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Arbuscular Mycorrhizal Fungi Symbiosis in Durum Wheat (*Triticum durum* Desf.) under No-tillage and Tillage Practices in a Semiarid Region

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ABSTRACT

Background: Arbuscular mycorrhizal fungi (AMF) are root symbionts that improve host plant growth and resilience against biotic and abiotic stresses allowing a sustain plant production particularly under harsh conditions.

Methods: The objective of this study is to compare the effect of conventional tillage and no-tillage practices on AMF symbiosis with durum wheat (*Triticum durum* Desf) through the evaluation of root colonization and AMF spore density in the soil of three different sites in a semi-arid region in Algeria. Two sites were conducted under rain-fed conditions and one was irrigated.

Result: Mycorrhizal root colonization varied according to the site and the tillage practice, while spore density differed between sites. Spore density was higher in sites under rain-fed conditions suggesting that water deficit stimulate sporulation, while root colonization seems to be limited by drought. No-tillage improved root colonization rates by 54.3% compared to conventional tillage system and this improvement, particularly the arbuscular percentage, was higher under drought conditions. In addition, root colonization rates showed a positive correlation with the organic matter content and pH in the soil and a negative correlation with the available phosphorus in the soil. These results indicate that no-tillage enhance the establishment of AMF symbiosis with durum wheat under semi-arid conditions.

Key words: Arbuscular mycorrhizal fungi, Drought, Durum wheat, No-tillage, Semi-arid.

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) presents the first field crop grown in Algeria. Mostly cultivated under rain-fed conditions, its production is often restricted by rainfall scarcity and irregularity that characterizes the Mediterranean climate. Arbuscular mycorrhizal fungi (AMF), thanks to their benefits, can help to overcome these constraints. AMF are root symbionts that promote the absorption of water and nutrients of the host plant in exchange of carbonic substances, which can affect positively plant performance (Smith and Smith, 2011). AMF have been recognized for improving plant growth and resilience against biotic and abiotic stresses, as they also improve soil health and structure (Thirkell *et al*. 2017), which gives them a promising role in improving and sustaining crop production, especially under stress conditions.

Conventionally, durum wheat cultivation is carried out on plowed fields because tillage improves soil structure, minimizes weed infestation, homogenizes soils and fertilizers, and thus improves productivity. However, tillage could affect the stability and the fertility of soil, which threatens the sustainability of production. For this reason, no-tillage has been suggested as part of conservation agriculture to sustain crop production (Chen *et al*. 2009). Promoted by the ecological services provided, the adoption of no-till is extending worldwide (Marandola *et al*. 2019). While in Algeria, its adoption is still low, but an increase of technical support and the awareness of farmers can increase its extension (Rouabhi *et al*. 2018a).

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Besides AMF and host plant traits, environmental factors including agricultural practices such as tillage system, can influence AMF symbiosis (Yang *et al*. 2014) and thus, the benefits that could be provided by these fungi. Therefore, information on the effect of different agricultural practices on AMF in different contexts is essential to optimize their role in sustainable production of crops. In this study, AMF symbiosis in durum wheat conducted with conventional tillage and with no-tillage was assessed through the evaluation of root colonization and spore density in three sites located in a semi-arid region.

MATERIALS AND METHODS

Study area and sampling

The study was carried out in Setif (Algeria) on three sites: Remada, Station and Benifouda (Table 1) during the season 2016/2017. At each site, two durum wheat plots (10 m × 50 m) were selected, one next to another: one under traditional tillage system (T) consisting of deep plough (40 cm) followed by secondary tillage with disk harrow (15 cm). The second plot was conducted under no-tillage (NT) practice or direct seeding. The region of the study is characterized by a semi-arid climate with fresh winter and an average annual rainfall of 400 mm (Rouabhi *et al*. 2018b). At the heading stage, three samples of roots and rhizospheric soil (0-20 cm) were collected from each field. The samples were taken 15 m apart along the diagonal.

Measures

The soil texture was determined with the wet sieving method using the USDA texture triangle. The pH and the electrical conductivity (EC) were measured on a soil suspension (soil/ water: 1/5) with a pH meter and conduct-meter. Available phosphorus (P) was determined according to the Olsen extraction method (Olsen *et al*. 1954). The organic carbon as the content of organic matter (OM) were measured according to the method of Anne (1945) and the total nitrogen (N) was determined according to Kjedahl Method (Bremner *et al*. 1982).

To evaluate root colonization, cleaned roots were soaked in a solution of KOH (10%) then stained with a solution of trypan blue (0.05%) according to the method described by Phillips and Hayman (1970). Then, following the method of McGonigle *et al*. (1990), total root colonization rate as well as arbuscular and vesicular colonization rates were measured with microscopic observation (x400) of 60 root fragments of 1 cm length from each sample.

AMF spores were extracted from the soil following the wet sieving method (Gerdemann and Nicolson, 1963) and counted under a binocular loupe (x40).

Data analysis

Using XLStat 2019, the significance effect of Tillage system, Site and their interaction (Tillage system*Site) on measured parameters was tested by a two-way variance analysis, the comparison between the means of different treatments was done using LSD test and the correlations between AMF root colonization rates and soil characteristics were calculated.

RESULTS AND DISCUSSION

Field climatic conditions and soil characteristics

The agricultural campaign (2016/2017) was characterized by a severe and long period of drought during the spring season: March, April, May (Fig 1). This drought coincided with the full growth phase of the studied species: durum wheat, when the need for water is high and could therefore affect the nutritional status of the plant and by consequence the association with AMF.

Except for the no-tilled (NT) plot at the Station site, which had a silty texture, the soil in all of the studied plots was clay-silty. EC ranged from 2.23 (ds/m) to 3.42 (ds/m), indicating a slight salinity of the studied soils. The pH ranged from 7.14 to 7.43 indicating a neutral soil in Benifouda and Station and slightly alkaline soil in Remada (Horneck *et al*. 2011). The OM content, which ranged between 2.1 and 3.14%, was slightly higher in the NT plots compared to the tilled ones, especially at Remada and Station sites.

The available P amounts (ppm) were low in Remada (8.48) and average in Benifouda (15.74) for both treatments, whereas at the Station site, they were average in the no-tilled plot (15.56) and high in the tilled plot (27.75) (Horneck *et al*. 2011). Concerning total N content, the levels recorded in Benifouda (0.23%) were higher than those in Station (0.15%) and Remada (0.16%) sites (Table 2).

AMF root colonization and Spore density

Even if it is not certain that AMF root colonization (RC) is translated into a better performance of the host plant (Thirkell *et al*. 2017), high RC rates have often been correlated with increased yields of wheat and other field crops (Zhang *et al*. 2019). This suggests that RC could be used to estimate the potential benefits from this symbiosis.

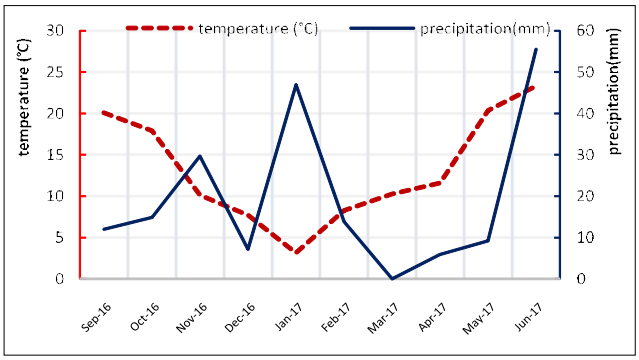


Fig 1: Ombrothermic diagram of the agricultural season 2016/ 2017 in Setif (Algeria).

Table 1: Location, altitude, wheat cultivar and the cultivation system (Rainfed/ Irrigated) of the studied sites.

Site	Remada	Station	Benifouda
GPS	36°8′42″ N	36°9′58″ N	36°15′13.3″ N
Coordinates	5°20′38″ E	5°21′32″ E	5°30′19.4″ E
Altitude	960 m	982 m	1204 m
Cultivar	Bousselem	Boutaleb	Sculptur
Rainfed/ Irrigated	Rainfed	Irrigated	Rainfed

The analysis of variance results (Table 3) showed a significant effect of the site and the tillage system on total colonization rate (TCR), arbuscular colonization rate (ACR) and vesicular colonization rate (VCR), while the combined effect (Site*Tillage) was significant only on TCR and ACR. The mean colonization rates were 31.6, 27.9 and 10.2% for TCR, ACR and VCR, successively. For both Tilled (T) and no-tilled (NT) plots, the TCR recorded in the site "Station" was higher than TCR in "Benifouda" by 59% and TCR in "Remada" by 74%, while the TCR recorded under NT system over all sites was 54% higher than TCR under T system.

Root colonization (RC) depends on the density and affinity of AMF and the host plant, as well as the environmental variables such as soil characteristics and agricultural practices (Gafur, 2014). In our study, RC was positively correlated with organic matter (OM) and pH, and negatively correlated with the available phosphorus amounts (P) in the soil (Table 4). The relation between AMF and OM seems to be synergetic. AMF contribute to the increase of OM in the soil by the AMF biomass generated from the carbon translocated from the host plant, which can reach 20% of the assimilated carbon (Graham, 2000), the microbiome biomass often associated with AMF (Turrini *et al*. 2018) and the increase of root biomass as a result of promoting plant growth. On the other hand, organic amendments (compost or manure) stimulate AMF development (Buto *et al*. 2016). In contrast to OM, the available P in the soil had a negative correlation with RC. Since it is the main element enhanced by AMF, P excess in soil-root interface or in plant organs can be detected by the host plant, which reacts by inhibiting RC and/or its progress (Ferrol *et al*. 2019). While the positive correlation of pH with RC could be explained by the decrease in P availability due to a rise in pH (Hopkins and Ellsworth, 2005).

Besides soil characteristics, agricultural practices also influence arbuscular mycorrhizal colonization. In general, intensive farming practices have a negative effect on AMF, in contrast to conservation agriculture practices (Säle *et al*. 2015). Tillage is important in the intensive crop system with many benefits. However, it can alter the physico-chemical and biological properties of the soil, including AMF (Siddiqui and Pichtel, 2008). Our findings confirmed the negative impact of tillage on AMF, where the no-tillage improved RC by 54% in durum wheat roots. These results are in accordance with the findings of Taibi *et al*. (2020) in similar edaphic and climatic conditions. Tillage affects RC in different ways, as turning over the soil, it buries propagules and/or exposes them to environmental stresses, which will dilute and destroy propagules, and consequently reduce the inoculum potential of the soil. By burying propagules below

Table 2: Physico-chemical characteristics of the studied soils in the different fields.

Site tillage	Remada no-tillage	Tillage	Station no-tillage	Tillage	Benifouda no-tillage	Tillage
Sand (%)	36.50	32.05	33.71	35.41	29.61	18.66
Silt (%)	35.89	31.14	40.25	32.29	39.10	35.59
Clay (%)	27.61	36.81	26.04	32.29	31.28	45.75
Texture	Clay-Silt	Clay-Silt	Silt	Clay-Silt	Clay-Silt	Clay-Silt
OM(%)	3.14	2.60	2.36	2.12	2.10	2.36
P_2O_5 (ppm)	7.40	10.28	15.56	27.75	16.36	15.12
N(%)	0,15	0.16	0.16	0.15	0.22	0.24
pH	7.43	7.39	7.36	7.33	7.16	7.14
EC (ds/s)	2.50	3.11	3.42	3.41	2.23	2.36

OM: Organic matter content, P_2O_5: Available phosphorus, N: Total nitrogen content, EC: Electrical conductivity.

Table 3: Means of total colonization rate (TCR), arbuscular colonization rate (ACR), vesicular colonization rate (VCR) and spore density (SD spores/g soil) in each plot and the significance effect of site, tillage system and their interaction site* tillage.

Site	Tillage	TCR	ACR	VCR	SD
Benifouda	No-tillage	34.52b	32.15a	9.63b	5.42b
	Tillage	19.41c	17.99b	5.04bc	3.97b
Remada	No-tillage	34.81b	34.37a	7.41b	7.58a
	Tillage	14.37c	13.48b	1.40c	8.23a
Station	No-tillage	45.19a	36.30a	20.04a	4.75b
	Tillage	40.44ab	34.07a	17.93a	4.34b
Factors			**Significance P**		
Site		<0.001	<0.001	<0.001	<0.001
Tillage system		<0.001	<0.001	0.008	0.465
Site* Tillage system		0.014	<0.001	0.481	0.308

Means with the same letter are not different according to LSD test (Alpha = 0.05).

Table 4: Correlations between root colonization rates, spore density and soil characteristics.

Variables	TCR	ACR	VCR	SD	P_2O_5	pH	CE	OM
TCR								
ACR	0.96***							
VCR	0.87***	0.77***						
SD	-0.31	-0.24	-0.47*					
P_2O_5	-0.52*	-0.44	-0.49*	-0.10				
pH	0.48*	0.39	0.57*	-0.76***	-0.25			
CE	0.01	-0.01	0.10	-0.46	0.25	0.33		
OM	0.57*	0.42	0.69**	-0.25	-0.70**	0.46	-0.03	
N%	-0.34	-0.31	-0.27	-0.33	0.46	0.16	0.59*	-0.35

TCR: Total colonization rate, ACR: Arbuscular colonization rate, VAR: Vesicular colonization rate, SD: Spore density, P_2O_5: Available phosphorus, EC: Electrical conductivity, OM: Organic mater content, *: Significant at 0.05, ** Significant at 0.01, ***: Significant at 0.001.

the depth of early seedling root growth, it delays colonization (Kabir, 2005). Kabir (2005) also speculated that in NT system, the new formed roots grow following the channels left by the old colonized roots that contains more propagules. The decrease of OM by tillage (Oehl *et al*. 2004), could also affect AMF development.

Root colonization of durum wheat in the irrigated site was higher than those in sites under rain-fed conditions. This suggests that water deficit may affect RC as it was reported by Mathur *et al*. (2018). Augé (2001), based on a literature review has concluded that long soil drought decreases RC. Drought can reduce the primary colonization by limiting spore germination (Wu and Zou, 2017), as it can affect the secondary colonization by inhibiting extra radical hyphal length (Neumann *et al*. 2009). In addition, RC could be affected by the carbon supply decrease, because of drought stress on the host plant growth.

Besides their dependence on the AMF species (Smith and Read, 1997), the storage character of the vesicles suggests that VCR indicates the amount of carbon transferred to AMF by the host plant (Busby *et al*. 2012). This can explain the high VCR at the irrigated site, where plants had a better nutritional status than sites exposed to drought.

Moreover, the improvement of RC by no-tillage varied between sites, particularly the arbuscular form (ACR) that ensures nutrients exchange (Hause and Fester, 2005). This suggests that NT effect depend also on other factors. In the irrigated site (Station), NT improved ACR by 4.76% while in Benifouda and Remada, the improvements were 75.41 and 154.95%, respectively. These results indicate that the NT effect was more important under drought conditions. The increase of water storage in the soil under no-tillage condition (Huang *et al*. 2012) could reduce drought impact on RC. In addition, the interesting effect of NT in Remada could be the result of the combined outcome of drought, the tillage practice and the bare fallow (preceding wheat culture), where the removal of host plants reduced arbuscular mycorrhizal establishment (Schipanski *et al*. 2014). Bowles *et al*. (2017) concluded from a meta analysis study that continuous ground cover by crops was as important as the decreasing soil disturbance for AMF.

Spore density (SD), ranged from 3.97 spores/g of soil in the plot under T system in “Benifouda” to 8.2 spores/g soil under T system in “Remada”. Unlike root colonization, tillage effect on SD was not significant despite the decrease by 6,76% (Table 3). NT usually increases spores abundance in soil (Lehman *et al*. 2019), which is important particularly for annual crops (Yang *et al*. 2014). On the other hand, the site effect was significant on SD (Table 3) with higher density in sites under-fed conditions. This could be explained by the induction of sporulation when soil moisture decreased, which is a specific characteristic allowing AMF adaption to arid conditions (Jacobson, 1997).

CONCLUSION

AMF root colonization in durum wheat varied according to site and tillage system, while spore density varied according to site. Drought seems to stimulate sporulation but reduces root colonization. No-tillage improved root colonization rates by 54% compared to conventional tillage and this improvement was higher under drought, particularly the arbuscular form. Moreover, root colonization rates showed a positive correlation with the organic matter content and pH in the soil and a negative correlation with the available phosphorus.

The positive association of no-tillage and AMF symbiosis and thus their positive effects, encourages their combination in a sustainable approach to produce durum wheat under semi-arid conditions.

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