation of soil fertility (Priyadharsini & Muthukumar, 2015).

Microorganisms play an important role in agricultural systems. Specifically, arbuscular mycorrhizal fungi (AMF) are potential components of sustainable management systems (Adesemoye & Kloepper, 2009). These fungi are biotrophic because they depend on their host to complete their life cycle. They establish symbiotic associations with the majority of terrestrial plants in a diversity of ecosystems (Graham, 2008; Smith & Read, 2008; Neumann & George, 2010), improve plant tolerance to both biotic stresses (e.g., pathogens) and abiotic stresses (e.g., drought, soil salinity, and pollution) (Banuelos et al., 2014; Ruiz-Lozano et al., 2012; Cicatelli et al., 2014; Songachan et al., 2011), and play a role in remediation (Mrnka et al., 2012).

Over the past few decades, companies throughout the world have manufactured and commercialized AMF inoculants using either single AMF species or mixtures of AMF species that may include plant-growth-promoting rhizobacteria or other symbiotic and/or biocontrol fungi (Gianinazzi & Vosátka, 2004). The industrial manufacturing of AMF as crop inoculants is relatively new, and, despite practical demonstrations of the efficiency of AMF, and crop producers have been slow to adopt them. Inoculation with effective microorganisms could lead to enhanced crop productivity and higher incomes for farmers.

The effectiveness of the AM symbiosis is highly dependent on the host plant genotype (Yücel et al., 2009). Differences in the level of association of AMF with particular genotypes were found in four Canadian spring wheat cultivars (Xavier & Germida, 1998). These hexaploid Canadian wheat genotypes differed in AM root mycorrhizal development levels and in their response to inoculation, which ranged from positive to negative. The selection of crop cultivars that have strong associations with AMF may be important in providing adequate soil fertility to the crop (Singh et al., 2012).

The aim of this study was therefore to evaluate the influence of AMF on biomass, root morphology and yield in spring wheat lines.

2. Materials and Methods

2.1 Arbuscular Mycorrhizal Fungi Strain

Two granular inoculants were applied at seeding as follows: 20 g of *Rhizoglomus irregulare* (MYKE PRO commercial inoculants; 1-propagule/g), or 1 g of *Glomus cubense* (living culture of the type-specimen DAOM 241198; 1000-spores/g) per pot. Liquid *G. cubense* inoculant (20-spores/mL) obtained from the National Institute of Agricultural Science in Cuba (Fernández et al., 2004) was applied, through water irrigation, 7 d after seed germination (25-mL per pot). Controls plants were not inoculated.

2.2 Experimental Design and Plant Material

This experiment was conducted in a greenhouse at Agriculture and Agri-Food Canada (Ottawa Research and Development Centre), in Ottawa, Ontario, Canada, under controlled conditions during the period of February to June 2015. The experiment was performed in a completely randomized design with a split- plot arrangement. Five lines of spring wheat (*Triticum aestivum* L.) (AW-774, AC Carberry, HY-162, Major and AAC Scotia), two granular AMF strains (*R. irregulare* and *G. cubense*), and liquid *G. cubense* mycorrhizal treatments were used. The main plot was AMF, and the subplot was spring wheat lines. Each treatment had six replicates for a total 120 pots. The soil used was sieved, homogenized, and sterilized (twice on successive days), and then mixed with pure washed sand (1:1, v/v). Seeds were sown by hand at a depth of 2.5 cm after a germination test had been performed. Six seeds were sown per pot, and one month after planting, two plants per pot were removed. After seeding, irrigation with tap water was applied to maintain soil moisture near the maximum water-holding capacity. Urea was applied two times at a rate of 5-g per pot after 30 days of plant growth.

2.3 Measurements and Analysis

At 120 days, two random test plants were uprooted carefully from each pot treatment. The roots were washed with tap water, and a fresh root portion (200 g) was used to estimate root colonization levels by the grid line intersect method (Giovannetti & Mosse, 1980) after the roots had been bleached with 10% KOH using the microwave oven (Dalpé & Séguin, 2013) and stained with acid fuchsin (Phillips & Hayman, 1970). The frequency and intensity of colonization indicators were determined according to the methodology described in Trouvelot et al. (1986).

Plant height, fresh and dry roots and plants biomasses were measured. Total length (cm) and width (cm^2) of roots were measured using WinRHIZO Pro image analysis software. The number of grains per spike, number of grains per plant, grain weight, and yield were determined. Grain protein was evaluated using Kjeldahl digestion and total Kjeldahl nitrogen analysis.

2.4 Statistical Analysis

Data were analysed using the GLM procedure of the SAS software package (SAS Institute, 1989), and the means were separated using the least significant difference (LSD) method at the 5% level.

3. Results and Discussion

The effects of mycorrhizal inoculation on different parameters of the spring wheat lines AW-774, AC Carberry, HY-162, Major, and AAC Scotia are shown in Tables 1 to 5, respectively. Mycorrhizal root colonization was different between spring wheat lines and between AMF treatments. The Major and AW-774 lines showed a higher level of colonization (58% - 59%), whereas AC Carberry and HY-162 showed a lower level (46% - 47%). The AAC Scotia line showed the lowest mycorrhizal colonization level (35%) in comparison with two other wheat lines.

Arbuscular mycorrhizal fungi interact at the root–soil interface in a coordinated manner. Their hyphae absorb and translocate water and nutrients from the soil to the plant to increase its growth and development (Dwivedi, 2015; Priyadharsini & Muthukumar, 2015). Wheat is colonized by mycorrhizae, but colonization depends on AMF strains and soil conditions. Studies conducted in India showed that the interaction between mycorrhizae and wheat depends on the variety, and thus it is important to evaluate mycorrhizal dependency in the crops being grown (Solaiman et al., 2014).

Table1. Effects of arbuscular mycorrhizal fungal inoculation of spring wheat line AW-774 on plant parameters.

Treatment	Yield (g/pot)	Plant height (cm)	Spike weight (g)	Tiller number	Fresh biomass (g)	Dry biomass (g)	Fresh root biomass (g)	Dry root biomass (g)	Root colonization frequency (%)	Root colonization intensity (%)	Root total length (cm)	Root total width (cm^2)
Control	4.77 c	50.87	6.52 c	6.33 c	1.21	1.19	14.55 c	0.40	1.00 c	0.01 c	413.50 b	16.82 c
Glomus cubense (Liquid)	7.70 a	57.47	9.73 a	9.67 a	1.30	1.29	23.72 a	0.47	58.00 a	2.45 a	923.30 a	42.52 a
Rhizoglomus irregulare (Solid)	6.75 b	52.88	8.55 b	7.50 b	1.23	1.23	17.58 b	0.44	35.50 b	0.58 b	836.90 a	37.23 a
Glomus cubense (Solid)	8.48 a	57.25	10.67 a	9.67 a	1.33	1.30	23.19 a	0.42	59.17 a	2.48 a	592.50 b	30.06 ab
F-value	27.94	1.52	30.45	28.67	1.54	1.45	61.50	1.40	379.39	66.70	9.15	10.22
LSD	0.89	7.82NS	0.96	0.91	0.13NS	0.13NS	1.67	0.07NS	4.13	0.46	226.59	10.27
SE	0.30	2.65	0.32	0.31	0.04	0.04	0.57	0.02	1.40	0.16	76.81	3.48

Note: Different letters within each block indicate significant differences ($p \leq 0.05$) according to the least significant difference (LSD) test. NS: not significant.

SE: standard error

Table 2. Effects of arbuscular mycorrhizal fungal inoculation of spring wheat line AC Carberry on plant parameters.

Treatment	Yield (g/pot)	Plant height (cm)	Spike weight (g)	Tiller number	Fresh biomass (g)	Dry biomass (g)	Fresh root biomass (g)	Dry root biomass (g)	Root colonization frequency (%)	Root colonization intensity (%)	Root total length (cm)	Root total width (cm^2)
Control	3.66 b	42.83 b	4.96 b	6.33 c	0.90 b	0.87 b	11.05 d	0.25 c	1.00 c	0.01 d	391.55b	16.51 b
Glomus cubense (Liquid)	5.22 a	49.40 a	7.06 a	10.17 a	1.17 a	1.15 a	19.52 b	0.34 a	47.50 a	2.23 a	383.84b	15.89 b
Rhizoglomus irregulare (Solid)	4.46 ab	44.12 b	6.35 a	7.00 c	1.05 ab	1.02 ab	14.16 c	0.31 b	36.67 b	0.60 c	494.60 a	23.26 a
Glomus cubense (Solid)	5.22 a	49.43 a	7.12 a	8.67 a	1.19 a	1.16 a	20.89 a	0.37 a	46.83 a	1.83 b	559.05 a	26.33 a
F-value	6.26	6.74	7.51	20.37	5.24	5.67	125.50	12.25	369.87	99.28	7.10	5.14
LSD	0.88	3.94	1.08	1.13	0.17	0.17	1.21	0.04	3.36	0.31	93.67	6.67
SE	0.30	1.34	0.37	0.38	0.06	0.06	0.41	0.01	1.14	0.10	31.75	2.26

Note: Different letters within each block indicate significant differences ($p \leq 0.05$) according to the least significant difference (LSD) test. NS: not significant.

SE: standard error

Table 3. Effects of arbuscular mycorrhizal fungal inoculation of spring wheat line HY-162 on plant parameters.

Treatment	Yield (g/pot)	Plant height (cm)	Spike weight (g)	Tiller number	Fresh biomass (g)	Dry biomass (g)	Fresh root biomass (g)	Dry root biomass (g)	Root colonization frequency (%)	Root colonization intensity (%)	Root total length (cm)	Root total width (cm^2)
Control	3.48 b	31.35c	4.79 b	6.33b	0.75 b	0.74 b	9.53b	0.19b	1.00 c	0.01 d	348.75	16.12
Glomus cubense (Liquid)	4.70 a	42.10a	5.90 a	8.33a	0.98 a	0.96 a	13.71a	0.26a	45.67 a	6.52a	384.23	18.82
Rhizoglomus irregulare (Solid)	3.68 b	36.50b	5.01 b	6.33 b	0.87 ab	0.85 ab	10.25 b	0.24 a	36.50b	0.60 c	390.50	15.82
Glomus cubense (Solid)	4.64 a	42.03a	5.94 a	8.50 a	1.00 a	0.98 a	13.05 a	0.24 ab	47.50 a	2.23 b	354.38	16.43
F-value	7.10	15.87	5.49	18.33	4.26	3.78	20.11	2.67	519.32	1.56	0.46	0.92
LSD	0.71	3.81	0.75	0.83	0.16	0.17	1.35	0.05	2.80	6.93	90.78NS	4.23NS
SE	0.24	1.29	0.25	0.28	0.06	0.06	0.46	0.02	0.95	2.35	30.67	1.44

Note: Different letters within each block indicate significant differences ($p \leq 0.05$) according to the least significant difference (LSD) test. NS: not significant.

SE: standard error

Table 4. Effects of arbuscular mycorrhizal fungal inoculation of spring wheat line Major on plant parameters.

Treatment	Yield (g/pot)	Plant height (cm)	Spike weight (g)	Tiller number	Fresh biomass (g)	Dry biomass (g)	Fresh root biomass (g)	Dry root biomass (g)	Root colonization frequency (%)	Root colonization intensity (%)	Root total length (cm)	Root total width (cm^2)
Control	4.73 c	50.21c	7.09 c	6.17 b	1.31 b	1.28 b	15.60 c	0.41 c	1.00 c	0.01 c	433.06 c	17.18c
Glomus cubense (Liquid)	7.45 a	79.37a	9.78 a	9.17 a	1.59 a	1.58 a	36.25 a	0.73 a	58.17 a	2.47 a	988.21 a	43.00a
Rhizoglomus irregulare (Solid)	6.38 b	56.90b	8.42 b	6.83 b	1.39 b	1.37 b	24.46 b	0.47 b	38.33 b	0.79 b	736.96b	33.76b
Glomus cubense (Solid)	7.87 a	79.62a	9.60 a	10. 17 a	1.60 a	1.59 a	35.95 a	0.74 a	58.00 a	2.45 a	1010.02a	48.04a
F-value	16.10	119.38	10.59	28.04	11.99	11.95	163.71	154.00	429.18	48.11	31.24	47.05
LSD	1.03	4.11	1.13	1.05	0.12	0.13	2.29	0.04	3.87	0.52	142.25	5.83
SE	0.35	1.39	0.38	0.36	0.04	0.04	0.78	0.01	1.31	0.18	48.22	1.98

Note: Different letters within each block indicate significant differences ($p \leq 0.05$) according to the least significant difference (LSD) test. SE: standard error

Table 5. Effects of arbuscular mycorrhizal fungal inoculation of spring wheat line AC Scotia on plant parameters.

Treatment[a]	Yield (g/pot)	Plant height (cm)	Spike weight (g)	Tiller number	Fresh biomass (g)	Dry biomass (g)	Fresh root biomass (g)	Dry root biomass (g)	Root colonization frequency (%)	Root colonization intensity (%)	Root total length (cm)	Root total width (cm)
Control	5.28b	40.67	7.81b	5.50	1.21	1.19	8.73 c	0.21 b	1.00 b	0.01 b	403.75ab	16.73ab
Glomus cubense (Liquid)	6.08a	41.87	9.09a	5.67	1.22	1.20	12,05 a	0.25 a	35.66 a	0.58 a	488.80 a	23.44a
Rhizoglomus irregulare (Solid)	5.62ab	41.00	7.69b	5.33	1.19	1.17	10.63 b	0.22 ab	35.50 a	0.56 a	485.05 a	21.66ab
Glomus ubense (Solid)	5.98ab	40.80	8.22ab	5.50	1.21	1.19	12.43 a	0.24 ab	36.50 a	0.59 a	285.10b	15.55b
F-value	1.91	0.19	3.82	0.39	0.05	0.05	15.25	1.86	298.69	197.06	3.79	2.65
LSD	0.77	3.67NS	0.95	0.64NS	0.13NS	0.14NS	1.26	0.04	2.98	0.06	144.69	6.90
SE	0.26	1.24	0.32	0.22	0.05	0.05	0.43	0.01	1.01	0.02	49.05	2.34

Note: Different letters within each block indicate significant differences ($p \leq 0.05$) according to the least significant difference (LSD) test. NS: not significant.

SE: standard error

The mycorrhizal colonization results showed differences between *G.cubense* and *R.irregulare*, which could be related to infectivity. Some studies demonstrated that fungal infectivity can be associated with differences in inoculum level and in the ability of the fungi to colonize roots (Solaiman et al., 2014). In our case, the inoculum concentration was different for both strains; such a difference can induce variations in fungal colonization and interfere with the relationship between inoculum level and infectivity. However, other research demonstrated a positive effect of the *G.cubense* strain in a different crop grown in red soil with low to high fertility (Rivera et al., 2007). The mycorrhizal colonization levels obtained in the present study are similar to those reported for rice cultivation in saline conditions (Fernández et al., 2011).

The root morphology results showed significant differences between wheat lines, but for the HY-162 line, no positive effect to AMF was found. A comprehensive analysis of root morphology variables for the Major wheat line showed that *G.cubense* was more effective than *R.irregulare*. The results for these variables were in agreement with those for fungal colonization. Although root morphological variables for the AAC Scotia line showed significant differences, the level of mycorrhizal colonization was low, with no significant differences from the levels for the other lines, and exceeded only the level for the control treatment.

Significant differences in plant growth indicators (plant height, fresh and dry weights of roots and biomass) between all five wheat lines under study were determinated. In two lines (AW-774 and AAC Scotia), inoculation with AMF strains showed no positive effect on plant height, but in the rest of the lines, significant differences were obtained in relation to the control treatment.

The tallest plants were achieved with the Major wheat line, at 79 cm, in the treatments inoculated with *G.cubense*, whereas inoculation of Major with *R.irregulare* produced a height of 56 cm. Inoculation with liquid and solid *G.cubense* stimulated plant height for the AC Carberry and HY-162 wheat lines, with values of 49 and 42 cm, respectively. Root dry weight differed between wheat lines and between inoculation treatments. The AW-774 wheat line did not show significant differences for this indicator, but levels were variable and exceeded the value for the control treatment.

Plant growth indicators can be used to evaluate benefits when microorganisms are applied, given that plant growth and development are stimulated significantly. Our results show differences depending on the AMF strains and spring wheat lines, but inoculant treatment responded to fungal colonization. The results were higher when *G.cubense* (liquid and solid) inoculum was applied. This response could be related to mycorrhizal effectiveness (Bonfante & Genre, 2010; Fernández et al., 2011). The positive effect of AMF on the height and development of the aerial part and root system of plants was previouly reported in various crops, including maize (*Zea mays*) (Sheng et al., 2011), tomato (*Solanum lycopersicum*) (Hajiboland et al., 2010), wheat (*Triticum aestivum*) (Stonor et al., 2014), rice (*Oryza sativa*) (Fernández et al., 2011) and pepper (*Capsicum annuum*) (Çekiçet al., 2012).

The yield of a crop is the end result of the interaction of several factors. In this study, differences between AMF strains and between wheat lines were found for yield components. Inoculation with liquid and solid *G.cubense* increased tiller number and spike weight in four of the wheat lines (AW-774, AC Carberry, HY-162, and Major). Inoculation with *R.irregulare* increased the levels of those indicators as well, but to a lower extent than inoculation with *G.cubense* did. Wheat yield was stimulated by AMF inoculation. Inoculation with liquid and solid *G.cubense* was more effective than inoculation with *R.irregulare* was, but both species showed better results than control treatment.

The effects of mycorrhizal inoculation on total nitrogen and grain protein in the spring wheat lines are shown in Table -6. In this case, AMF inoculation did not have a positive effect for both indicators, and only the HY-162 line showed significant differences. This response could be related to the fertilization and mineral requirements of each wheat line.

Table 6. Effects of arbuscular mycorrhizal fungal inoculation of spring wheat lines on total nitrogen (N) and grain protein.

Treatment[a]	AW-774		AC Carberry		HY-162		Major		AAC Scotia	
	Total N (%)	Grain protein (%)	Total N (%)	Grain protein (%)	Total N (%)	Grain protein (%)	Total N (%)	Grain protein (%)	Total N (%)	Grain protein (%)
Control	3.27	18.63	3.50b	20.03	3.87ab	22.10ab	3.80	21.66	3.51	19.96
Glomus cubense (Liquid)	3.27	18.63	3.70a	21.10	3.98a	22.70a	3.67	21.00	3.70	21.10
Rhizoglomus irregulare (Solid)	3.29	18.73	3.60ab	20.53	3.60c	20.56c	3.37	19.20	3.60	20.53
Glomus cubense (Solid)	3.43	19.56	3.64a	20.80	3.68bc	21.00bc	3.49	19.90	3.45	19.70
F-value	0.61	0.62	2.45	2.24	6.88	6.94	1.78	1.77	0.39	0.37
LSD	0.32NS	1.87NS	0.17	0.98NS	0.21	1.21	0.46NS	2.70NS	0.58NS	3.35NS
SE	0.10	0.58	0.05	0.30	0.07	0.37	0.14	0.83	0.18	1.03

Note: Different letters within each block indicate significant differences ($p \leq 0.05$) according to the least significant difference (LSD) test. NS: not significant. SE: standard error

The results showed a positive effect of inoculation in comparison with the control treatment. Liquid and solid *G.cubense* inoculant produced better results than inoculation with R.*irregulare*. Fungus indicators were in

agreement with root morphological parameters because of the effect induced by AMF activity. Yield increased significantly in the mycorrhizal treatments.

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