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Effects of Tillage System and Cultivation Year on Secondary Metabolites and Antioxidant Capacity of Durum Wheat under Rainfed Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NC, SL, SA, FBJ and MBH designed the study, performed the statistical analysis and wrote the protocol. Authors NC, SL and SA wrote the manuscript. Authors LM and AC installed the trials and applied necessary management practices. All authors read and approved the final manuscript.

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ABSTRACT

One of the proposed solutions to face climate change impact and to maintain food production sustainability is conservation agriculture. This study tries to determinate the effect of conventional tillage (CT) and no tillage (NT) on secondary metabolites such as total phenolics content (TPC), total flavonoids content (TFC) and antioxidants capacity (DPPH %) in relation to natural

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mycorrhization of durum wheat during the tillering stage for three cultivation years. The experiment was conducted in a referential farm (Krib, Siliana, North West Tunisia). The results showed that TPC, TFC and DPPH% were not influenced by tillage system (T). However, cultivation year (Y) had a significant effect on the studied parameters independently of tillage system. In addition, for the first cultivation year, tillage system (T) had significantly influenced the mycorrhization rate (MR%) and NT presented the highest mycorrhization rate (24%). DPPH% showed high significant positive correlations with MR% and TPC. For partial correlation based on Tillage system, high positive correlations were noted between DPPH%, MR% and TPC. Considering the partial correlation based on cultivation year, only a significant positive correlation between TPC and TFC was observed. In conclusion, durum wheat quality was not affected by tillage system and there are not reasons against no tillage adoption in this region for a sustainable wheat production.

Keywords: Tillage system; cultivation year; durum wheat; secondary metabolites, antioxidants capacity.

1. INTRODUCTION

Durum wheat (*Triticum turgidum* Desf.) is the main cultivated and consumed cereal crop in Mediterranean area, covering up to 2/3 of total world production [1]. Fiber, proteins and mineral elements high contents of durum wheat is in favor of considering it as main staple food in human diet [2]. As well, durum wheat is the main ingredient to prepare the Tunisian dish “couscous”. However, country demand far exceeds the production which satisfies just 30% of demand. Durum wheat production is about 68% of cereal production obtained from 46% of the cereal area [3]. In addition, cereal monoculture practices in conventional agriculture threaten wheat production sustainability.

Around the world, conventional agriculture requires soils tillage twice a year before cereals sowing to control weed and to prevent crust formation. However, these practices added to climate change and soils vulnerability engendered erosion [4], soil moisture loss and soil organic matter reduction [5]. Consequently, conservation agriculture appeared such an alternative to conventional agriculture. Then, no tillage was adopted for the first time in 1999 in north west Tunisia under rainfed conditions. A recent study of Bahri et al. [6] tried to map priorities zones for conventional agriculture adoption and designated 260000 ha of Tunisian agricultural area characterized by semi-arid and sub-humid climate.

The adoption of conservation agriculture has resulted research to focus on its effect on several parameters such as growth and yield parameters of sowing species. A variation of no tillage effect on grain yield was observed [7]. Furthermore, research on soil demonstrated that tillage system could affect soil moisture, physical properties and organic matter [8]. Previous works related the tillage system effect to considered species, cultural practices, rainfall, environmental conditions and several others parameters interactions on the interaction between many others parameters [9-10]. As well, no tillage is acknowledged to recover soil organic matter and moisture, to improve soil physico-chemical properties and to boost biological processes [5,7]. Nevertheless, few research activities have considered the tillage effects on plant antioxidants composition and mycorrhization. This is in spite of the tillage effects observed on protein and gluten content [11], sucrose content [8] and hormone activity [12].

Under normal growth conditions, oxidative stress is very low, while biotic and abiotic stresses result in an increase of oxidative stress in plant cells. To confront oxidative stress, plants develop a complex defense system based on antioxidant [13]. Antioxidants are substances capable of delaying, retarding or preventing oxidation processes [14]. Two classes of antioxidants were identified by Amarowicza and Pegg [15], primary antioxidants which are responsible of oxidation reactions inhibition and secondary antioxidants which react in indirect way. Phenolic compounds are primary antioxidants that gathered phenolic acids, flavonoids, lignans, stilbenes and tannins. Cereals antioxidants contents could vary according to the genotype, the environment and possibly genotype-environment interactions [16-17]. Several studies tried to determinate total phenolics content and total flavonoids content in wheat due their capacity to inhibit herbivory, to act against UV radiation, and to reinforce plant defense system against insects and microbes [18-19].

Furthermore, no tillage system under semi-arid conditions results in an increase of arbuscular mycorrhizal fungi (AMF) spores in soil [20]. Actually, a stimulation of roots-fungi symbiotic reaction was observed for cereals cultivated under no-tillage [21-23]. Thus, fungus contributes to water and nutrients uptake of the host plant whilst plant provides carbon matter [24].

This study aims to determine the effect of tillage system and cultivation year on total phenolic content, total flavonoids content and antioxidant capacity of durum wheat in relation to mycorhization during tillering stage in North West Tunisia.

2. MATERIALS AND METHODS

2.1 Trials Description

This trial was installed at the referential farm for direct drilling (Krib, Siliana) situated in northwestern Tunisia (36°22'24"N; 9°10'26"E; elevation = 460m). Krib is characterized by annual precipitation of about 450 mm. It had a specific microclimate ranged between superior semi arid and sub-humid. For cultivation years, the annual means of temperatures and rainfall of the experimental site are showed in Fig. 1. The soil was sandy clay and relatively poor in organic matter (2.1%) and slightly alkaline (pH=7.6). The trial was installed since 1999-2000 growing season and the sampling was achieved in tillering stage for the three cultivation years 2015-2018. The biannual crop-rotation was durum wheat (*Triticum turgidum* Desf.) cultivar 'Karim' with seeding rates (160 kg.ha-1) and faba bean (*Vicia faba* L. *minor*). Two tillage systems were tested: conventional tillage (CT) versus no-tillage (NT). For CT, reversible moldboard ploughing to 30-40 cm depth was applied followed by secondary tillage with offset 15-20 cm and a direct driller was used for NT plots. For NT, glyphosate (3 $l.ha^{-1}$) was applied to control weeds. The seeding rates of durum wheat were 160 kg.ha-1. At sowing, durum wheat received 100 $kg.ha^{-1}$ of Di-Ammonium Phosphate. Then, ammonium nitrate (150 kg.ha-1) was used at early tillering and (150 kg.ha-1) at stem elongation stages.

2.2 Sampling and Measurements

For three cultivation years 2015-2018, a sampling was performed during tillering stage. Areal parts were dried, milled, sieved and stored for TPC, TFC and DPPH scavenging effects determination. After cleaning, roots were conserved in ethanol 50% to trypan blue coloration.

2.3 Extraction

Ground plant material (0.5 g) were put in 25 ml of methanol (80%) then shaken during 2 h and the solid phase was discarded using a Whatman filter paper. For each treatment, four extracts were prepared and stored until analysis.

2.4 Determination of Total Phenolic Content (TPC)

A method based on Folin–Ciocalteu reagent, proposed by Singleton and Rossi [25] was used for TPC quantification. At 720 nm, the spectrophotometer was used to measure absorbance of different extract and a blank after 1 h. Gallic acid (GA) was used for the standard curve (0–1000 ppm) and TPC was expressed as milligram of gallic acid equivalent (GAE) per gram of dry weight.

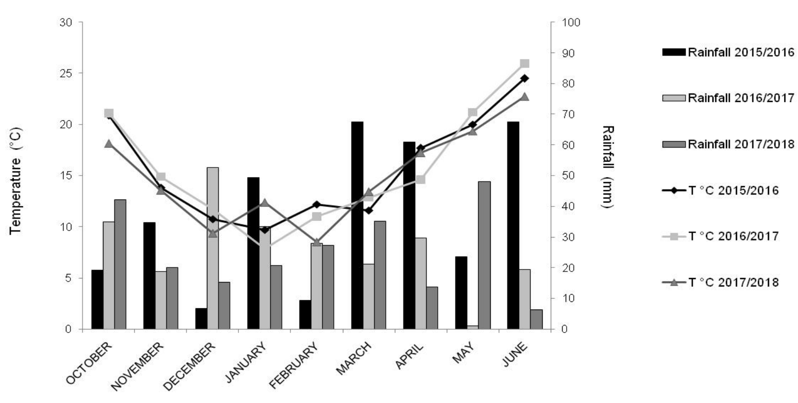

Fig. 1. Temperatures (°C) and rainfall (mm) recorded in the region of Krib from October to June during the cultvation year 2015-2018

2.5 Estimation of Total Flavonoid Content (TFC)

The colorimetric method proposed by Zhishen et al. [26] and modified by Chaieb et al. [27] was used to determine TFC of durum wheat samples at 510 nm against a blank. Rutin was used for the calibration curve and TFC was expressed as milligram of rutin equivalents (RE) per gram of dry weight.

2.6 DPPH Scavenging Effect

Samples antiradical capacity estimation is based on the DPPH reduction. As DPPH is stable, it is generally used for samples free radical-scavenging ability evaluation. Thus, the method of Chen et al. [28] with some modifications was used for DPPH determination. For each sample, the methanolic extract (10 µL) was mixed with 3 mL of 0.06 mM DPPH in methanol. After the incubation step in darkness (30 min), the absorbance was measured at 517 nm against methanol blank. The DPPH radical inhibition percentage was calculated based on the expression of Maisuthisakul et al. [29], that below:

DPPH radical scavenging capacity (%) = [A0-(A1-As)]/A0*100]

2.7 Trypan Blue Coloration

To evaluate Arbuscular Mycorrhizal Fungi (AMF) root colonization, for three cultivation years: from 2015 to 2018 during tillering, 3 plants/plot were sampled. A KOH solution (5%) was used to clarify wheat roots at 90°C during 20 minutes. To facilitate colorant fixation, HCl (2%) solution was used to emerge roots during 5 min. After filtration, Trypan blue was used to stain roots based on the method described by Phillips and Hayman [30]. For mycorrhization rates calculation the method of Mc Gonigle et al. [31] was used.

2.8 Statistical Analysis

The results were statistically analysed by Social Sciences software (SPSS 20.0, SPSS Inc., Chicago, IL, USA) to identify treatment effects and interactions (Two-way MANOVA and PEARSON correlation). DUNCAN post hoc test was used to check differences between variables at the level of significance P = 0.05.

3. RESULTS AND DISCUSSION

3.1 Secondary Metabolites

3.1.1 Total phenolic content (TPC)

Phenolic compounds play a major role in plant resistance, as they have nematicide, fungicide and insecticide effects. Analysis of variance of TPC showed that the effect of tillage system is no significant. While cultivation year had a significant effect on this parameter and the highest TPC was noted for the first cultivation year (Y1).

These results are in accordance with those of Chaieb et al. [32] who studied two durum wheat genotypes under semi-arid conditions and found that neither tillage systems nor crop rotation had significant effects on TPC. In similar, Strake et al. [33] reported that management practices did not affect phytochemicals concentrations of wheat. On the contrary, Asami et al. [34] noted that management practices affected TPC of wheat. Simić et al., 2020 [35] tried to test the effect of tillage and cultivation year on maize and found that, phenolic content showed significant variations according to tillage system and growing season.

3.1.2 Total flavonoid content (TFC)

In cereal, flavonoids are one of the main groups of phenolic compounds [15]. Therefore, analysis of variance of TFC showed that the effect of tillage system is no significant. While cultivation year had a significant effect on this parameter and the highest TPC was noted for the first cultivation year (Y1).

These results are in agreement with those noted by Chaieb et al. [32] who noted that neither tillage system nor crop rotation had significant effects on TFC of two durum wheat genotypes cultivated under semi-arid conditions. Likewise, management practices did not show any significant effect on phytochemicals concentrations of wheat [33]. In contrast, others studies reported that management practices affected TFC of wheat [34].

3.2 Antioxidant Capacity (DPPH)

Phenolic compounds act in plant as antioxidants to face biotic and abiotic attacks.

Then, we estimated the antiradical capacity of the samples based on the reduction of DPPH. For all cultivation years, tillage system had no showed significant effect on DPPH. However, this parameter presented significant difference according the cultivation year (Y). For CT and NT, the first cultivation year (Y1) showed higher DPPH % than Y2 and Y3.

In agreement to our results, Costanzo et al. [36] found that tillage system had no significant effect on antioxidants capacity of wheat. In contrast, many results reported that antioxidants capacity depends on many factors such environment, genotype, management practices and their interactions, in addition to the applied test and the extraction solvents [37-38]. Huseynova [39] reported that under stress conditions, an increase of antioxidants concentration is observed and antioxidants play a determinant role to protect plant against oxidative stress. In addition, Hai-cheng et al. [40] found that antioxidants concentration of winter wheat tillers could vary even according to tiller position.

3.3 Mycorrhization Rate

Mycorrhization is commonly known to affect plant chemical and biochemical composition. Consequently, this study tried to elucidate its relationship with antioxidants in durum wheat as affected by tillage system and cultivation year. Results of durum wheat mycorrhization are showed in Table 1. Both tillage system (T) and cultivation year (Y) had affected significantly mycorrhization rate (MR%). For the first cultivation year, CT and NT showed high significant difference and the highest mycorrhization rate was observed for NT with a value of 24%. Despite of the fact that tillage system did no present significant effect on MR for the second and the third cultivation years, higher rates were noted for NT compared to CT. Negative effect of soil disturbance on MR% was also noted by Kabir [41]. In similar, Roldan et al. [42] found that NT resulted in higher level of mycorrhizal propagules in the soil for maize and bean compared to tilled soils. Furthermore, mycorrhization rate of durum spring wheat was investigated under different tillage systems in Chile by Curaqueo et al. [43]. They noted that despite of the fact that tillage system had not significant effects on MR%, higher values were presented by NT compared to CT. In Algeria, an increase of durum wheat mycorrhization rate was observed after three years of NT adoption Hadj Youcef Taibi et al. [44]. Several studies revealed that tillage disturbs soils and slows their biological processes such as mycorrhization under CT versus NT [5]. Besides, climate conditions could affect the establishment plant-fungi symbiosis [45].

Table 1. Effects of tillage system on total phenolic content (TPC), total flavonoids content (TFC), antioxidant capacity (DPPH%) and mycorrhization rate (MR%) of durum wheat in Krib during 2015-2018 cultivation years

	Tillage System	TPC (mg/g–MS)		TFC (mg/g–MS)		DPPH (%)		MR (%)	
		CT	NT	CT	NT	CT	NT	CT	NT
Cultivation Year									
Year 1 2015/16		11.99 ± 1.78 a[1] A[2]	12.43 ± 1.78 a A	9.00 ± 2.32 a A	6.36 ± 2.50 a A	78.54 ± 18.04 a A	67.40 ± 22.12 a A	6.66 ± 1.57 b B	24 ± 6.74 a A
Year 2 2016/17		3.30 ± .45 b A	4.51 ± 1.95 b A	4.33 ± 1.80 b A	8.17 ± 4.56 a A	47.75 ± 3.68 b A	51.68 ± 3.40 ab A	20.44 ± 5.06 a A	25.77 ± 5.79 a A
Year 3 2017/18		5.03 ± .66 b A	5.90 ± .36 b A	9.75 ± 1.29 a A	10.16 ± .60 a A	38.63 ± 11.05 b A	29.34 ± 2.74 b A	30.22 ± 8.25 a A	35.11 ± 5.75 a A

[1] *In each column, values not followed by the same minuscule letter for the same treatment are significantly different (p=.05).*

[2] *In each line, values of the same parameter not followed by the same majuscule letter are significantly different (p=.05)*

3.4 Correlation between Mycorrhization Rate, TPC, TFC and Antioxidants Capacity

As shown in Table 2, mycorrhization rate (MR) showed high significant negative correlation with DPPH. TPC presented high significant positive correlation with DPPH%. For partial correlation based on tillage system (Table 3, a), other correlations were noted. MR% showed significant negative correlations with DPPH%. Highly significant correlations were noted between DPPH% and TPC. Considering the partial correlation based on cultivation year (Table 3, b), MR% had no showed significant correlations with the studied parameters. Total phenolic content (TPC) had showed significant correlation with TFC (r=0.667). These results are in agreement with those of Jaroszewska et al. [46] who found that mycorrhization results indecrease of antioxidants compounds. In contrast, Kaur and Garg [47] reported that mycorrhization rate is positively correlated to antioxidants compounds. Our results could be explained by the fact that mycorrhization rate and antioxidants correlation is influenced by the environment, management practices and their interactions [39-40]. These results could be explained by the fact that rainfall and temperatures interact with other environmental factors and influences secondary metabolites concentrations. Then, TPC, TFC and antioxidants vary under abiotic and biotic stress and consequently their correlations.

Table 2. Correlation coefficients among mycorrhization rate, total phenolic content, total flavonoids content and antioxidants capacity of durum wheat under two different tillage systems in Krib for three cultivation years 2015-2018

	MR%[a]	TPC	TFC	DPPH%
MR%	1			
TPC	-.365	1		
TFC	.196	.202	1	
DPPH%	-.592**	.619**	-.213	1

** Significant correlation p=.05.*
*** High significant correlation p=.01.*
[a]MR%, mycorrhization rate; DPPH%, Antioxidants Capacity; TFC, Total Flavonoids Content; TPC, Total Phenolic Content

Table 3. Partial correlation based on tillage system (part a) and cultivation year (part b) among mycorrhization rate and Total Phenolic Content, total flavonoids content and antioxidants capacity of durum wheat

Table 3. (Part a)

Tillage System	MR%[a]	TPC	TFC	DPPH%
MR%	1			
TPC	-.463	1		
TFC	.173	.193	1	
DPPH%	-.596*	.645**	-.203	1

** Significant correlation p=.05.*
*** High significant correlation p=.01.*
[a]MR%, mycorrhization rate; DPPH%, Antioxidants Capacity; TFC, Total Flavonoids Content; TPC, Total Phenolic Content

Table 3. (Part b)

Cultivation Year	MR%[a]	TPC	TFC	DPPH%
MR%	1			
TPC	.124	1		
TFC	.005	.667**	1	
DPPH%	-.227	.074	.088	1

** Significant correlation p=.05.*
*** High significant correlation p=.01.*
[a]MR%, mycorrhization rate; DPPH%, Antioxidants Capacity; TFC, Total Flavonoids Content; TPC, Total Phenolic Content

4. CONCLUSION

In this study, we aim to understand the effect of tillage system and cultivation year under rainfed conditions on secondary metabolites and antioxidants capacity of durum wheat and their relation to natural mycorrhization. The results showed that tillage effect on TPC, TFC and DPPH% of wheat is dependent of cultivation year. In addition, tillage system did not affect antioxidants concentration of wheat then the capacity of plant to defend itself against biotic and abiotic stress. As no tillage adoption did not negatively affect durum wheat quality such as secondary metabolites and antioxidants capacity. Then, durum wheat quality will not be a limiting factor to no tillage adoption and upscaling in North West Tunisia. This practice will permit soil conservation and will reinforce wheat production sustainability.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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