Original Research Article

Spore density and arbuscular mycorrhizal colonization in sunflower

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ABSTRACT

The objective of this study was to evaluate the number of spores and mycorrhizal root colonization in Cerrado soil (Red-Yellow Latosol) cultivated with different genotypes of sunflower. Sampling of rhizospheric soil occurred in three periods: sowing, flowering and sunflower harvest. The experimental design was a randomized complete block design with four replications. To proceed the evaluations different sunflower hybrids were selected: M 734, Agrobel 960 and Helio 358, in 2009, and M 734, Embrapa 122 and HLA 860 H.O. in 2010. The measured parameters were number of total spores in 50 g of soil in three periods: sowing, flowering and harvesting and the arbuscular mycorrhizal colonization was evaluated only in the final of the experiment. The mean number of spores was 4,94 g $soil^{-1}$ and 4,64 g $soil^{-1}$ in 2009 and 2010, respectively. The maximum spore production occurred during the flowering period and mycorrhizal colonization was not influenced by the genotype. The mycorrhizal colonization rate ranged from 21 to 28% in 2009 and from 28 to 48% in 2010. The number of spores varies from 153 to 342 in 2009 and 147 to 320 in 2010, and the maximum production occurs, on average, in the flowering period. Lower soil phosphorus levels favors arbuscular mycorrhizal colonization.

Keywords: Helianthus annuus L.; soil; arbuscular mycorrhizal fungi, root colonization.

1. INTRODUCTION

Soil quality and the viability of improvements through chemical, physical and biological management are essential factors for success in agricultural production. In this context, the study and the use of soil microbial population has shown the way to link sustainability to efficiency.

The symbiotic association between plant and fungi is called mycorrhiza. Root colonization by arbuscular mycorrhizal fungi (AMF) generates several improvements; the plant provides photosynthates to the fungus, and this, through the branching and extension of the mycelium, increases the area of nutrient absorption for the plant [1]. Thus, AMFs can be used as an alternative to reduce the use of agricultural inputs, mainly fertilizers of chemical synthesis.

The influence of arbuscular mycorrhizal fungi acts not only on soil particles aggregation but also on plant growth, providing essential nutrients [2] and improving their ability to withstand adverse conditions.

Studying AMF inoculation in sunflower, it was observed an increase in chapter diameter, achenes weight and yield. These traits were related to better development of plants through their association with AMFs, due to higher absorption of nutrients such as P, K and Fe [3].

Sunflower (*Helianthus annuus* L.) cultivation has aroused interest, especially in Brazilian Midwest, due to its broad adaptability to edaphoclimatic conditions, suitability for crop rotation and usage as edible oil, biodiesel, ornamental crop, animal feed, etc. [4,5].

The present work aimed to evaluate three sunflower genotypes on the basis of their root mycorrhizal colonization at three different growth stages in Cerrado Biome soils.

2. MATERIAL AND METHODS

The experiment was carried out at Santa Luzia Farm, in Campo Verde (MT-Brazil), latitude 15°45'12"S and longitude 55°22'44"W. Soil in the experiment was Red-Yellow Latosol with the following properties: clayed texture, acidic pH, 50% average base saturation, absence of aluminum and high organic matter content (Table 1). Soybean and corn were most crops grown under minimum soil tillage practiced over more than ten years. Over both cropping seasons considered (2009 and 2010), the preceding crop of sunflower was soybean.

Table 1. Chemical and physical properties of soil under sunflower cultivation in the 2009 and 2010 harvests at Farm Santa Luzia, Campo Verde – MT, Brazil

Year	pH $CaCl_2$	P	K	Ca	Mg	Al	H	OM	CTC
		mg dm^{-3}			$cmol_c dm^{-3}$			gdm^{-3}	$cmol_c dm^{-3}$
2009	5,1	21,8	76	3,2	0,9	0	4,4	37,8	8,7
2010	4,9	8,0	80	3,3	0,7	0	5,5	39,9	9,7

	Bases saturation (V%)	Sand	Silt	Clay	Saturation (%)			
			g kg^{-1}		Ca	Mg	K	H
2009	49,3	196	133	671	36,7	10,5	2,3	50,7
2010	43,3	172	200	628	33,9	6,8	2,1	56,7

P: phosphorous; K: potassium; Ca: calcium; Mg: magnesium; Al: aluminium; OM: organic matter; CTC: cation exchange capacity; H: hydrogen.

The experimental design used was a randomized complete block with four replications. Every plot was composed of four rows of 6.0 meters, with 0.8 m of inter-row spacing and 0.3 m spacing within rows (19.2 m^2). Two central rows (9.6 m^2) were weighted at harvest to determine crop yield. NPK and boron fertilizers were applied 30 days after sowing respectively at the following rates: 30-80-80 and 2.0 kg ha^{-1}. The 2010 cropping season was rainy compared to 2009, with respectively 974 and 442 mm of total precipitation (Table 2).

Table 2. Rainfall distribution (in mm/month) over 2009 and 2010 cropping seasons in Campo Verde - MT, from February to July, in 2009 and 2010

Year	February	March (S)	April	May (F)	June	July (H)	Total
2009	262	132	16	10	22	0,2	442,4
2010	385	206	325	55	3	2	974,0

S: sowing; F: flowering; H: harvest.

Three different sunflower hybrids were evaluated on the basis of their response to fungi colonization. Hybrids M 734, Agrobel 960 and Helio 358 were used in 2009, whereas M 734, Embrapa 122 and HLA 860 H.O., in 2010. Rhizospheric soil sampling was done at harvest over 0-20 cm depth and at three different growth stages, namely sowing (first half of March), flowering (60 days after sowing) and harvest (after maturation).

Both parameters evaluated were total number of spores in soil, and arbuscular mycorrhizal colonization. The extraction of spores was carried out by the wet sift methodology [6], in which the soil was processed in a sieving systems (0.42 and 0.053 mm mesh) and centrifuged with water at 2800 rpm for 4 min. Subsequently, samples were re-suspended in 50% sucrose solution, centrifuged and washed. Spores were counted using a stereomicroscope in petri dishes.

To check the mycorrhizal colonization, crop roots were washed, clarified with KOH (10%), acidified with diluted HCl [7] and stained with trypan blue [8]. Ten segments of 1-2 cm in length were selected for slide assembly. Determination of root colonization percentage was made using an optical microscope (40x).

The analysis of variance was calculated using Sisvar 5.8 software package, and significant differences between means were determined following Tukey test at 5%.

3. RESULTS AND DISCUSSION

Regarding the factor year, there was no significant difference in the number of spores of AMF (Table 3). This could be explained by a general improvement in soil fertility resulting from the practice of minimum soil tillage over more than 10 years. According to Carrenho et al [9], the dissemination of mycorrhizal propagules is much more affected during initial phases of land use.

Table 3. Quantification of spores of arbuscular mycorrhizal fungi in Cerrado Biome soil, under sunflower cultivation, in two years and three periods, in Campo Verde – MT, Brazil

Year	Genotype	Sowing	Flowering	Harvest	Average
		n° spores 50 g soil^{-1}			
2009	M 734	153 bB	296 aA	267 aA	247 a
	Agrobel 960	185 abB	342 aA	233 abB	
	Helio 358	262 abAB	311 aA	174 aB	
	Average	200 B	317 A	225 B	
2010	M 734	234 abAB	270 aA	147 bB	232 a
	Embrapa 122	191 abA	254 aA	216 abA	
	HLA 860 H.O.	271 aAB	320 aA	184 abB	
	Average	232 AB	281 A	182 B	
	CV (%)		11,60		

Means followed by different letters, uppercase in line and lowercase in column, differ from each other, by the Tukey test (P =0.05). CV: coefficient of variation.

Spore density at the flowering stage was higher over both cropping years of study, with on average 317 and 281 in 2009 and 2010, respectively (Table 3). Maximum spore production may occur at flowering and final growth stages of host crop as reported by Smith and Read

[10]. They also reported that increase in the amount of spores could be related to higher production of internal crop resistance structures in response to drought.

The spore density of AMFs is generally higher in agricultural systems, and variations may occur due to edaphoclimatic factors, growing time, agricultural practices as well as the implanted crop [2].

Smith and Read [11], studying the Cerrado biome, verified that the arbuscular mycorrhizal fungi contribute to the growth of cultivated plants in annual cropping and pasture systems, and also that the number of spores of the native fungi varies, with the crop and the cultivation system being determinant for the enrichment of the mycorrhizal fauna.

The interaction between the genetic factors and the period was significant, demonstrating that the genetic material influences the sporulation process. However, the variations were low, indicating a stabilization of the mycorrhizal fungi sporulation.

In a study performed with a maize crop, it was verified that spore densities varied from 301 to 608, whereas in a soybean cultivated soil the values ranged between 239 and 287 [12], similar to those obtained in the present work with sunflower. Mycorrhizal dynamics involving root colonization and sporulation occur in different ways in different crops due to the compatibility between AMF and the genetic characteristics of plants [13]. In addition, environmental, climatic and edaphic factors generate changes in the symbiotic process [14].

In sugarcane, the occurrence of AMF increased when the crop was preceded by sunflower [15]. Likewise, sunflower favored the inoculum potential of AMF in the soil and the subsequent corn growth [16]. Annual crops, green manures, and forage species present a high degree of mycorrhizal dependency, acting as soil conditioners by multiplying the native mycorrhizal community [17,14]. In this sense, the sunflower is an option to benefit the soil mycorrhizal population in crop rotation/succession systems.

For the mycorrhizal colonization rate, a variation ranging from 21 to 28% was observed in 2009, and from 28 to 48% in 2010 (Table 4) (Figure 1), with no difference between genotypes. According to Janos [18], mycorrhizal dependence can be defined as the plant responsiveness to mycorrhization through increased growth, which may be related to the fertility and amount of phosphorus present in the soil.

Table 4. Average percentage of AMF colonization in soil under sunflower cultivation, in Campo Verde - MT, Brazil, in 2009 and 2010

Year	Genotype	Mycorrhizal colonization (%)	Average
2009	M 734	28 a	24 b
	Agrobel 960	21 a	
	Helio 358	22 a	
2010	M 734	38 a	38 a
	Embrapa 122	48 a	
	HLA 860 H.O.	28 a	
CV (%)		16,24	

Means followed by different letters in the column differ from each other, by the Tukey test (P =.05). CV:coefficient of variation.

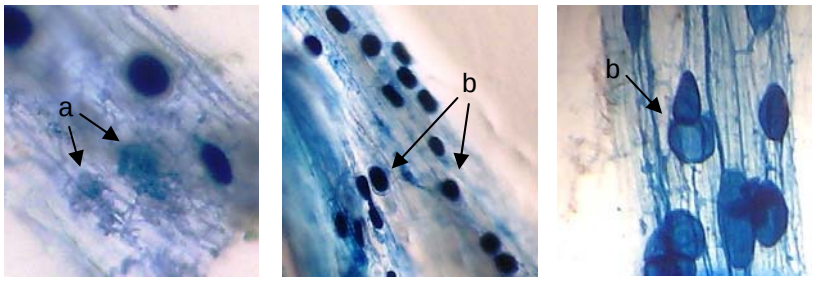

Figure 1. Sunflower root colonization by arbuscular mycorrhizal fungi (AMF). Fungal structures: arbuscules (a) and vesicles (b).

Silva et al [19] found an AMF colonization between 11 and 54% for arboreal species; from 33 to 49% for different crop rotation/succession systems [20]; from 31 to 71% when associated to the cassava crop in different localities [21]; and from 40 to 75% in banana plants [22].

Regarding the factor year, in 2010 there was a greater mycorrhizal colonization, which can be explained by the lower phosphorus content in the soil (Table 1). The effect of the increase in phosphorus availability and decrease in plant-mycorrhiza symbiosis is negative [23] and emphasized in the literature [24]. Therefore, the reduction in the P content may lead to an increase in plant colonization. When evaluating different doses of P_2O_5 in the mycorrhiza colonization, it was verified that doses higher than 30 mg P kg soil^{-1} decreased AMF colonization in sunflower [25].

By studying sunflower hybrids, it was verified that higher doses of P decreased sporulation and AMF colonization [22]. In the same crop, another study reported a colonization percentage within 66 to 71%, and spore density about 155 to 294; the soil, however, had a lower phosphorous content if compared with the present work [26]. Contrasting the results by Balota et al. [26] with those of this research, a lower colonization was capable of producing similar or higher quantities of spores.

In the same way, when comparing the results by Alves et al. [27] with the present work, it was observed that a lower colonization was able to produce similar or higher quantities of spores; therefore, the efficiency in mycorrhiza species perpetuation was superior.

In general, the AMF-plant relationship can be mediated by nutrient levels, present in the soil, since these fungi increase the root exploration area, contributing to a greater absorption of nutrients by the plant. As the increase in soil phosphorus decreases the root mycorrhizal colonization and the plant dependence to mycorrhization [28] in soils with low levels of phosphorus, typical of the Cerrado biome, the AMF favors sunflower cultivation [25].

Besides, there is evidence that the mycorrhizal-sunflower ratio enables greater plant resistance to heat, showing an interesting impact in Cerrado production systems, which is characterized by high temperatures [29].

Moreover, the potential of AMFs as biofertilizer for oleaginous crops is reinforced, especially for soils with low fertility, since this practice allows reaching adequate levels of production with less use of synthetic fertilizers, making the production system more sustainable [26].

Therefore, colonization and mycorrhizal sporulation vary according to the sunflower genotype and the evaluation period. There was intense AMF activity in the flowering period, a moment in which the plant requires a high nutritional supply for grain production.

4. CONCLUSION

The number of spores varied from 153 to 342 in 2009 and 147 to 320 in 2010, and the maximum production occurs, in average, in the flowering period.

Mycorrhizal colonization in sunflower is not influenced by the genotype, and the average percentage was 24 (2009) and 38 (2010).

Lower soil phosphorus levels favor arbuscular mycorrhizal colonization.

COMPETING INTERESTS

We declare that no competing interests exist.

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