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Effect of spontaneous arbuscular mycorrhizal colonization in bread wheat varieties on the incidence of foliar diseases and grain yield

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This study aimed to determine the ability of different wheat genotypes to form a symbiosis with arbuscular mycorrhizal fungi (AMF) present in the field and the effect of such a symbiosis on disease severity and grain yield. A bioassay was performed during an agricultural cycle under field conditions in a randomized block factorial design. The factors used were application of fungicide (two levels: with and without fungicide) and wheat genotypes (six levels). Arbuscular mycorrhizal colonization, green leaf area index, and severity of foliar diseases were evaluated in the tillering and early dough stages. At maturity, the number of spikes per square metre the number of grains per spike, and the thousand-kernel weight were determined to estimate grain yield. In addition, the spores of Glomeromycota present in the soil were identified by morphological techniques. Spores belonging to 12 fungal species were recovered. Genotypic variability was found for arbuscular mycorrhization, with the cultivars Klein Liebre and Opata exhibiting the highest colonization values. The results obtained show a beneficial effect of mycorrhizal symbiosis on foliar disease resistance and grain yield in the controls, but the results varied in the case of fungicide treatment. A greater understanding of the ecological role of these microorganisms in agricultural systems can lead to more sustainable agronomic practices.

Keywords. Glomeromycota; mycorrhizal colonization; severity of foliar diseases; *Triticum aestivum*

1. Introduction

Bread wheat (*Triticum aestivum* L.) is a staple food of 35% of the world's population, representing approximately 20% of the total calories consumed. Since the 1960s, wheat grain yield has increased with time in keeping with world population growth. Argentina ranks among the ten leading world producers, with Buenos Aires province having the largest agricultural area devoted to the primary production of wheat (Ministry of Agriculture, Livestock and Fisheries, 2019: *https://www.magyp.gob.ar/sitio/areas/estimaciones/tableros/tablero-ultivos.php?accion=imp*). Annually, diseases of this crop cause harvest losses of 10–28% (Figueroa *et al*. 2017; Savary *et al*. 2019). Among them, foliar diseases such as yellow rust (*Puccinia striiformis* f. sp. *tritici* West), leaf rust or orange rust (*Puccinia triticina* Eriks), and yellow spot (*Pyrenophora tritici-repentis* [Died.] Drechs, anamorph *Drechslera tritici-repentis* [Died.]) are significant (Dean *et al*. 2012).

Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota (Schüssler *et al*. 2001), form symbiotic associations with the roots of most plants of agricultural interest. This symbiosis promotes greater efficiency in nutrient uptake, particularly of low-mobility nutrients such as phosphorus, thus increasing crop nutrition and growth (Smith and Read 2008). Due to their ability to associate with most crop plants and to produce favorable changes in their hosts, AMF have potential economic importance in agriculture. AMF may play an important role in resistance to

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biotic stress factors such as root, stem, and leaf pathogens (Pozo *et al*. 2013). This protective effect has been attributed to different mechanisms associated with an increase in mineral nutrition (Gernns *et al*. 2001; Mustafa *et al*. 2016) and the induction of systemic resistance to diseases (Jung *et al*. 2012). It is possible to compensate for yield loss in a crop affected by pathogens if plants establish symbiosis with effective mycorrhizal fungi (Gernns *et al*. 2001).

Several authors have reported positive effects of symbiosis with AMF on wheat crop development and grain yield, especially under limiting conditions such as drought, low nutrient availability, and salinity (Pellegrino *et al*. 2015; Smith *et al*. 2015; Zhang *et al*. 2019). The genetic diversity of wheat has been shown to determine great variability in its response to mycorrhizal symbiosis, as observed in a comparative evaluation of the interaction of different genotypes with AMF. Genotypes can respond differently to mycorrhization in terms of growth, colonization, nutrient uptake, and grain yield (Pellegrino *et al*. 2015; Fiorilli *et al*. 2018; García de León *et al.* 2020). It has also been proposed that the year of cultivar release could determine the responsiveness to mycorrhization, with the oldest cultivars benefiting the most from mycorrhizal symbiosis (Hetrick *et al*. 1993). However, more recent studies have shown variable results (Lehman *et al.* 2012; Lehnert *et al*. 2017; Zhang *et al*. 2019; García de León *et al.* 2020).

The studies conducted with wheat plants and AMF have mostly used cultured fungal species. However, it is interesting to study how wheat cultivars respond not only to inoculation with cultured fungal taxa but also to AMF communities that are naturally present in agricultural soils (García de León *et al.* 2020). In this study, spontaneous arbuscular mycorrhizal colonization was evaluated in six wheat genotypes grown under field conditions in andisol during an agricultural cycle. In addition, plant growth parameters, the severity of foliar diseases, and grain yield were measured. The objective of this study was to determine the ability of the different genotypes to form a symbiosis with the AMF present in the field, their influence on foliar disease resistance, and their impact on grain yield.

2. Materials and methods

2.1 *Experimental design*

Bioassay was performed at the Julio Hirschhorn experimental station (La Plata: 34°59′ S, 57°59′ W) of the Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. A randomized block factorial design with three replications was used with combinations of the following factors: (a) fungicide application (with and without fungicide, hereinafter called fungicide treatment and control, respectively) and (b) genotypes. Six wheat genotypes were used: a modern commercial variety, Klein Liebre, of an intermediate cycle, with moderate susceptibility to foliar diseases; three genotypes derived from a population of double haploids from the cross of the Synthetic W7984 wheat (Synthetic) (Altar84/*Aegilops tauschii* [219] CIGM86.940) × Opata M85 (Opata), which is of spring habit, from CIMMYT (genotypes A, B, and C)); and the parental genotypes of this population (Synthetic and Opata), which differed in their percentages of mycorrhizal colonization in previous tests. Miravis Triple Pack (Elatus Ace® [propiconazole + benzovindiflupyr] [600 cm^3/ha] and Miravis [pydiflumetofen] [200 cm^3/ha]) were added to the fungicide treatments at the EC23 (tillering, main stem, and three tillers) and EC39 (flag leaf ligule just visible) stages (Zadoks *et al*. 1974). The applications were performed with a CO_2-pressurized sprayer equipped with four nozzles spaced 0.5 m apart on a bar. All flat fan tips worked with a pressure of 5 bar and spray volume of 100 L/ha. The inclusion of this treatment was motivated by the need to propose a management condition typical of commercial wheat cultivation which involves using chemical treatments to counteract the development of fungal diseases. Fertilization was carried out with phosphorus at sowing with 50 kg/ha of P as triple calcium phosphate, and N as urea, with 50 kg/ha of N applied at sowing, and 50 kg/ha at the EC22 stage (tillering, main stem, and two tillers) (Zadoks *et al*. 1974).

2.2 *Mycorrhizal colonization*

To evaluate mycorrhizal colonization, fine roots in good condition were selected from 5 plants per treatment at the stem elongation (first node detectable) (EC31) and early dough grain development (EC82) stages (Zadoks *et al*. 1974). The roots were then stained according to the method of Phillips and Hayman (1970). Colonization percentages were evaluated according to Cabello *et al*. (2013). The presence of the characteristic structures of arbuscular mycorrhizal symbiosis was verified by quantifying the internal mycelium (IM), entry points (EP), arbuscules (Ar),

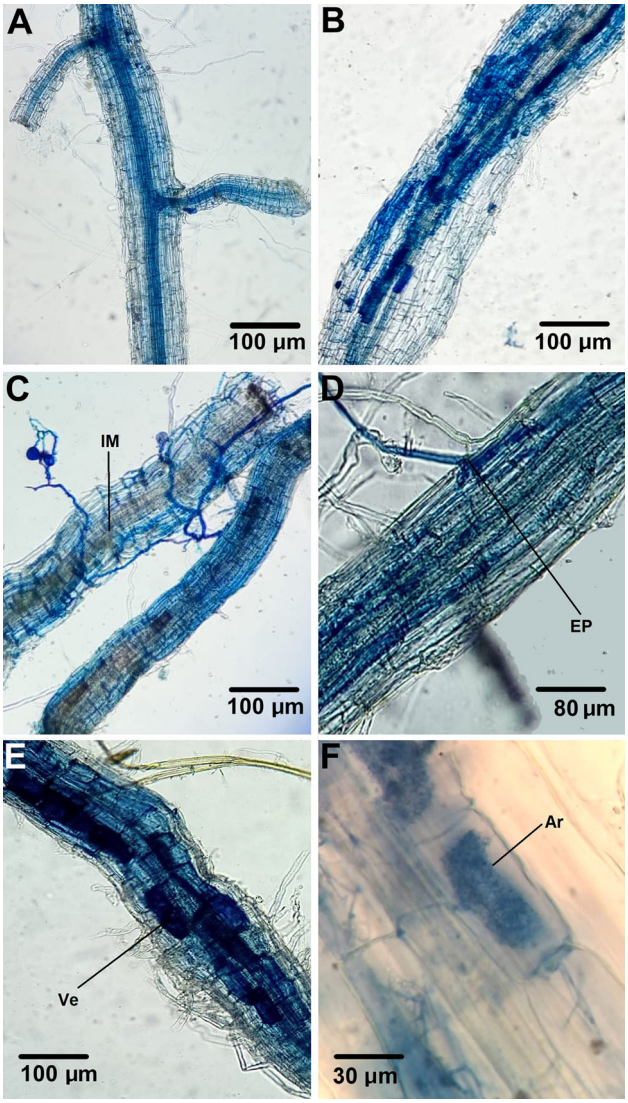

Figure 1. Arbuscular mycorrhizal fungi in cleared and stained wheat roots. (**A**) Non-colonized root (100×); (**B**) colonized root (100×); (**C**) spores, external mycelium, and internal mycelium (IM) (100×); (**B**) entry point (EP) (400×); (**E**) vesicles (Ve) (100×); (**F**) arbuscles (Ar) (600×).

vesicles (Ve), and coils (Co). Percentages were calculated for the presence of each structure and total colonization.

2.3 *AMF community*

In order to count and identify AMF spores, rhizospheric soil samples were taken at sowing, EC31, and EC82 stages by random composite sampling. Six subsamples were taken from 3 m^2 areas and mixed to form a composite sample. AMF species were isolated and identified by wet sieving and decanting 100 g of soil using sieves of different mesh sizes (450, 105, 75, 30 µm), followed by decanting (Gerdemann and Nicolson 1963) and sucrose gradient centrifugation (Walker *et al.* 1982). The isolated spores were transferred to Petri dishes and inspected under a dissecting microscope. Spore phenotypic characteristics such as hyphal attachment, spore size, color, and shape were used to distinguish different morphotypes. Spores were mounted permanently on slides in polyvinyl alcohol–lactic acid–glycerol (PVLG) and Melzer's reagent and examined under an optical microscope. Spores were identified to the genus and species levels based on the analysis of their spore wall structure, Melzer's reaction, and other taxonomically informative characteristics, and comparisons with those originally described (Błaszkowski 2012), and online references of species descriptions of INVAM at West Virginia University, USA (*http://invam.wvu.edu*). AMF morphospecies were classified using the methods proposed by Schüßler and Walker (2010) and Wijayawardene *et al.* (2020). Specific richness and diversity, and evenness indices, were determined for each sample.

2.4 *Foliar diseases, green leaf area, and grain yield*

The severity of the most important foliar diseases occurring in EC31 and EC82 under natural infection was evaluated in all green leaves from 10 tillers per treatment. Severity was determined by visual estimation and expressed as a percentage of the leaf surface covered with lesions (necrosis and chlorosis). The area under the disease progress curve (AUDPC) was calculated according to Shanner and Finney (1977). The total leaf area index was estimated by counting tillers in 2 linear metres and measuring the length and width of all leaves with at least a portion of green tissue in a total of 7 tillers and considering a form factor of 0.835 (Miralles and Slafer 1997). The green leaf area index (GLAI) was calculated using severity estimates. The green leaf area duration (GLAD) was also determined according to the trapezoidal formula: $GLAD = \Sigma [GLAI_i + GLAI_i{+}1/2] \times (t_i{+}1 - t_i)$, where $(t_i{+}1 - t_i)$ is the interval between two consecutive evaluations. At maturity, the tillers were counted in 3 linear metres, and 20 spikes were harvested from each plot to determine the number of grains per spike (GPS), thousand-kernel weight (TKW), number of spikes per square metre (SPSM), and grain yield.

Table 1. Means and standard deviations of percentages of internal mycelium (IM), entry points (EP), arbuscules (Ar), vesicles (Ve), coils (Co), and total mycorrhizal colonization in roots of wheat in EC31 and EC82 stages, with and without fungicide application

EC31		IM	EP	Ar	Ve	Co	Total
Genotype A	Without fungicide	24.97±5.00	12.18±1.11	6.62±2.21	3.85±0.55	2.22±2.21	26.08±3.88
	With fungicide	16.63±3.33	10.95±1.01	6.08±0.55	3.87±0.53	0±0	16.63±3.33
Genotype B	Without fungicide	28.85±4.45	4.42±4.41	9.40±5.00	2.75±2.75	1.10±1.10	30.50±6.10
	With fungicide	19.97±11.10	4.9±2.78	7.2±7.20	2.2±0	1.65±1.65	19.97±11.10
Genotype C	Without fungicide	14.97±1.66	6.6±0	8.30±0.56	0±0	2.20±0	14.97±1.66
	With fungicide	18.86±1.10	10.40±1.56	6.63±0	4.40±1.10	0±0	18.867±1.10
Klein Liebre	Without fungicide	37.77±8.90	13.87±2.76	11.63±0.53	9.98±7.78	0±0	40.53±11.67
	With fungicide	22.75±10.55	4.98±1.68	13.32±11.12	0.55±0.55	1.1±1.10	22.75±10.55
Opata	Without fungicide	41.63±5.00	19.4±1.66	11.62±1.68	7.17±1.66	1.1±1.10	41.63±5.00
	With fungicide	12.2±4.43	6.07±0.56	4.97±2.76	2.2±0	0.55±0.55	12.77±5.00
Synthetic	Without fungicide	26.07±16.10	17.75±11.12	14.98±12.78	4.4±2.20	6.65±6.65	27.73±15.53
	With fungicide	17.75±4.45	3.32±1.11	9.97±1.10	1.1±1.10	0±0	19.97±4.43
				ANOVA results			
Genotype		**	***	***	***	ns	***
Fungicide		***	***	***	***	ns	***
Genotype×Fungicide		**	**	ns	***	*	**
EC82		IM	EP	Ar	Ve	Co	Total
Genotype A	Without fungicide	61.08±12.22	9.41±2.79	19.93±8.83	23.32±1.11	1.10±1.10	66.63±6.66
	With fungicide	43.40±1.10	13.30±0.15	8.83±0	15.50±4.45	0±0	52.18±1.11
Genotype B	Without fungicide	50.18±6.45	24.97±0.56}	21.08±5.55	7.73±1.10	0±0	56.63±0
	With fungicide	37.73±8.90	26.65±8.88	16.63±7.76	9.95±1.11	3.86±3.86	37.73±8.90
Genotype C	Without fungicide	59.97±0.03	16.08±3.88	16.10±0.56	7.19±0.55	0±0	60.53±0.53
	With fungicide	43.30±3.33	18.87±7.76	13.32±4.45	9.95±1.11	2.20±2.20	45.52±1.11
Klein Liebre	Without fungicide	69.95±1.11	32.73±0	48.87±13.3	17.2±6.10	1.10±1.10	71.07±0
	With fungicide	48.87±0	21.08±1.11	15.52±2.21	14.35±3.20	1.10±1.10	49.98±1.11
Opata	Without fungicide	71.63±6.10	15.53±0	24.37±2.16	28.85±8.88	1.10±1.10	73.85±3.88
	With fungicide	48.87±2.20	28.85±0.01	18.85±7.78	15.52±4.45	0±0	49.98±3.31
Synthetic	Without fungicide	49.97±5.56	29.97±3.33	23.32±1.11	18.87±3.33	0±0	54.42±7.78
	With fungicide	49.98±1.11	14.98±2.78	8.87±0	8.83±0	1.10±1.10	51.10±0
				ANOVA results			
Genotype		***	***	***	***	ns	***
Fungicide		***	ns	***	*	ns	***
Genotype×Fungicide		*	***	**	**	*	**

*p<0.05; **p<0.01; ***p<0.001; ns= non-significant.

Two-way ANOVA results according to genotype and fungicide factors, and their interaction in wheat plants at EC31 and EC82 stages (n=9).

2.5 *Data analysis*

InfoStat 2016 software (Di Rienzo *et al.* 2016) was used for data analysis. Normality and homoscedasticity of variances were verified using the Shapiro–Wilk and Levene tests. Colonization, severity, GLAI, and grain yield values were subject to bifactorial ANOVA considering the genotype, fungicide treatments, and their interaction as factors. Comparisons of means were made with Tukey's test. Pearson correlation analyses were performed between mycorrhizal colonization data and severity, GLAI, and grain yield components. In all analyses, confidence interval α=0.05 was used.

3. Results

3.1 *Mycorrhizal colonization*

The presence of fungal structures (IM, EP, Ar, Ve, and Co) corresponding to arbuscular mycorrhizal colonization was observed in all the genotypes and stages evaluated (figure 1). In both stages, the application of fungicide significantly reduced the percentages of fungal structures. In addition, differences were found in the percentages of fungal structures for the different genotypes (table 1).

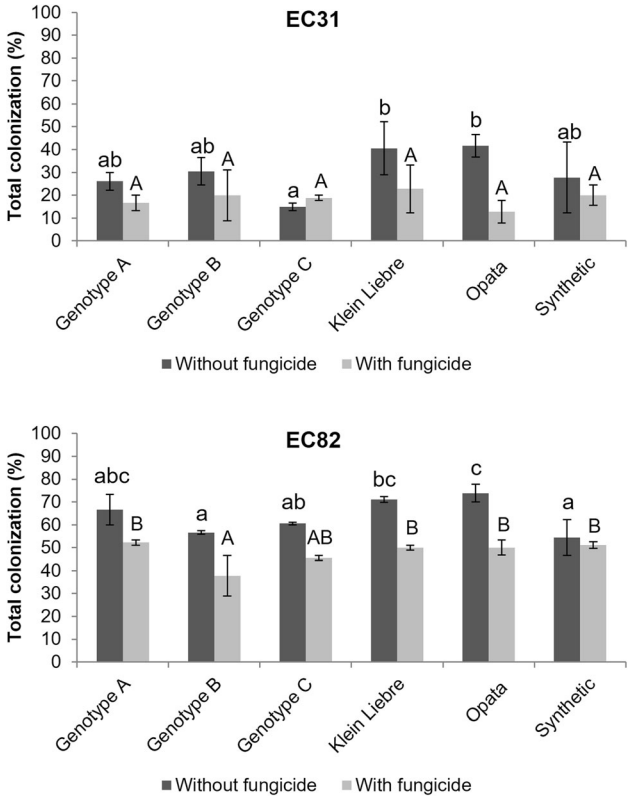

Figure 2. Percentages of total mycorrhizal colonization in wheat plants in EC31 (top) and EC82 (bottom) with and without fungicide application. Different letters indicate significant differences according to Tukey's test: lowercase letters, without fungicide; uppercase letters, with fungicide. Values are means ± standard deviation.

Colonization percentages were higher in EC82 than in EC31. Total colonization increased between stages by 33.61±9.15% in the controls and 29.25±7.75% in the fungicide treatment. This trend was observed in all genotypes and all fungal structures. In the controls, the lowest values of total colonization in the EC31 stage were found in genotype C and the highest in Klein Liebre and Opata (F=3.88, p=0.0253), while in EC82, the lowest percentages of total colonization occurred in genotypes B and Synthetic, and the highest in Opata (F=9.33, p=0.0008). In the fungicide treatment, no differences were found in the percentages of total colonization between genotypes in EC31 (F=0.73, p=0.6165). In EC82, the lowest values were observed in genotype B while the highest were found in genotype A, Klein Liebre, Opata, and Synthetic (F=5.60, p=0.0068) (figure 2).

3.2 *AMF community*

Spores belonging to 12 species of Glomeromycota were recovered. The most abundant family was Glomeraceae (7 species), followed by Acaulosporaceae (3 species), and Gigasporaceae (2 species). Table 2 shows the species found in the different treatments, as well as the diversity, evenness, and richness indices. *Funneliformis mosseae* and *Scutellospora calospora* species were recovered in all the stages and treatments sampled. Specific richness and diversity indices

Table 2. Species of arbuscular mycorrhizal fungi, specific richness (S), evenness index (E), and the Shannon–Wiener diversity index (H) in soil samples in a wheat crop field at sowing times, EC31, and EC82 stages, with and without fungicide application

		EC31		EC82	
AMF species	Seed time	Without fungicide	With fungicide	Without fungicide	With fungicide
Acaulospora delicata	x	-	x	x	x
Acaulospora mellea	-	-	-	-	x
Funneliformis mosseae	x	x	x	x	x
Glomus sp.	x	-	-	x	-
Glomus microaggregratum	x	x	x	-	x
Glomus clarus	-	x	-	x	-
Claroideoglomus etunicatum	-	-	-	-	-
Septoglomus constrictum	x	-	-	-	-
Rhizophagus intrarradices	-	-	-	x	-
Acaulospora scrobiculata	-	-	-	x	-
Racocetra sp.	x	x	x	-	x
Scutellospora calospora	x	x	x	x	x
S	2.75±2.21	2.25±0.50	2.75±1.25	3.5±0.50	2.33±0.57
E	0.68±0.46	0.88±0.11	0.65±0.46	0.83±0.28	0.88±0.14
H	0.37±0.31	0.31±0.10	0.33±0.22	0.45±0.13	0.30±0.03

Values are means ± standard deviation.

Table 3. Mean and standard deviation of green leaf area index (GLAI) evaluated at EC31 and EC82 stages, green leaf area duration (GLAD), the area under the disease progress curve (AUDPC), thousand-kernel weight (TKW), number of spikes per square metre (SPSM), number of grains per spike (GPS), and grain yield of wheat plants with and without fungicide application

		GLAI EC31	GLAI EC82	GLAD	AUDPC	TGW (g)	SPSM	GPE	Grain yield (ton/ha)
Genotype A	Without fungicide	1.66±0.25	1.42±0.35	120.99±21.30	2306.70±240.43	27.72±5.15	548.16±11.84	0.24±0.02	3.61±1.94
	With fungicide	1.95±0.07	1.92±0.04	161.31±6.10	1880.52±16.87	36.49±0.08	600.00±20.00	0.32±0.01	5.76±1.44
Genotype B	Without fungicide	1.49±0.07	1.23±0.24	120.02±9.41	3224.44±274.98	38.40±4.08	405.00±5.00	0.13±0.01	5.36±0.25
	With fungicide	1.93±0.20	2.30±0.12	179.31±8.70	1972.40±17.04	34.61±8.85	585.00±5.00	0.30±0.00	6.68±2.46
Genotype C	Without fungicide	1.31±0.18	1.25±0.32	112.40±9.21	2166.91±604.98	28.30±0.99	396.67±33.34	0.19±0.04	2.56±0.06
	With fungicide	1,71±0.07	2.02±0.25	152.76±1.31	1977.91±120.96	31.85±4.36	445.00±5.00	0.23±0.03	3.64±1.08
Klein Liebre	Without fungicide	1.94±0.02	2.90±0.02	170.31±1,30	2084.62±460.62	32.61±2.98	503.33±13.33	0.26±0.09	6.12±0.39
	With fungicide	2.18±0.32	2.44±0.02	183.08±6.30	1490.21±254.38	33.03±0.77	593.33±6.67	0.41±0.07	9.00±0.36
Opata	Without fungicide	0.92±0.01	1.18±0.27	79.04±8.28	1472.08±331.92	34.73±3.63	466.67±33.34	0.32±0.05	6.03±1.60
	With fungicide	1.58±0.03	1.91±0.31	132.32±1.85	812.96±205.54	36.83±1.24	575.00±8.34	0.74±0.18	8.27±0.91
Synthetic	Without fungicide	1.00±0.07	1.09±0.32	79.97±6.70	2171.38±76.34	26.76±3.52	476.67±6.66	0.22±0.00	3.60±1.31
	With fungicide	1.83±0.14	2.38±0.11	155.27±1.04	647,74±274.68	34.51±2.03	558.00±8.00	0.99±0.47	5.01±1.41
					ANOVA results				
Genotype		***	**	***	***	ns	***	***	***
Fungicide		***	**	***	***	**	***	ns	**
Genotype × Fungicide		*	***	**	*	ns	***	ns	ns

*$p<0.05$; **$p<0.01$; ***$p<0.001$; ns= non-significant.

Two-way ANOVA results according to genotype and fungicide factors, and their interactions (n=9).

Table 4. Pearson correlation coefficients of the percentages of total mycorrhizal colonization, entry points (EP), arbuscules (Ar), vesicles (Ve), and internal mycelium (IM) with the green leaf area index (GLAI) in the EC31 and EC82 stages, the green leaf area duration (GLAD), the percentage of severity of foliar diseases (severity), the area under the disease progress curve (AUDPC), number of grains per spike (GPS) and thousand-kernel weight (TKW), and grain yield in wheat genotypes with and without fungicide application (n=18)

Variables			Without fungicide		With fungicide	
Growth stage	Mycorrhizal colonization	Growth disease severity and yield	r-coefficient	p-value	r-coefficient	p-value
EC32	Total colonization	TGW	0.6900	0.0015	−0.5482	0.0185
	Total colonization	GPS	0.7651	0.0002	-	-
	EP	Severity	−0.5220	0.0263	0.5469	0.0188
	EP	AUDPC	−0.4877	0.0401	0.4836	0.0420
	Ar	GLAI	-	-	0.5319	0.0231
	Ar	TGW	-	-	−0.4859	0.0409
	Ve	AUDPC	-	-	0.5429	0.0199
	Ve	GPS	0.4875	0.0402	−0.5977	0.0088
	Ve	TGW	-	-	−0.5635	0.0149
	Ve	GPS	-	-	−0.5977	0.0088
	Ve	SPSM	-	-	−0.5129	0.0295
	Ve	Yield	0.6107	0.0071	−0.5682	0.0139
EC81	Total colonization	GLAI	0.6212	0.0059	-	-
	Total colonization	Severity	−0.5275	0.0245	−0.5635	0.0149
	Total colonization	AUDPC	−0.5801	0.0116	-	-
	Total colonization	TGW	-	-	0.5093	0.0309
	IM	Severity	−0.6036	0.0080	−0.7390	0.0005
	IM	AUDPC	-	-	−0.6530	0.0033
	IM	TGW	-	-	0.524	0.0256
	Ar	Seveity	−0.4830	0.0423	-	-
	Ar	GPS	0.5868	0.0105	0.5184	0.0275
	Ar	Yield	0.6312	0.0050	0.5516	0.0176
	Ve	GLAI	0.5300	0.0237	-	-
	Ve	AUDPC	−0.6792	0.0019	-	-
	Ve	GPS	-	-	0.5114	0.0300
	Ve	SPSM	0.5773	0.0121	-	-
	Ve	Yield	-	-	0.5581	0.0161

Only the combinations of variables with significant r-coefficients are shown in the table.

showed no significant differences resulting from fungicide application or the stage evaluated.

3.3 *Foliar diseases, green leaf area, and grain yield*

GLAI evaluated in different periods showed differences between genotypes and was affected by fungicide application. The interaction between these factors was significant. GLAD also showed differences between genotypes and was affected by fungicide application, having the lowest values in the controls. The interaction between factors was significant (table 3). In the controls, the lowest GLAD values were found in the Opata and Synthetic genotypes and the highest ones in Klein Liebre (F=19.82, p<0.0001), while in the treatment with fungicide, the lowest values were found in Opata and the highest in Klein Liebre and genotype B (F=16.44, p=0.001).

Regarding severity, the percentage of lesions covered with foliar diseases was predominantly due to yellow rust, followed by yellow spot, in the different stages evaluated. The severity percentage differed between genotypes and was affected by fungicide application as it was lower in the fungicide treatment. The interaction between factors was significant, except in EC31. AUDPC also exhibited differences between genotypes and was affected by fungicide application, with the lowest values occurring in the fungicide treatment. The

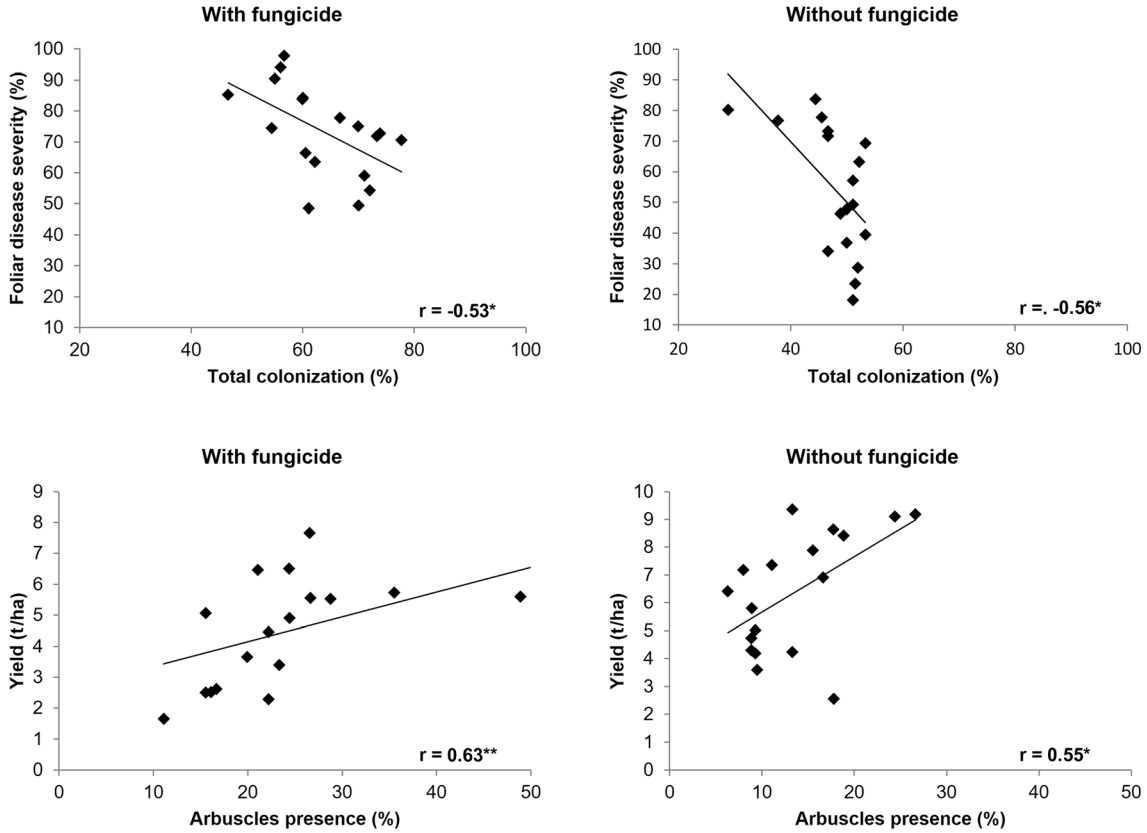

Figure 3. Correlation between the total mycorrhizal colonization (%) and the severity of foliar diseases (%) (top) and between the presence of arbuscules (%) and the grain yield (ton/ha) in wheat in EC82 without fungicide application (left) and with fungicide application (right). The Pearson correlation coefficient (r) and its significance level are presented (*p<0.05; **p<0.01; ns=not significant).

genotype×fungicide interaction was significant (table 3). In the controls, the lowest values of AUDPC were found in the Opata and Klein Liebre genotypes and the highest in genotype B (F=5.67, p=0.0065). In contrast, in the fungicide treatment, the lowest values were found in Opata and the highest in Klein Liebre and genotype B (F=16.44, p=0.001).

Grain yield differed between genotypes and was lower in the controls. Genotype D showed the lowest grain yield value, while the Klein Liebre and Opata genotypes showed the highest values. The interaction between the factors was not significant. TKW showed no difference between genotypes and was affected by fungicide application, being lower in the control. The interaction between factors was not significant. SPSM was lower in the controls, differed between genotypes, and exhibited a positive effect of the interaction between factors. In the control, the highest values were found in genotype A and the lowest in genotype C (F=23.16, p<0.0001). In the fungicide treatment, the same trend was observed (F=96.24, p<0.0001). GPS differed between genotypes, was lower in genotype C and higher in Klein Liebre, and was affected by the application of fungicide, exhibiting the lowest values in the controls, with no interaction between the factors (table 3).

The statistically significant values obtained in the Pearson correlation analysis performed with the percentages of mycorrhizal colonization, severity, GLAI, grain yield, and its components are shown in table 4. In the controls, the percentages of total colonization and different fungal structures in both the stages evaluated showed a positive correlation with grain yield and vegetative growth, and a negative correlation with the severity of foliar diseases (figure 3). On the other hand, in the fungicide treatment, this trend was not as clear because in EC31 the presence of entry points was positively correlated with severity while the presence of vesicles was negatively correlated with grain yield. In addition, both negative and positive correlations were found with vegetative growth, which varied between the different fungal structures and stages evaluated.

4. Discussion

This study evaluated the spontaneous arbuscular mycorrhizal colonization in different wheat genotypes and its effect on foliar diseases and grain yield. Mycorrhizal colonization was found in all genotypes, and all fungal structures were present at the roots. This agrees with the results of a field study conducted by Schalamuk *et al.* (2003), who reported an increase in colonization percentages from tillering to early dough stages.

Numerous studies have reported the effect of fungicides used in agricultural systems on AMF development (Hernández-Dorrego and Pares 2010; Buysens *et al.* 2015; Hage-Ahmed *et al.* 2018). In our study, we used the Miravis Triple Pack (propiconazole + benzovindiflupyr + pydiflumetofen), which has been shown to control foliar diseases (Jecke *et al.* 2022). Propiconazole, one of the most frequently used systemic fungicides, is an inhibitor of membrane sterol biosynthesis that does not have a significant impact on AMF development when used at recommended field rates (Schmitz *et al.* 1991; Frey *et al.* 1994; Kling and Jacobsen 1997). Pydiflumetofen and benzovindiflupyr are carboxamides that act as inhibitors of succinate dehydrogenase and complex II. Their use in wheat is recent, and till date no study has analyzed their effect on mycorrhization. It has been reported that under field conditions, the application of foliar fungicides has no direct impact on fungi; due to their mode of application and scarce basipetal transport in plant tissues, they do not reach the soil in high concentrations (Hage-Ahmed *et al.* 2018). However, our results indicated that fungicide application significantly reduced mycorrhizal colonization in all stages. Therefore, it is of great interest to study the effect of this type of fungicide on AMF.

Regarding the variability of the different wheat genotypes in their interaction with AMF, a bioassay performed by Hetrick *et al.* (1993) using 20 genotypes revealed that those released before 1950 benefited most consistently from AMF inoculation, while those released after 1950 showed variable responses. A meta-analysis conducted by Zhang *et al.* (2019) showed that the most recent wheat varieties tended to have decreased response to mycorrhization regarding grain yield. These authors proposed that current agronomic and improvement practices may have favored the development of cultivars adapted to agricultural systems with high levels of fertilization and, therefore, less dependence on mutualists. However, other studies have shown contrasting results (García de León *et al.* 2020). Lehman *et al.* (2012) performed a meta-analysis and found no evidence that the new wheat genotypes have lost their responsiveness to mycorrhization. In our study, we were able to verify the existence of genotypic variability in arbuscular mycorrhization, since there were significant differences between the different genotypes. The highest percentages of colonization were, in general, observed in Opata, and to a lesser extent in Klein Liebre, two modern commercial genotypes. The total colonization values of around 75% observed in these genotypes at the EC82 stage indicate their ability to establish symbiosis with AMF, according to Lehman *et al.* (2012). In addition, higher colonization values were found, in general, in Opata and Synthetic parental genotypes than in their derivatives, genotypes A, B, and C.

The AMF richness (12 species) found in this study is in agreement with similar studies conducted in agricultural systems which registered between 8 and 20 species (Land and Schönbeck 1991; Douds and Millner 1999; Jansa *et al.* 2002; Oehl *et al.* 2003; Schalamuk *et al.* 2003). The Glomeraceae family, which predominated in the sampled sites, has been well represented in agroecosystems (Oehl *et al.* 2003). The representatives of this family are characterized by short life cycles and rapid biomass production, which renders them tolerant to disturbances (Chagnón *et al.* 2013). In particular, *Funneliformis mosseae* and *Claroideoglomus etunicatum* have been recorded both in agricultural soils in the region (Schalamuk *et al.* 2003) and in other parts of the world (Jansa *et al.* 2002; Oehl *et al.* 2003). *Funneliformis mosseae* has exhibited great adaptability to this type of environment, remaining in agricultural soils even after decades of conventional tillage, probably due to its rapid infection capacity (Menéndez *et al.* 2001). *Scutellospora calospora*, belonging to Gigasporaceae, another species present in all the samples, has also been recorded in soils of agricultural systems in different parts of the world (Oehl *et al.* 2005; Priyadharsini *et al.* 2012). Both specific richness and diversity were similar in all treatments and exhibited changes neither over time nor in association with fungicide application. According to Hage-Ahmed *et al.* (2018), spores present in soil can resist exposure to inhibitory substances, such as fungicides, for a certain period by lying dormant until the inhibitory substance has been immobilized, reduced, metabolized, or degraded.

Both GLAD and severity were affected by fungicide application and varied between genotypes, indicating genotypic variability in foliar disease resistance. The Klein Liebre genotype, which exhibited high GLAD values in both treatments, with and without fungicide

application, had the lowest AUDPC values in the treatment without fungicide, similar to Opata. Besides, Opata showed the lowest GLAD values and, together with Klein Liebre, was the genotype least affected by diseases in the fungicide treatment and the controls. Both genotypes displayed the highest total colonization, as well as the highest grain yield values in both treatments. The increase in GLAD associated with lower infection percentages leads to greater radiation absorption during the crop cycle, implying greater generation of biomass and, consequently, an improvement in the grain yield components (Simón *et al*. 2020).

In the controls, mycorrhizal colonization was positively related to GLAD and grain yield, and negatively related to severity in both stages. In contrast, in the treatment with fungicide, colonization was positively related to severity and negatively related to grain yield in the earliest stage. However, this trend was reversed in the later stage, since in EC82, colonization was negatively related to severity and positively related to grain yield. These results may indicate a beneficial effect of AMF on the genotypes evaluated, in agreement with Pellegrino *et al*. (2015), who conducted a meta-analysis of studies from around the world, and concluded that there is a strong mutualism relationship between wheat and AMF. Such mutualism is reflected in improved nutrient uptake and grain yield. This effect may invigorate the plants, decreasing AUDPC and increasing GLAD. These authors found a beneficial effect of mycorrhizal colonization both in experiments with AMF inoculation and in those in which the soil was used as a source of native fungal propagules. Regarding the negative relationship between colonization and grain yield in the fungicide treatment observed in EC31, Gaitán *et al*. (2005) stated that when an endophyte, such as AMF, protects the host plant from pathogens and simultaneously consumes carbon compounds, its net effect on the plant will depend on which of these variables is critical at a given time. In this case, the application of fungicide could reduce the beneficial effects of mycorrhization. Our results indicate that resistance to foliar diseases may be induced by spontaneous mycorrhization under field conditions. New studies are required to delve into the resistance abilities of the genotypes evaluated, with emphasis on the identity of the pathogen and the AMF strains involved. The diverse results obtained in different studies indicate that the induction of resistance against pathogens depends on multiple mechanisms that may operate simultaneously (Fiorilli *et al*. 2018). From this point of view, it is essential to conserve the native AMF community and promote mycorrhizal symbiosis through adequate agricultural practices.

Interestingly, colonization by AMF may not necessarily translate into increased grain yield or better plant performance in measurable parameters (Thirkell *et al*. 2020). However, the literature suggests that proper management of the native AMF community can benefit the nutrient uptake of the crop as well as its resistance to abiotic stress factors and pathogens (Pellegrino *et al*. 2015; Mustafa *et al*. 2016; Fiorilli *et al*. 2018; García de León *et al*. 2020). A better understanding of the ecological roles of these microorganisms in agricultural systems can bring us closer to more sustainable agronomic practices.

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