Journal of Fundamental and Applied Sciences

ISSN 1112-9867

Available online at http://www.jfas.info

Research Article

# DYNAMICS OF WHEAT ROOT COLONIZATION BY ARBUSCULAR MYCORRHIZAL FUNGI UNDER CONTRASTING SOIL TILLAGE SYSTEMS AND ITS IMPACT ON GRAIN YIELD

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Received: 07 October 2020 / Accepted: 11 November 2020 / Published online: 01 January 2021

## ABSTRACT

In this work, we investigated the effect of the soil tillage farming system on the variability of arbuscular mycorrhizal fungi (AMF) root colonization of a local durum wheat variety, conducted during five years of experimental trial in a semi-arid region of eastern Algeria. No tillage farming system improved the AMF symbiotic activity from the fourth year of testing with the appearance of high root percentage of arbuscules at tillering and grain filling stage. Compared to that, the conventional tillage was marked by the observation of a high root percentage of vesicles especially at grain filling. Despite inter-annual fluctuations, the no tillage farming system improved the average yield of the five trial years by 20%. Multiple regressions analyses using the stepwise selection method were made to formulate an equation linking grain yield and the AMF symbiotic components specific to each tillage farming system.

**Keywords:** Arbuscular mycorrhiza; Soil tillage Farming system; Triticum durum; Yield.

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doi: http://dx.doi.org/10.4314/jfas.v13i1.25

## 1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) establish mutualistic symbiotic associations with the roots of most land plants [31] and provide many different agro-ecosystem services, such as an efficient use of fertilizers and soil nutrients, protection against biotic and abiotic stresses and improved soil aggregation [15]. They increased plant nutrition in legume crops by means of extensive mycelial networks which spread from colonized roots and efficiently absorb and translocate mineral nutrients from the soil to the host plants [3,4,12].

Mycorrhizal fungi have the potential to be considered a major component of sustainable agro-ecosystems and their presence in environments where rainfall is scarce, can make plants more resistant to water stress and strengthen their capacity to use the nutrients naturally present in the soil which would reduce the use of chemical fertilizers [1; 20] Although many data corroborate the AMF positive effect on plant growth, however, the role of AMF colonization rate on crop yield and economics of farming is still uncertain, particularly in wheat [28]. Durum Wheat (*Triticum durum*) is a major food crop that is widely grown around the world under diverse climatic conditions [29]. It is classified as a non-mycorrhizal or mycorrhizal plant species depending on the wheat cultivar [19; 16; 14]. Positive and negative effects of AMF seem to influence wheat crops [8]. The different cropping practices used in the management of organic and conventional wheat producing farms influence its mycorrhizal symbiosis and affect some of the benefits conferred by mycorrhizal fungi [7].

The crop rotation and soil tillage, the main elements of the conservation agriculture, influence mycorrhizal root colonization [10]. A greater proportion of the roots being colonized by AMF in the non-tilled soil early in the season and cause a higher uptake of P and Zn elements by plants than under conventional tillage and this may eventually translate to yield-increases [17]. Soil disturbance imposed by tillage is particularly detrimental to AMF hyphae if the soil is tilled in the fall and the hyphae are detached from the host plant which reduces mycorrhizal infectivity [18]. The effect of no-till cultivation on mycorrhizal colonization of roots and wheat yield has been the subject of several studies at long and short term field experiment and in different agro-ecological zones and have shown that tillage practices affected the colonization dynamics and the lack of tillage supplied the most appropriate conditions for the establishment of the symbiosis between the plant and the fungus [13,30], but this benefit of enhanced AMF colonization, did not translate into enhanced growth and yield. This impact on the yield can be related to the installation and the dynamics of the mycorrhizal symbiosis influenced by input system, the effective control of weeds and with climatic conditions [13].

In Algeria, wheat is mainly grown under rainfed conditions, where its culture is suffering from the hardness of the semi-arid climate conditions. The adoption of the No-Till wheat cropping interested local farmers because it recorded less work time and fuel consumption in comparison with conventional tilled wheat [27]. This practice minimizes the soil disturbance and providing residue soil cover, is found to increase the soil fertility and water use efficiency, thus helping cereal farmers to sustain the crop yield over a longer term [9]. For this, it was considered useful to conduct a study in a semi-arid cereal region for five years to examine the effect of two tillage systems (without tillage and conventional tillage) on the evolution of the arbuscular mycorrhizal fungi (AMF) root colonization of durum wheat and yield production.

## 2. MATERIAL AND METHODS

### 2.1 Site description and experimental design

The field trials were carried out from 2008 to 2013, at the agronomic experimental station of the Technical Institute of Field Crops (ITGC) of Sétif (latitude, 36°08′ N; longitude, 5°20′ E and altitude 962m). In general, this region is considered as a representative of the semi arid cereal area in Algeria. The climate is Mediterranean and semi-arid, characterized by cold wet winters and hot dry summers. During the experiment, the average annual rainfall did not exceed 400 mm with the dry season usually noted from May to September. Field experiments were conducted over an area of 1080m$^2$. The wheat cultivar "Boussalem", one of the most important varieties cultivated in such regions, was used in the present study. The experimental design was based on the application of two-soil management treatment: 1) No-tillage system (NTS) and 2) Conventional tillage system (CTS). Each experiment plot had 180 m$^2$ (6 m × 30 m) of area with three replicates per treatment. In the plots of the No-tillage system, sowing was carried out directly in the residues of the previous crop but in the conventional tillage system treatment, the sowing was carried out after tillage with a moldboard plough followed by a cover crop and a disc harrow. The both farming systems not differed in fertilization strategy and plant protection management. The previous crop has been wheat for all experimental period.

### 2.2 Plants and soil Sampling

For each treatment, two soil samples per plot and replicate were taken at 10 cm depth for further soil physicochemical studies.

To estimate the components of AMF root colonization, the roots of 6 plants per plot and per stage (tillering, grain filling and grain maturity of wheat vegetative growth cycle), were sampled in 2011, 2012 and 2013. In total, 324 plants were then collected.

**2.3. Root processing**

Roots were soaked during 5 min in an $H_2O_2$ (30%) bath to remove pigments, cleared in KOH (10%) and stained with Trypan blue (0.05%) [25]. Root mycorrhizal rates were calculated using a magnified grid line intersects method [21]. 108 segments of ∼1 cm long stained root samples were mounted on slides in lacto glycerol and examined for AMF structures under a light microscope (Olympus 41209). Estimations of AMF colonization were done by the magnified intersection method [21]. Intersections were counted in the following categories: negative (no fungal material in root), arbuscules, vesicles and hyphae. This made it possible to quantify the following pourcentage: $H\% = (G – p)/G \times 100$; $Arb\% = (q + s)/G \times 100$; $Ves\% = (r + s)/G \times 100$ (1)

Where, G: total number of intersections; p: no mycorrhizal structure; q: presence of arbuscules; r: presence of vesicles; s: presence of arbuscules and vesicles.

**2.4. Soil properties**

The soil characteristics which are presented in the Table 1, were determined using the Robinson method, Organic matter (OM) content was calculated from the measurement of organic carbon (OC) using the ANNE method, the total nitrogen (N) by Kjeldahl method, the pH of the soil (pH water) measured with a pH meter. Available phosphorus (Olsen Phosphorus) was determined by the Olsen method [24]. The total and active calcium carbonate measurements were made respectively by the methods of the Bernard and Drouineau calcimeter.

**2.5. Statistical analysis**

Statistical analyses were conducted using the software package Statbox 2012. We also checked the normality of data distribution, transformed the data by a square-root procedure as necessary. The variance analysis with a p-level of 0.05 was conducted, including means comparisons according to the test of NEWMAN and KEULS with a p-level of 0.05. Pearson correlation coefficients between the mycorrhizal root colonization, Abuscules and Vesicles percentage estimated at three growing stage under two farming system (no-till and conventional tilling) and yield were estimated. In addition, the coefficients of regression were used to estimate the effect of conventional tillage (CTS) and no-till farming system (NTS) on total mycorrhizal root colonization, Abuscules and Vesicles percentage evolution over years.

To identify the traits that were significant covariates on yield production, multiple regressions analyses using the stepwise selection method were made. The yield was the dependent variable and total mycorrhizal root colonization, Abuscules and Vesicles percentage were the independent variables.

## 3. RESULTS

### 3.1. Soil properties

The soils of experimental plot were characterized by clay loam texture with strong alkalinity (pH 8.26) and extremely low available P concentrations (Table 1). No-till system improved soil organic matter content but low nitrogen content for both treatments.

**Table 1.** physical and chemical characteristics of the Soil in the experimental plot

| Physical characteristics % | | | Silt | Clay | Sand | CE (mmho/cm) |
|---|---|---|---|---|---|---|
| | | | 34.66 | 34.66 | 30.66 | 0.175 |
| Chemical characteristics | | pH (water) | $CaCo_3$ (%) | | Actif $CaCo_3$ ( % ) | Available $P_2O_5$ ppm |
| | | 8.26 | 51.58 | | 21.25 | 7.33 |
| Organic characteristics | | OM % | C org % | | N % | C/N |
| 2011 | NTS | 2.6 | 1.51 | | 0.13 | 11.62 |
| | CTS | 1.9 | 1.11 | | 0.12 | 9.25 |
| 2012 | NTS | 3.15 | 1.83 | | 0.16 | 11,44 |
| | CTS | 2.12 | 1.23 | | 0.155 | 7,94 |
| 2013 | NTS | 3.72 | 2.16 | | 0.175 | 12.34 |
| | CTS | 2.62 | 1.52 | | 0.17 | 8.94 |

OM: organic matter C org: organic carbon NTS: no-tillage system CTS: conventional tillage system

A gain in organic matter content varying between 23.1 t / ha and 23.94 t / ha was respectively recorded at 2012 and 2013 after five years of experimentation. whereas in conventional tillage plots, the gain in soil organic matter content varied between 9.24 t / ha to 21 t / ha during the same study period (fig.1).

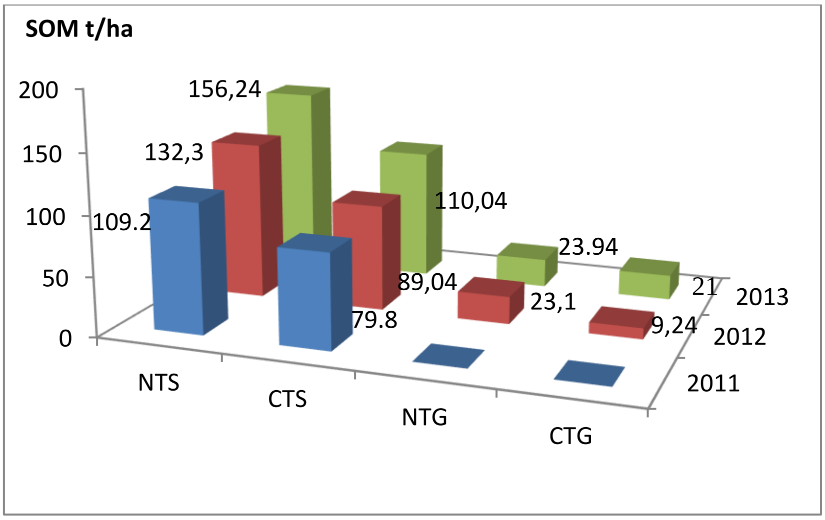


**Fig.1.** Effect of tillage farming system on the soil organic matter amount. NTS: no-tillage system; CTS: conventional tillage system; NTG: no-tillage gain; CTG: conventional tillage gain. Means were obtained from 3 replicates in 2011, 2012 and 2013. Significant differences between NTS and CTS are reported according to the Newman-Keuls test (P <0.05)

### 3.2. Presence of arbuscular mycorrhizal fungi

Microscopic observations of stained root segments showed AMF structures such as Intraradical hyphae, vesicles and arbuscules, with occasionally intraradical spores (fig. 2).

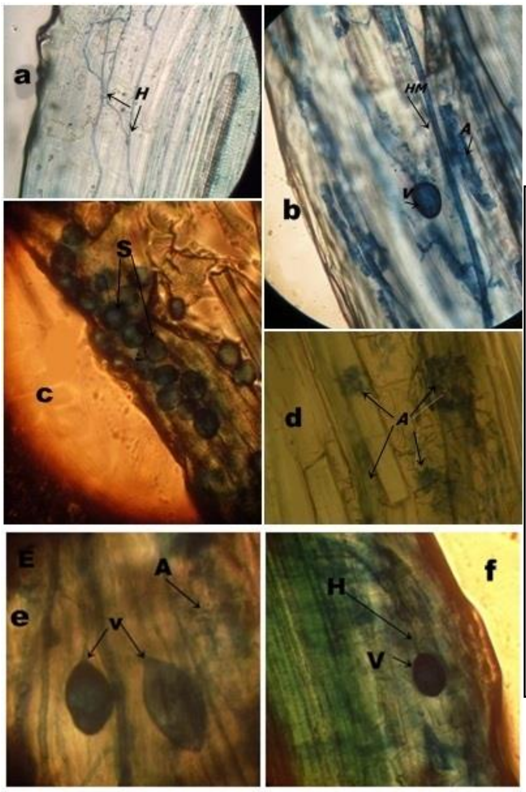


**Fig.2**. AMF structures in roots of Durum Wheat Microscopic observation (×40) in:

-Conventional tillage and no-till, grain filling stage 2012 (a and f)

-No-till system at various years of study at grain filling stage (b; 2011), (c; 2012); (d and e; 2013). hyphae (H); vesicles (V), arbuscules (A), spores (S)

### 3.3. Importance and evolution of AMF symbiotic components

The symbiotic behavior illustrated by the evolution of the AMF symbiotic components (H%, A% and V%) showed a year effect in relation with duration of the test and a growth stage effect which proved to be statistically very significant for the all parameters studied in both treatments (Table 2a). A very highly significant farming system effect was statistically reported; it had a significant interaction with the year and stage growth effect (Table 2b).

**Table 2 a**. Mean squares from the analysis of variance of *total mycorrhizal root colonization*, *Abuscules* and *Vesicles percentage* estimated at three growing stage under two farming system (no-tilling and conventional tilling)

| | | No-tillage | | | Conventional tillage | | |
|---|---|---|---|---|---|---|---|
| | Df | H% | A% | V% | H% | A% | V% |
| Year(Y) | 2 | 524.48*** | 1354.93*** | 6137.07*** | 1151.22** | 4735.66*** | 3331.44*** |
| Rep(E) | 6 | 6.26$^{ns}$ | 24.45$^{ns}$ | 76.30$^{ns}$ | 26.23$^{ns}$ | 191.69$^{ns}$ | 30.85$^{ns}$ |
| Growing stage (G) | 2 | 1984.50*** | 1015.52*** | 2878.12*** | 4572.181*** | 776.09** | 1841.59*** |
| Y x G | 4 | 428.01*** | 1648.82*** | 1377.62*** | 631.54** | 1105.72*** | 747.21** |
| Error | 12 | 9.80 | 45.70 | 49.73 | 76.30 | 69.12 | 87.37 |

ns, *, ** = no significant effect, significant effect respectively at (P <0.05) and P <0.01)

**Table 2 b.** Mean squares from the combined analysis of variance of total mycorrhizal root colonization, Abuscules and Vesicles percentage estimated at three growing stage under two farming system (no-till and conventional tillage)

| | H% | A% | V% | Yield |
|---|---|---|---|---|
| Year (Y) | 1581.62*** | 3466.83*** | 7677.47*** | 98.98*** |
| Rep(E) | 16.40$^{ns}$ | 82.45$^{ns}$ | 69.67$^{ns}$ | 7.44* |
| Farming system (F) | 609.18*** | 688.74** | 3130.76*** | 21.78** |
| Growing stage (G) | 6277.67*** | 1677.96*** | 2779.58*** | - |
| F x G | 279.01** | 113.64$^{ns}$ | 1940.13*** | - |
| Y x F | 94.08$^{ns}$ | 2623.77*** | 1791.04*** | 21.11** |
| Y x G | 1037.27*** | 1312.60*** | 1145.71*** | - |
| Y x F x G | 22.28$^{ns}$ | 1441.94*** | 979.11*** | - |
| Error | 37.66 | 72.66 | 62.34 | 1.51 |

ns, *, ** = no significant effect, significant effect respectively at (P <0.05) and P <0.01)

AMF root colonization (H%) was detected in all collected samples, from both treatments. Maximum levels of root length colonization occurred during tillering and grain filling stages (H%: 97% - 100%) and decreased at grain maturity. The annual regression coefficients were heterogeneous for the studied treatments and stages. A significant regression was observed during grain maturity stage with a higher and negative coefficient of regression for conventional tilling (CTS -30.11 ± 3.162) compared to no-tilling (NT -18.37 ± 6.045) (Table 3). This reduction lowered root length colonization from 85% and 87% in 2011 to 48.76% and 26.85% reached in 2013 for the no-tilling and conventional tilling system respectively.

The no-tillage observations showed a high root percentage of arbuscules at the tillering and during grain filling stages and which decreased at the grain maturity (Table 3). Positive regression coefficients for this parameter were observed (b=16.51±0.753 and b=14.48±0.045) for respectively the two first stages. The increase in the root percentage of arbuscules started in 2012 which corresponded to the fourth experimental year.

Compared to that, the conventional tillage was marked by the observation of a high root percentage of vesicles especially at grain filling. At this stage, positive regression coefficient for this parameter were observed (b=10.18±0.463). The difference between no-till and conventional tillage in the evolution of this parameter started from the fourth year of experimentation.

**Table 3.** Effect of conventional tillage (CTS) and no-till farming system (NTS) on total mycorrhizal root colonization, Abuscules and Vesicles percentage estimated at three growing stage through three cropping seasons, significant regressions (b ± s.e.) and coefficient of determination ($R^2$)

| | No-Tilling | | | Conventional Tilling | | |
|---|---|---|---|---|---|---|
| Years | Tillering | Grain filling | Grain maturity | Tillering | Grain filling | Grain maturity |
| ***Total mycorrhizal root colonization H%*** | | | | | | |
| 2011 | 98.26 | 99.65 | 85.50 | 99.31 | 100.00 | 87.08 |
| 2012 | 100 | 99.38 | 85.27 | 93.10 | 100.00 | 66.45 |
| 2013 | 97.22 | 99.38 | 48.76 | 90.28 | 97.22 | 26.85 |
| *b* ± es | -0.52±0.753 ab | -0.13±0.045 a | **-18.37±6.045** ab | -4.51±0.565 b | -1.39±0.463 ab | **-30.11±3.162** c |
| $R^2$ | 0.14 | 0.75 | 0.75 | 0.95 | 0.75 | 0.97 |
| ***Abuscules percentage A%*** | | | | | | |
| 2011 | 12.49 | 19.79 | 3.12 | 0.90 | 1.38 | 3.95 |
| 2012 | 64.19 | 48.91 | 2.15 | 28.23 | - | 1.38 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2013 | 80.56 | 48.76 | 2.78 | 18.05 | 21.75 | 1.54 |
| *b* ± es | **16.51±0.753** a | 14.48±0.045 abcd | -0.17±6.045 b | 8.57±0.565 abc | 10.18±0.463 d | -1.20±3.162 bc |
| $R^2$ | 0.91 | 0.75 | 0.12 | 0.38 | 0.99 | 0.70 |
| ***Vesicles percentage V%*** | | | | | | |
| 2011 | 68.40 | 75.34 | 63.19 | 51.45 | 56.24 | 45.48 |
| 2012 | 23.45 | 16.66 | 29.16 | 12.03 | 66.66 | 12.80 |
| 2013 | 11.72 | 26.54 | 39.81 | 38.27 | 74.99 | 21.91 |
| *b* ± es | -28.34±0.753 a | -24.40±0.045 ab | -11.69±6.045 ab | 9.38±0.565 b | **10.18±0.463** b | -11.78±3.162 ab |
| $R^2$ | 0.91 | 0.75 | 0.12 | 0.38 | 0.99 | 0.70 |

### 3.4. Wheat grain yield

Significant year effect was observed for the wheat grain yield in the both treatments (P <0.01). A significant effect of the tillage system (P <0.05) and the interaction between year and tillage system (P <0.01) (Table 2b) gave an average yield over five years of study of 24.4 and 19.7qx/ha respectively for the no-tillage and conventional tillage system thus a gain of 20%.

### 3.5. Identification of significant covariates traits on yield production

The multiple regression analysis of grain yield according to the measured traits revealed that in the no-till farming system, it was very significantly depended on arbuscules percentage of grain filling stage (cumulated $R^2$ = 0.63) and on vesicles percentage of grain maturity (cumulated $R^2$ = 0.81) (Table 4). However, emergence of arbuscules at grain filling stage had a negative effect on grain yield (b=-0.19). Vesicles percentage of grain maturity stage was the only factor affecting grain yield in a conventional tillage system conditions with lower impact ($R^2$ = 0.57) than for the no-tillage system.

**Table 4.** Multiple regression analyses, using the stepwise procedure, for each trait on yield under both no-tilling and conventionel tilling farming systems. Only significant coefficients (*b*) are shown along with their accumulated coefficients of determination ($R^2$)

| **No-till system** | | | **Conventionel tillage system** | | |
|---|---|---|---|---|---|
| Traits | *Cumulative $R^2$* | *P value* | Traits | *Cumulative $R^2$* | *P value* |
| A%2 | 0,63 | 0,006 | | | |
| V%3 | 0,81 | 0,033 | V%3 | 0,57 | 0.019 |
| *Coefficients* | | | | | |
| | *b* | *e.s* | | *b* | *e.s* |
| Constante | 26,46 | 2,08 | Constante | 19,10 | 0,98 |
| A%2 | -0,19 | 0,04 | | | |
| V%3 | 0,09 | 0,03 | V%3 | 0,10 | 0,03 |

The regression analysis model therefore made it possible to formulate an equation linking grain yield and the AMF symbiotic components specific to each tillage farming system.

**3.6. Relationship between variables**

Pearson correlation coefficients between the mycorrhizal root colonization, Abuscules and Vesicles percentage estimated at three growing stage for both treatments (no-till and conventional tillage ) and grain yield showed in the no-till farming system, a significant and positive correlation between AMF root colonization percentage (H%) and arbuscules percentage (A%) (r= 0.69*) at tillering stage. At grain maturity stage AMF root colonization percentage (H%) was significantly and positively correlated with vesicles percentage (V%) (r= 0.75*) and negatively with arbuscules percentage (A%)( r= -0.95** ). Under the conditions of the conventional farming system, a positive correlation was observed between AMF root colonization percentage (H%) and vesicles percentage (V%) (r= 0.94**) and negative with the percentage of arbuscules (A%) (r= -0.96**) at grain filling stage.

Analysis of the interference between the AMF symbiotic components and the grain yield showed in no-till farming system, a significant and negative correlation between grain yield and arbuscules percentage (A%2) (r=**-0.82****) at grain filling stage. At all stages, a significant and positive correlation between yield and percentage of vesicles (V%) was observed. In the conventional farming system, the yield is significantly and positively correlated only with percentage of vesicles (V%) (r= **0,75*)** at grain maturity stage.

**4. DISCUSSION**

The effect of the no-till farming system appeared in the fourth year of testing; it contributed to increases of the soil organic matter (SOM), en relation to presence of plant residues (mulch).

This parameter is the central indicator of soil quality and health, which is strongly affected by agricultural management, similarly [32] reported that, after twenty years of no-till operations, the soil organic carbon increases by 16% in humid temperate regions and by 10% in dry temperate regions, compared to conventional seedling.

The presence of different mycorrhizal structures in the root samples collected from both treatments confirmed durum wheat interaction with soil mycorhizal inoculum and the mycotrophic status of this durum wheat variety [23]. Similarly, some characteristics of the study area soil such as aridity (a rainfall close to 400 mm), low total phosphorus level and alkaline soil pH contributes to the AMF installation [11].

Many studies reported temporal and seasonal variation of AMF dynamic [22; 30]. These authors reported that the kinetics of colonization of roots by AM fungi showed an increase from the tillering stage to a peak reached at the earning (flowering) stage and fall thereafter until the grain filling stage. Our results showed similarly with a reduction at grain maturity stage. However, the no-till farming system increased the accumulation of mycorrhizal propagules such as root infected percentage at the end of the cycle which will affect the installation of mycorrhization at the following year through the no disruption of the hyphal network [13].

In the no-till farming treatment, the arbuscules are the main components of AMF root colonization at tillering stage; these symbiotic structures ensure the exchanges between both symbiotic partners [6]. Their emergence at this stage in the root cortex of the no-till farming plants is likely to reflect early good AMF activity, as put forward by [17], and be related to P absorption and root health [7].

In the case of conventional tillage, the arbuscules percentage was low, and the vesicles percentage are main component of AMF root colonization. The vesicles are carbon storage structures in particular lipids, obtained from the host plant; it is also a mean of conservation and propagation of AMF [6]. Their formation is abundant at the end of the growth cycle of the host plant [15].This model was verified for the no-tillage, but for the conventional tillage system, the formation of vesicles occurring at the grain filling stage reflected a negative partnership between the host plant (durum wheat) and the AMF.

[5] and [26] indicated that no-tillage systems in the dry regions of Africa increase yields compared with CTS. Angar et *al*. [2], reported that durum wheat yields, during ten years of no tillage in a semi-arid zone, increased by 0.35 t/ha, compared to conventional tillage. Our results showed an average gain of 0.47 t / ha.

This yield was strongly impacted by the vesicles percentage formed at the end of the cycle. Vesicles are resting structures their number is increased in old or dead roots and corresponded to mycorrhizal soil infectivity (MSI) [30].

Emergence of arbuscules at grain filling stage was a limiting factor of grain yield, this negative relationship between the yield and the activity of the mycorrhizal symbiosis stated in no-tillage farming system highlighted the notion of competition for C assimilates between plant and AM fungal sinks and AMF symbiosis cost [8].

## 5. CONCLUSION

The results showed the symbiotic behavior of the local variety Boussalem in response to the two tillage farming system treatments during five years of experimental trial in a semi-arid area of eastern Algeria. This variety presented a high AMF root colonization in both treatments.

Compared to conventional tillage, the effect of the no-till farming system appeared in the fourth year of testing and it improved the following parameters;

- The soil organic matter (SOM) in relation to the presence of the mulch thus allowing a gain of soil carbon.
- The symbiotic exchange activity in relation to the high root percentage of arbuscules present from the start of the vegetative cycle. Observing these structures during the growing stage suggests this is a dynamic process.
- The mycorrhizal inoculum produced at the end of the cycle and based on roots colonized by hyphae and vesicles thus affecting the mycorrhizal soil infectivity.

The characteristics of the study region gave specificity to the mycorrhizal symbiosis. In these study conditions the grain yield depends in the no-till soil farming system on the cost of mycorrhizal activity and the amount of inoculums produced at the end of the cycle.

This preliminary study should be complemented by other investigations relating to the study of mycorrhizal biodiversity of cultivated soil and the factors influencing the mycorhzation of the wheat plants such as the sowing date, the presence of weeds and the wheat variety behavior in the different agro-ecological zones.

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**How to cite this article**:
Hadj Youcef Taibi H, Smail-Saadoun N, Djemel A. Dynamics of wheat root colonization by arbuscular mycorrhizal fungi under contrasting soil tillage systems and its impact on grain yield. J. Fundam. Appl. Sci., 2021, *13(1), 468-483*.