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Original article

Response to arbuscular mycorrhizal fungi inoculation in different spring wheat (*Triticum durum* L.) lines

Respuesta a la inoculación de hongos micorrízicos arbusculares en diferentes líneas de trigo (*Triticum durum* L.) de primavera

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ABSTRACT : With the aim to evaluate the response to arbuscular mycorrhizal fungi inoculation in different lines of spring wheat this research was developed under controlled conditions. Seeds from five spring wheat (*Triticum durum* L.) lines (AW-774, AC Carberry, HY-162, Major and AAC Scotia), with 98 % germination, were used and disinfected before sowing. The INCAM-4 strain: *Glomus cubense* (in solid and liquid formulation), the Canadian commercial inoculant MYKE®PRO (*Rhizoglomus irregulare*) and a control without inoculation were used. Treatments were distributed with an arrangement of divided plots under a completely randomized design, the main plot being the inoculation of AMF and the five lines of spring wheat as sub-plot. The evaluations were carried out at 120 days after sowing and variables related to mycorrhizal functioning (frequency and intensity of colonization), with the growth and development of the plants and the crop yield were determined. The results showed a positive effect of *Glomus cubense* inoculation in both formulations (solid and liquid). A differential response in AMF inoculation was observed among spring wheat lines. The Major and AW-774 lines showed significant increases in the variables studied compared to the rest.

Key words: cereal, mycorrhiza, yield.

RESUMEN : Esta investigación se desarrolló con el objetivo de evaluar la respuesta a la inoculación de hongos micorrícicos arbusculares (HMA) en diferentes líneas de trigo de primavera, en condiciones controladas. Se utilizaron semillas de cinco líneas de trigo (*Triticum durum* L.) de primavera (AW-774, AC Carberry, HY-162, Major y AAC Scotia), con un 98 % de germinación, que se desinfectaron antes de la siembra. Se utilizó la cepa de HMA INCAM - 4: *Glomus cubense* (en formulación sólida y líquida), el inoculante comercial canadiense MYKE®PRO: *Rhizoglomus irregulare* y un control sin inoculación. Los tratamientos se distribuyeron con un arreglo de parcelas divididas bajo un diseño completamente aleatorizado, siendo la parcela principal la inoculación del HMA y como sub-parcelas las cinco líneas de trigo de primavera. Las evaluaciones se realizaron a los 120 días después de la siembra y se determinaron variables relacionadas con el funcionamiento micorrícico (frecuencia e intensidad de la colonización), con el crecimiento y desarrollo de las plantas y el rendimiento del cultivo. Los resultados mostraron un efecto positivo de la inoculación de *Glomus cubense*, con ambas formulaciones (sólido y líquido). También, se observó una respuesta diferencial de las líneas de trigo de primavera a la inoculación de HMA. Las líneas Major y AW-774, mostraron incrementos significativos en las variables estudiadas, comparadas con el resto.

Palabras clave: cereal, micorrizas, rendimiento.

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INTRODUCTION

Today's agriculture requires new approaches to sustainability and sustainability, supported by research, innovation and technology, in order to ensure food security (1). In this context, arbuscular mycorrhizal fungi (AMF) represent a group of soil microorganisms that establish symbiosis with numerous plant species of agricultural interest (2).

Among the main benefits of this symbiosis are direct effects on mineral nutrition, especially on the absorption of macro and micronutrients (3, 4), the induction of tolerance to biotic (e.g. pathogens) (5) and abiotic (e.g. drought and salinity) stress conditions (6, 7), their participation in phytoremediation processes (8) and their contribution to the stability of soil aggregates (9).

Of special interest is the symbiosis established between AMF and cereals (10, 11), specifically with wheat (*Triticum* spp.), a crop that represents the third cereal with the highest production worldwide, as it is an important source of vegetable protein, cultivated under diverse edaphoclimatic conditions (12).

Spring wheat cultivars differ from winter cultivars in having a higher protein content, grain hardness and better physicochemical properties of the mass, which favors its milling process and gives its flour a superior quality (13). Some studies have demonstrated the effectiveness of mycorrhizal symbiosis in wheat plants, highlighting that a better nutrition in the plant increases its yield; therefore, it is suggested that AMF can be part of the strategies that lead to a better food security (14).

In view of these criteria, the objective of this study was to evaluate the response to the inoculation of arbuscular mycorrhizal fungi in different spring wheat lines, under controlled conditions.

MATERIALS AND METHODS

General characteristics of the experiment

The experiment was conducted under controlled greenhouse conditions at the Ottawa Agricultural Institute, Canada. Treatments were distributed in a split-plot arrangement under a completely randomized design, with the main plot being the AMF inoculation and the five spring wheat lines as sub-plots. A mixture of commercial organic substrate: Lambert (Canadian *Sphagnum* peat moss with pH = 7.1) and washable sand, in a 1:1 (v/v) ratio, which was autoclaved at 121 ºC for two hours, in cycles of three continuous days, was used. Six seeds were sown per pot and seven days after plant emergence, the plants were thinned and three plants were left per pot.

Plants were grown under controlled conditions with an average temperature ranging between 20 - 22 °C, 80 % relative humidity and photoperiod adjusted to 16 hours light/8 hours dark. Urea was applied at a rate of 5 g per pot, which was divided in two moments of the crop cycle: 3 g 15 days after sowing and the rest 30 days later, according to the established norms for these wheat lines in greenhouses (15). Six observations were made per treatment for a total of 120 pots.

Description of plant material

Plant material consisted of seeds from five spring wheat (*Triticum durum* L.) lines (AW-774, AC Carberry, HY-162, Major, and AAC Scotia), with 98 % germination and certified by the Canadian Food Inspection Agency variety registration office (15). Seeds were sown in 5 kg pots (0.22 m high and 0.24 m top diameter).

Description of fungal material

INCAM - 4 strain: *Glomus cubense* (Y. Rodr. & Dalpé) (16), in solid and liquid formulation, from the collection of the National Institute of Agricultural Sciences (INCA) of Cuba and registered in the National Mycological Herbarium of Canada, Ottawa, with code DAOM 241198, was used, in addition to the Canadian commercial inoculant MYKE®PRO (*Rhizoglomus irregulare*) (Blaszk., Wubet, Renker & Buscot) (17).

The application of the solid formulation (SF) of *G. cubense*, with an average concentration of 20 spores per gram of inoculant, consisted of 1 g per pot, while in the case of the commercial product MYKE®PRO, with a purity of 1 propagule per gram of inoculant, 20 grams per pot were inoculated. Both applications were made at the time of planting.

For the application of the inoculant in liquid formulation (LF), a hydraulic dosing injection pump for water-soluble products (ECOFERTIC) was used, at a pressure of 0.15 MPa and with a delivery of 5 mL per pot in each irrigation. The pump was adjusted to the drip irrigation system with hoses as distribution lines and drippers spaced at 30 cm, with output for four pots. A single application of this formulation was made at the time of planting, at a concentration of 20 spores per mL, in a final volume of 150 mL of the product.

In the treatments inoculated with the solid formulation (SF) of *G. cubense*, the commercial product MYKE®PRO and the control, irrigation was carried out manually, at the frequency and volume of water used in the inoculated treatment by drip irrigation. In the case of the treatment inoculated with the liquid formulation, irrigation was carried out using the automated system. In both cases, irrigation was in correspondence with the phenology of the spring wheat lines (15).

Evaluations and statistical analysis

The experiment was extended for 120 days after sowing (DAS) of seeds and the following indicators were evaluated:

- Frequency and intensity of fungal colonization (%): 250 mg of secondary roots were taken from each sample, which were carefully washed, dried in an oven at 70 ºC until constant mass, stained, clarified (18) and quantified (19).

- Plant growth and development: Spike length (cm), fresh and dry mass of aerial part and root (g) and root volume (m^3) were determined. Fresh mass was determined by weighing on a digital technical balance (Acom JW-1, precision level 0.1 g), while dry mass was determined after drying the samples at 70 ºC in the oven to constant mass. Roots were washed with abundant water to remove soil particles. Subsequently, they were placed in a scanner and the root volume was determined with the WinRhizo-Pro program for Windows.
- Crop yield: The number of ears per plant, average grain mass (g) and crop yield (g per m^2) were determined.

The assumptions of normality and homogeneity of variance were verified. Statistical processing of the data was carried out by means of multivariate principal component analysis and hierarchical full linkage clustering. The clustering obtained from these analyses was verified using a Discriminant Factor Analysis. The IBM SPSS version 19.0 statistical package was used.

RESULTS

The Principal Component analysis allowed identifying which of the evaluated variables were most related to the observed differences, not only in the growth and development of the wheat lines, but also in their response to AMF inoculation. As a result of the analysis, two components were found that showed a cumulative variance of 85.51 % (Table 1).

Table 2 shows the variables that showed the highest correlation coefficients with both components.

In the case of component 1, the variables with the highest incidence were those related to plant growth (spike length, aerial and root dry and fresh mass and root volume), crop yield (number of spikes per plant and yield) and colonization frequency; while with component 2, AMF colonization intensity was related.

Figure 1 shows the clustering of the treatments, after the application of both multivariate methods (Principal Components and Full Linkage Hierarchical Clustering).

Clustering of treatments was the result of the order of their appearance from the Complete Ligament Hierarchical Cluster Analysis (Figure 2) performed. For the distribution of the treatments, the variables with the highest correlation in component 1 were taken into account.

Group I was represented by the treatments inoculated with *G. cubense*, in both formulations, in the Major wheat line. Close to it was group II, identified by the combinations of *G. cubense* (solid and liquid) and *R. irregulare* in the AW-774 and Major lines. Group III consisted of treatments inoculated with *G. cubense* (solid and liquid) and *R. irregulare* in wheat lines HY-162 and AC Carberry. A little distant was group IV, with the control treatments and the one inoculated with *R. irregulare* in the same lines of the previous grouping. Finally, group V consisted of the control treatment in lines AW-774, AAC Scotia and Major and the inoculation of *G. cubense* (solid and liquid) and *R. irregulare* in line AAC Scotia.

The multivariate analysis allowed finding, in an integral way, a positive effect of mycorrhizal inoculation in the different spring wheat lines, based on significant increases in the inoculated treatments in relation to the absolute control, highlighting the treatments with *G. cubense*, in both formulations, in the Major line (group I) with the best performance.

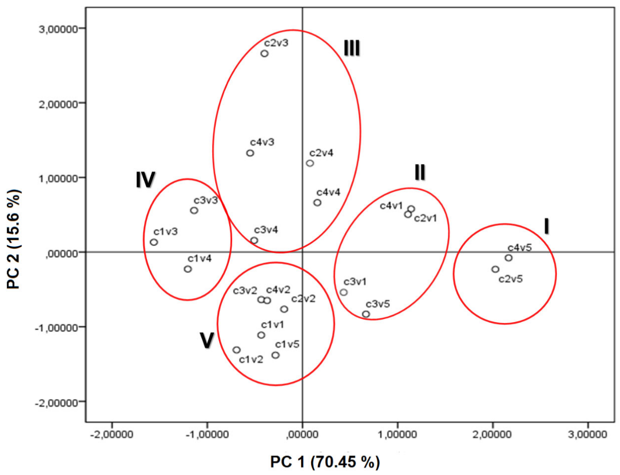
The discriminant factor analysis showed 100 % coincidence between the groups formed. Table 3 shows the means of the variables evaluated by each group formed. It was observed that the highest values of the indicators evaluated were located in-group I, with a tendency to decrease in groups IV and III, while group V was represented by the treatments with lower values.

Table 1. Eigenvalue and explained variance plot for the Principal Component method

Components	Eigenvalue	Variance explained (%)	Cumulative variance (%)
1	7.75	70.45	70.45
2	1.66	15.06	85.51

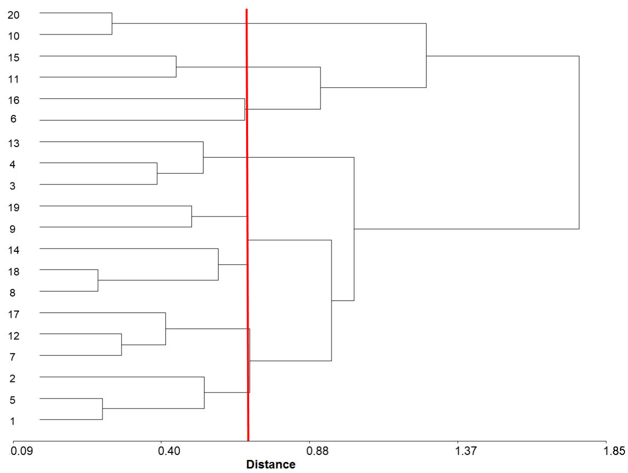
Table 2. Correlations between the initial variables and the first two components of the principal component analysis

Variables	Component 1	Component 2
Mass Grains (g)	0.697	0.597
Length of spike (cm)	0.947	-0.090
Fresh air mass (g)	0.901	-0.355
Aerial Dry Mass (g)	0.904	-0.349
Fresh Root Mass (g)	0.949	0.057
Root Dry Mass (g)	0.913	-0.116
Frequency (%)	0.705	0.532
Intensity (%)	0.393	0.819
Root Volume (m^3)	0.901	-0.046
Yield (g per m^2)	0.904	-0.099
Number of Spikes	0.849	-0.246



c1: Control; c2: *Glomus cubense* (liquid); c3: *Rhizoglomus irregulare*; c4: *Glomus cubense* (solid); V1: AW-774; V2: AAC Scotia; V3: HY-162; V4: AC Carberry; V5: Major. Matrix of principal components 1 and 2 (PC1 and PC2). Clustering: I (c4V5, c2V5); **II** (c2V1, c3V1, c3V5, c4V1); **III** (c2V3, c2V4, c4V3, c4V4); **IV** (c1V3, c1V4, c3V3, c3V4); **V** (c1V1, c1V2, c1V5, c2V2, c3V2, c4V2)

Figure 1. Distribution of treatments, according to the Principal Components and Full Linkage Hierarchical Cluster Analysis, in the indicators evaluated



Vertical line (red color) in the graph indicates the distance at which the clustering was established. 1: C1V1, 2: C1V2, 3: C1V3, 4: C1V4, 5: C1V5, 6: C2V1, 7: C2V2, 8: C2V3, 9: C2V4, 10: C2V5, 11: C3V1, 12: C3V2, 13: C3V3, 14: C3V4, 15: C3V5, 16: C4V1, 17: C4V2, 18: C4V3, 19: C4V4, 20: C4V5. C1: Control; C2: *Glomus cubense* (liquid); C3: *Rhizoglomus irregulare*; C4: *Glomus cubense* (solid); V1: AW-774; V2: AAC Scotia; V3: HY-162; V4: AC Carberry; V5: Major

Figure 2. Clustering of treatments with the application of the multivariate method Hierarchical Complete Ligament Clustering

DISCUSSION

Although some studies refer to the nonspecific character of mycorrhizal symbiosis due to the diversity of families of the plant kingdom, as they are susceptible to colonization (20), each plant species, and even cultivars or varieties within the same species, have a different degree of mycorrhizal dependence (21).

Some authors evaluated the feasibility of an autotrophic cultivation system in the production of spores and its capacity to reproduce the life cycle of the fungus under *in vitro* conditions in different European and Andean cultivars of potato (*Solanum tuberosum* L.) and found a differential response among them. It could be related to physiological and genetic characters of each cultivar that determined different degrees of dependence (22).

Similarly, in other investigations, the effect of *R. irregulare* inoculation was evaluated in 5 wheat cultivars and they found a different mycorrhizal dependence in each cultivar, with variable colonization percentages that were correlated with the rest of the indicators evaluated (dry biomass, grain mass and phosphorus content in the grain) (23).

The elements described above, together with the aforementioned differential behavior of the wheat lines evaluated in this study, confirm that, although AMF develop non-specific associations with their host plants, the degree of mycorrhizal dependence tends to vary for some plant-fungus combinations.

Table 3. Means of the variables for each group formed from the discriminant factor analysis

Variables Evaluated	Averages per Group				
	I	II	III	IV	V
Mass Grains (g)	9.67	8.92	8.42	6.50	5.75
Length of spike (cm)	74.49	56.13	45.75	44.24	38.70
Fresh air mass (g)	1.60	1.31	1.22	1.08	0.89
Aerial Dry Mass (g)	1.58	1.30	1.06	1.20	0.86
Fresh Root Mass (g)	36.10	22.24	16.79	12.33	11.25
Root Dry Mass (g)	0.74	0.45	0.30	0.28	0.25
Frequency (%)	58.58	47.75	46.88	18.79	18.44
Intensity (%)	2.46	1.57	3.20	0.30	0.29
Root Volume (m^3)	45.52	35.89	19.37	18.56	17.93
Yield (g per m^2)	381.05	364.67	269.17	246.03	190.07
Number of Spikes	9.69	9.34	7.73	6.05	5.28

CONCLUSIONS

The treatments inoculated with *G. cubense*, regardless of the type of formulation used, showed a superior performance to that observed when *R. irregulare* was applied, in all the varieties evaluated. Similarly, it was observed that the spring wheat lines showed a different degree of response to inoculation, with the Major and AW-774 lines standing out with higher yield values, specifically.

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