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Effect of arbuscular mycorrhizal fungi on drought tolerance in durum wheat

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Abstract

In this study, we evaluated arbuscular mycorrhizal fungi inoculation effect on three durum wheat cultivars grown under well-watered and post heading water deficit conditions. Inoculation improved water use efficiency and drought tolerance. This improvement was shown by a lower proline content, and an increase in the following parameters as soluble sugars content, leaf area development, relative water content, leaf specific weight, root and shoot biomass, spike fertility, and grain yield. Response to inoculation varied by genotype, suggesting a genotypic effect which is involved in root colonization and inoculation response, and may play an important role in maximizing plant profit from this symbiosis. In this study, root colonization was proportional to inoculation response that could be used as a selection index for a better cultivar-inoculum combination to maximize durum production under water stress conditions.

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Introduction

Durum wheat (*Triticum Durum* Desf.) is one of the most important staple foods (Trematerra and Throne, 2012). Like other large field crops, the main concern is to achieve a consistent balance between increasing demand and the depletion of natural resources. In addition to groundwater scarcity, climate change has increased the frequency and severity of drought (Chahbar and Belkhodja, 2016; Lizarazo *et al*., 2016) which makes water availability the main limiting factor in durum wheat production, especially in Mediterranean-type climatic conditions where it is mainly cultivated (Soriano *et al*., 2018). Therefore, combining all available resources to improve water use efficiency and cope with drought is a necessity. Arbuscular Mycorrhizal fungi (AMF) are important soil microorganisms from the Glomeromycota phylum (Smith and Read, 2008). They are root symbionts that associate with most of the plants by colonizing cortical root cells and forming highly branched structures called arbuscules (Brundrett and Tedersoo, 2018). These arbuscules present the interface of exchange between the two symbiotic partners (Essiane-Ondo *et al*., 2019). In exchange for photosynthetic sugar, AMF facilitates plant absorption of water and nutrients from the soil (Smith and Read, 2008), which can affect plant performance (Smith and Smith, 2011). Besides, AMF has been linked to increasing plant diversity, growth (Van Der Heijden *et al*., 1998; Hartnett and Wilson, 1999), tolerance to pathogens, drought, salinity, and improved soil health and structure (Newsham *et al*., 1995; Augé, 2001; Smith and Read, 2008; Igiehon *et al*., 2017). Thus, AMF-plant symbiosis displays the potential to contribute significantly to the improvement of durum wheat production and its stability, particularly under water deficit conditions.

Several studies are seeking to understand the functional process of this symbiosis and the identification of factors influencing this relationship between the two partners to optimize the plant's carbon investment (Müller ad Harrison, 2019). Besides, the recent emergence of the AMF-based bio-fertilizer manufacture requires numerous trials to assess their impact and define the limits of their application (Sharma *et al*., 2017). In this study, we tested the AMF inoculation effect on three durum wheat cultivars under well-watered and post heading water deficit conditions. Here we aim to evaluate the impact of root colonization by AMF on plant growth, grain yield, drought tolerance, and genotype response.

Materials and methods

Experiment installation and conduction

The experiment was conducted in an unheated greenhouse at a temperature of 20±10 °C, the relative humidity of 45±15%, and natural light conditions at Ferhat Abbas Setif 1 University. Plastic pots filled with 2.2 kg of sieved and sterilized soil were used. The soil has a clayey-silty texture with a content of 1.5% organic matter and a pH (water) of 7.6. Four sterilized seeds were sown in each pot, then at the three-leaf stage, they were thinned to two plants. Each pot received 1g of mono ammonium phosphate at the three-leaf stage, 0.5 g of urea twice at five-leaf and tillering stage, and 0.5 g of potassium nitrate at the stem elongation stage.

In this experiment, three factors were tested. The First was genotype where three cultivars Megress (Algeria), Gtadur (Mexico), and Vitron (Spain) were used, the second was irrigation with two levels. Using the gravimetric method, soil humidity was maintained at field capacity for watered treatment and 45% of field capacity starting from the booting stage till maturity for stressed treatment. The third factor was inoculation in which TEMIS® (500 propagates/g), a product of Inoculumplus (www.inoculumplus.eu) was used as AMF inoculum. In addition to mineral support, it contains a mixture of five AMF species (*Funneliformis mosseae, Rhizophagus, intraradices, Funneliformis geosporum, Claroideoglomus claroideum, and Glomus Sp*.). Moisten seeds were covered by the inoculum powder for inoculated plants (MP) with the recommended dosage and nothing added to seeds for non-inoculated plants (NMP). The experiment was set out as a randomized complete design with six pots in each one of the twelve treatments.

Harvest and measurements

At the flowering stage and after 15 days of stress application, plants in half of the pots were harvested then shoot fresh and dry weights, as root fresh and dry weights were measured. Also, leaf area was measured using image analysis software ImageJ (Schneider *et al.*, 2012), relative water content was calculated following Barrs and Weatherley (1962) method, and leaf specific weight was calculated using the equation (Leaf Specific Weight= Leaf dry weight/Leaf area). Proline content in leaves and roots dry matter was measured according to the method described by Trolls and Lindsley (1955), and total soluble sugars content in leaves and roots dry matter was measured according to the method described by Dubois *et al.* (1956).

From each one of the MP treatment, cleaned root samples were cleared in 10% KOH solution and stained with acidified ink (5% Schaeffer black ink, 5% acetic acid, 90% distilled water) (Vierheilig *et al.*, 1998). Then, by microscopic observation, total colonization rate (TCR), as well as arbuscular (ACR) and vesicular (VCR) rates were determined according to McGonigle *et al.* (1990) method.

At the maturity stage, plants in the rest of the pots were harvested and shoot biomass, spike number per plant, seed number per spike, thousand seed weight, and grain yield per plant were measured. The number of spores in the rhizospheric soil (spore density) was counted by observation under a stereoscope after extraction from soil using sucrose gradient method Ianson and Allen (1986).

Data analysis

ANOVA test was used to compare the effect of irrigation, inoculation, and their interactions on different genotypes tested. LSD Test was used to compare treatment means. For AMF related measures, we compared only between inoculated plants (MP). The number of measures was N=4 in each treatment. Person Correlation test performed to study relations between different measures. Significance was considered for ($P \leq 0.05$). Data were analyzed using XLStat 2019 software.

Results and discussion

Root colonization rates and spore density

The only genotype affected TCR and VCR, while both genotype and irrigation affected ACR (Table 1).

Table 1. Means of Total colonization rate (TCR %), Arbuscular colonization rate (ACR%), vesicular colonization rate (VCR%) by AMF, and spore density (SD spore/g of soil) of three durum wheat genotypes under watered (W) and stressed (S) conditions.

Genotype	Irrigation	TCR	ACR	VCR	SD
Vitron	W	36,51 ab	29,84 bc	26,03 ab	69,55 a
	S	32,70 b	24,76 c	18,41 b	76,00 a
Megress	W	48,57 a	39,05 ab	34,92 ab	25,00 d
	S	46,03 ab	38,73 ab	38,73 a	58,00 b
Gtadur	W	51,53 a	48,15 a	29,42 ab	29,75 cd
	S	50,05	38,84 ab	42,01 a	35,75 c
SEM		3,61	2,82	4,16	1.33
Signification			P		
Genotype		< 0,001	< 0,001	0,002	< 0,001
Irrigation		0,382	0,040	0,394	< 0,001
Genotype*Irrigation		0,949	0,291	0,064	< 0,001

SEM: standard error of the mean. Means followed by the same letter are not significantly different at 0.05 level Test LSD.

Recorded root colonization rates could be considered high for Gtadur and Megress, and Medium for Vitron (Becerra *et al.*, 2009). Even that AMF-host plant specificity is moderate but exists (Eom *et al.*, 2000; Sepp *et al.*, 2019). This specificity depends on the environment (Öpik *et al.*, 2009), plant species and even cultivar (Bertheau *et al.*, 1980, Tawaraya, 2003) which can explain the difference in root colorization

rate between genotypes. Stress modified the nutritional status of the host-plant and consequently, it reduced the formation of new arbuscules that are known to be ephemeral. The reduction of ACR was by 12.57%. Irrigation, genotype, and their interaction significantly influenced spore density (Table 1). The highest spore density was in Vitron rhizospheric soil followed by Megress than Gtadur. The stimulation of spore production by a decline in soil moisture (Jacobson, 1997, Becerra *et al.*, 2009) explains the 20.17% increase of spore density by stress.

Table 2. Means of physiological, biochemical, and morphological measures at flowering stage and agronomic measures at maturity stage of each treatment.

Treatments			Flag leaf			Proline		Soluble sugars		Plant morphology					Agronomic measures			
G	In	Ir	LA	LSW	RWC	Roots	Leaves	Roots	Leaves	SFW	SDW	RFW	RDW	SBM	SN	SNS	TSW	GY
Vit	NM	W	5,85 bc	5,14 ab	85,76 ab	.74,66 ab	162,57 a	60,2 c	109,943 bc	10,08 cd	4,00 cd	5,26 c	1,44 de	5,53 bcd	2,75 bc	15,50 bc	34,28 cdef	0,75 bc
		S	6,14 bc	5,71 ab	72,96 abcd	85,27 ab	84,31 def	66,8 abc	110,968 bc	7,73 bc	3,62 bcd	3,26 b	1,07 cd	4,89 cd	1,75 ab	13,83 cd	21,73 g	0,63 bcd
	M	W	6,42 abc	7,03 a	89,75 a	70,57 b	78,82 def	40,9 cd	125,372 bc	15,15 e	6,47 e	11,98 f	3,13 h	7,29 bc	3,50 cd	18,33 ab	43,95 bc	1,43 a
		S	5,24 c	5,97 ab	64,35 d	74,38 ab	113,45 bcde	71,9 abc	169,300 a	11,33 d	5,13 de	7,51 d	1,94 f	7,35 b	3,25 cd	13,83 cd	40,19 bcd	0,78 b
Meg	NM	W	8,41 abc	5,68 ab	77,11 abcd	77,94 ab	61,48 f	64,6 bc	70,731 d	7,45 bc	2,61 abc	5,72 c	0,90 bc	4,53 d	1,50 ab	7,50 f	59,04 a	0,63 bcd
		S	7,09 abc	5,41 ab	64,23 d	63,78 b	116,41 bcd	11,1 d	96,423 cd	4,73 ab	1,93 ab	3,34 b	0,59 ab	3,67 d	1,00 a	6,20 f	60,60 a	0,59 bcd
	M	W	9,65 a	5,94 ab	90,34 a	81,66 ab	129,48 abc	108,3 a	93,294 cd	15,75 e	6,25 e	8,23 d	1,68 ef	7,62 b	4,50 d	12,00 cd	45,42 b	0,70 bc
		S	8,99 ab	6,51 a	84,09 abc	84,98 ab	144,83 ab	38,4 cd	110,424 bc	15,65 e	6,78 e	10,05 e	2,05 fg	5,71 bcd	3,25 cd	11,50 de	46,80 b	0,69 bc
Gtd	NM	W	5,24 c	5,12 ab	85,72 ab	98,32 ab	85,57 def	56,9 c	113,408 bc	11,15 d	2,89 abc	3,87 b	0,88 abc	4,00 d	1,50 ab	8,00 ef	25,22 fg	0,31 cd
		S	5,63 c	3,91 b	65,78 cd	119,57 a	93,60 cdef	105,7 ab	133,842 bc	3,17 a	1,36 a	1,86 a	0,45 a	3,97 d	1,00 a	5,67 f	28,95 efg	0,25 d
	M	W	9,14 ab	5,41 ab	80,11 abcd	85,73 ab	77,55 ef	44,4 cd	111,722 bc	9,00 cd	3,16 abc	7,93 d	1,32 cde	12,65 a	2,50 bc	17,83 ab	38,55 bcde	0,94 b
		S	9,11 ab	5,10 ab	70,70 bcd	77,95 ab	69,82 f	48,2 cd	145,439 ab	11,83 d	6,41 e	10,77 ef	2,41 g	12,02 a	3,50 cd	20,83 a	29,49 defg	0,91 b
SEM			0,672	0,433	3,771	9,22	7,651	0,878	3,03	0,62	0,39	0,645	0,09	0,51	0,276	0,791	2,29	0,092
Signification										P								
G			< 0,001	0,002	0,424	0,008	< 0,001	0,422	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	0,004	< 0,001	< 0,001	< 0,001
In			< 0,001	0,002	0,04	0,174	< 0,001	0,666	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,0001	< 0,001	0,071	< 0,001
Ir			0,289	0,263	< 0,001	0,597	< 0,001	0,28	< 0,001	< 0,001	0,9	< 0,001	0,01	0,027	0,013	0,01	0,022	0,006
G*In			0,001	0,788	0,002	0,017	0,828	< 0,001	0,050	< 0,001	< 0,001	< 0,001	0,49	< 0,001	0,002	< 0,001	< 0,001	< 0,001
G*Ir			0,477	0,34	0,216	0,553	< 0,001	< 0,001	0,892	0,17	0,01	< 0,001	< 0,001	0,241	0,017	0,012	0,016	0,012
In*Ir			0,609	0,946	0,73	0,569	0,009	0,232	0,130	< 0,001	< 0,001	< 0,001	< 0,001	0,594	0,126	0,233	0,602	0,149
G*In*Ir			0,538	0,075	0,081	0,217	0,007	0,028	0,135	< 0,001	< 0,001	< 0,001	< 0,001	0,46	0,021	0,002	0,006	0,051

Leaf area (LA cm²), Leaf specific weight (LSW mg/cm²), Relative water content (RWC %), Proline content in Roots and leaf (µg/100mg dry matter), soluble sugars in roots and leaf (mg/g dry matter), shoot fresh weight (SFW g), shoot dry weight (SDW g), root fresh weight (RFW g), root dry weight (RDW g), shoot biomass (SBM g), spikes number per plant (SN), Seed number per spike (SNS), thousand seed weight (TSW g), and yield per plant (GY g). Means with the same letter are not significantly different. SEM: Standard error of mean. Significance effect of Factors: Genotype (G) (Vitron (Vit), Megress (Meg) and Gtadur (Gtd)), Inoculation (In) (inoculated (M) and non-inoculated (NM)), and Irrigation (Ir) (watered (W), stressed (S)) in addition to their interactions is expressed by p-value.

Flag leaf physiological and biochemical measurements

Photosynthesis alteration and related physiological dysfunctions in the flag leaf during filling grains stage are the reason for yield loss caused by stress (Bruce *et al.*, 2007; Zlatev and Lidon; 2012). Thus, flag leaf status assessment is important to evaluate plant sensibility and/or affection by water stress and predict its impact on yield.

Genotype and inoculation significantly influenced leaf area and leaf specific weight, and their interaction also influenced leaf area (Table 2). A decrease of the Leaf area, which represents the active photosynthetic surface (Santander, 2017), will affect photosynthesis activity and consequentially grain yield. Our results showed that inoculation improved the leaf area by 26.59%. The improvements were by 67.91% in Gtadur, and 20.28% in Megress, while Vitron

recorded a slight decrease of 2.71%.

Leaf specific weight can be used as an indicator of production (Basile, 1986) because it correlated with the photosynthetic activity (Frey and Moss, 1976) and its significant and proportional affection by water stress intensity (Chahbar and Belkhodja, 2016). Therefore, the 5.3% improvement by inoculation in our experiment, suggest that the inoculated plants (MP) are more productive and less affected by stress than non–inoculated plants (NMP).

Relative water content is an indicator of leaf hydration status (Clarke and McCraig, 1982; Chaves *et al.*, 2002). It is controlled by the balance between water loss and absorption (Rachmilevitch *et al.*, 2006). In our study, relative water content was influenced by inoculation, irrigation, and their interaction (Table 2). Stress reduced relative water content by 16.49%, while inoculation increased it by 6.64%, most of it in Megress (23%).

The higher relative water content in MP, suggests that they are less affected by stress. This improvement could be attributed to better water absorption and/or lower water loss by transpiration, osmotic adjustment and maintaining cellular turgescence (Schonfeld *et al.*, 1988; Siddique *et al.*, 2000). Similar results are found by Porcel and Ruiz-Lozano (2004) and Mathur *et al.* (2018).

Table 3. Significant correlations between plant-related measurements and AMF-related measurements.

Variables		r	Variables		r	Variables		r	Variables		r	Variables		r
SFW	SDW	0,92***	LSW	SDW	0,69*	GY	RFW	0,82**	VCR	SFW	0,73**	ACR	GY	0,62*
SFW	RFW	0,82**	LSW	RFW	0,69*	GY	RDW	0,85***	VCR	SDW	0,83***	VCR	SPN	0,72**
SFW	RFW	0,81**	LSW	RDW	0,7*	GY	RPC	-0,67*	VCR	SN	0,82***	VCR	SBM	0,76**
SDW	RFW	0,88***	SNS	SDW	0,66*	GY	SBM	0,63*	VCR	LA	0,7*	VCR	SNS	0,63*
SDW	RDW	0,91***	SNS	SN	0,67*	GY	SNS	0,81**	VCR	RFW	0,88***	VCR	GY	0,58*
RFW	RDW	0,93***	SNS	RFW	0,73**	TCR	SFW	0,7*	VCR	RDW	0,74**	SD	SFW	0,67*
SFW	SBM	0,68*	SN	RFW	0,83***	TCR	SDW	0,77**	ACR	VCR	0,96***	SD	SDW	0,76**
LSW	RPC	-0,6*	SN	RDW	0,82**	TCR	SN	0,82**	ACR	SD	0,72**	SD	SN	0,69*
LSW	GY	0,71*	SN	SBM	0,59*	TCR	LA	0,66*	ACR	SFW	0,67*	SD	LSW	0,68*
RP	RSS	0,6*	SNS	SBM	0,83***	TCR	RFW	0,85***	ACR	SDW	0,72**	SD	RFW	0,81**
SN	SFW	0,88***	GY	SFW	0,66*	TCR	RDW	0,71**	ACR	SN	0,78**	SD	RDW	0,83***
SN	SDW	0,92***	SNS	RDW	0,78**	TCR	ACR	0,99***	ACR	LA	0,68*			
LSW	SFW	0,73**	SNS	RFW	0,73**	TCR	VCR	0,98***	ACR	RFW	0,83***			
RWC	SFW	0,71**	GY	SN	0,66*	TCR	SD	0,75**	ACR	RDW	0,68*			

Plant related measures: Leaf area (LA), Leaf specific weight (LSW), Relative water content (RWC), Proline content in roots dry matter (RP), soluble sugars in roots dry matter (RSS), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), shoot biomass (SBM), spikes number per plant (SN), Seed number per spike (SNS), and grain yield per plant (GY). AMF related measurements: total colonization rate (TCR), Arbuscular colorization rate (ACR), Vesicular colonization rate (VCR), and Spore density (SD). r: coefficient of correlation. Significant correlation at $\alpha = 0.05$ (*), at $\alpha = 0.01$(**), and at $\alpha = 0.001$ (***).

Excessive production of proline is common in stressed plants (Sithtisarn *et al.*, 2009). It serves as an osmoprotector and nutritional source, as it has certain regulatory functions (Joyce *et al.*, 1992; Szabados and Savouré, 2010; Chun *et al.*, 2018). Its production in the photosynthetic tissues, justify its higher concentration in leaves than roots. Genotype and inoculation significantly affected roots' proline content, while genotype, irrigation, inoculation, and their interactions, except (Genotype* Inoculation), had a significant effect on proline in leaves (Table 2). Proline content in roots was 8.52% lower in MP than NMP. Inoculation did not have a direct significant effect, but depending on genotype there was a reduction of 24.82%, and 09.37% in Gtadur and Vitron, while an increase of 17.58% was observed in Megress roots' proline. Stress increased proline content in leaves by 35.34%, and it was 43.68% for

MP and 24.28% NMP. The effect of stress depended also on genotype, there was an increasing of 70.33% for Vitron, and 63% for Megress, and a reduction of 8.04% recorded in Gtadur. Inoculation reduced proline in leaves by 29.37%. This decrease could be explained by a lower stress level in MP (Aroca *et al.*, 2008). Some studies like Ruíz-Sánchez *et al.* (2011), Yooyongwech *et al.* (2013), and Chitarra *et al.* (2016) found contrasting results to ours, however, several others found that AMF inoculation reduces the accumulation of proline in the host-plants (Wu and Xia, 2006; Aroca *et al.*, 2008; Ruiz-Sánchez *et al.*, 2010; Bhosale and Shinde, 2011; Zou *et al.*, 2013; Hazzoumi *et al.*, 2015).

Drought, generally, increases soluble sugars concentration (Chaves and Oliveira, 2004), as it may decrease in a case of severe drought (Pinheiro *et al.*, 2001). Soluble sugars act as osmoprotectors (Augé, 2001), interact with hormones, and modify the expression of genes related to photosynthesis (Chaves and Oliveira, 2004). As proline, soluble sugars content in roots was less than that of the leaves. In roots, soluble sugars seem to be significantly influenced by the interactions (Genotype*Inoculation), (Genotype*Irrigation) and (Genotype*Irrigation*Inoculation), while in leaves it was influenced by genotype, irrigation, and inoculation (Table 2). Stress reduced roots' soluble sugars by 71.39% in Megress but increased it by 37.07% and 51.83% in Vitron and Gtadur successively. In leaves, inoculation raised soluble sugars content by 18.93%. Stress also increased soluble sugars in leaves by 22.73% and this increase was higher in MP (28,69%) than NMP (16,03%%). These results confirmed that AMF can influence the composition and abundance of organic solutes, including soluble sugars (Porcel *et al.*, 2003; Sheng *et al.*, 2011). Numerous studies reported that MP show high concentrations of soluble sugars than NMP (Paradis *et al.*, 1995; Wu and Xia, 2006; Zhu *et al.*, 2010; Chen *et al.*, 2014), and they are in accordance with our findings for both soluble sugars in roots and leaves (excluding Vitron for soluble sugars content in roots). yet, contrasting results also were found (Zhang *et al.*, 2010; Yooyongwech *et al.*, 2013).

Plant morphology

In the shoot system, all the three factors and their interactions had a significant effect on fresh and dry weights except irrigation on the dry weight (Table 2). Vitron and Megress had a higher fresh and dry weight than Gtadur. MP was higher by 78% for fresh weight and by 108% for dry weight than NMP. These increases were: 49%, 158%, and 45% for fresh weight, 52%, 187%, and 126% for the dry weight for the genotypes Vitron, Megress, and Gtadur successively. Stress reduced fresh weight significantly by 21%, but not the dry weight. This decline of fresh weight was by 24% for NMP and 16% for MP.

In the root system, all factors and interactions had a significant effect on root fresh and dry weight except for (Genotype*Inoculation) for the dry weight (Table 2). Inoculation improved root fresh weight by 142% and dry weight by 135%. Depending on Genotype, these improvements were 129% and 102% for Vitron, 102% and 151% for Megress, and 226% and 180% for Gtadur of fresh and dry weights successively, and depending on irrigation they were 235% and 204% for stressed plants and 89% and 90% for watered plants in the same order. Stress reduced both fresh and dry weights by 14% and 09%. The short period between stress application and measures, in addition to its timing, was not enough to affect shoot dry weight.

These results showed that morphological characters of the root system and shoot system were improved by AMF inoculation, which confirms their role in growth stimulation (Thirkell *et al.*, 2020). The MP is less affected by drought than NMP, suggesting that AMF root colonization enhances growth and water use efficiency in all growth conditions (Kucey and Janzen, 1987), and improves drought tolerance. AMF improve access to the limited water reserve in soil (Begum *et al.*, 2019) directly, by an extra-radial mycelium pathway offering a higher absorption surface (Li *et al.*, 2013) and with lower carbon cost (Jakobsen *et al.*, 2005; Schnepf *et al.*, 2008), which

enhance water and nutrients uptake efficiency. Also, AMF may cause morphological and physiological alterations in colonized roots (Fusconi, 2014), as they can modify soil physicochemical characteristics (Cavagnaro, 2008). Moreover, AMF improves drought tolerance by physiological alterations in shoot organs and tissues (Bárzana *et al.*, 2012), such as modifying hormonal signals (Fan and Liu, 2011), osmotic adjustment, gazes exchange, water use efficiency, protection against oxidative damages (Rapparini and Peñuelas, 2014). Consequently, the shoot system, like the root system, shows better growth. Our findings are consistent with many studies like Al –karaki *et al.* (2004), Garmendia *et al.* (2017), Thirkell *et al.* (2020), and Bernardo *et al.* (2019).

Agronomic measurements

Genotype, inoculation, and their reciprocal action significantly influenced all agronomic measures except seed weight that was not under the influence of inoculation apart, on the other hand, Irrigation and its interaction with genotype influenced all parameters except shoot biomass (Table 2). Inoculation improved shoot biomass by 98.03%, spike number per plant by 99.55%, and seed number per spike by 66.37%. These improvements depended on genotype, and they were in order for Gtadur, Megress, and Vitron, 275%, 100%, and 26.72% for spike number, 209.82%, 62.54% and 40.52% for shoot biomass and 182.93, 71.53 and 9.66 for seed number per spike. On the other hand, stress-reduced spike number, shoot biomass, and Seed number per spike by 17.66%, 9.64%, and. 9.22% successively. Except for the slight increase of 2.58% in Gtadur, the reductions of seed number by stress in Megress and Vitron were 9.23%, and 18.23% successively. The seed weight of Megress was higher than Vitron and Gtadur was the lowest. Stress reduced seed weight by 7.59% and this reduction was 8.35% for Gtadur and 20.84% Vitron while there was a slight increase for Megress by 2.81%.

Vitron had the highest grain yield followed by Gtadur than Megress. Inoculation improved yield by 72.73%, and depending on genotype it was 230.06% for Gtadur, 60.76% for Vitron, and 40% for Megress. On the other hand, stress-reduced yield by 19.27%, and the reduction depended also on genotype: 7.52% for Gtadur, 3.85% for Megress, and 35.52% for Vitron.

These results validate the precedent findings that show an improvement of growth and drought tolerance by AMF inoculation. Here inoculation improved shoot biomass, grain yield, and its components spike number and seed number. Several studies found similar results like Pellegrino *et al.* (2015), Manske *et al.* (2000), and Panwar (1993) and suggest that AMF exploitation could be an effective approach to increase yields sustainably (Ortaş, 2017).

Significant correlations (Table 3) showed that morphological characters were positively related. Grain yield was positively related, in addition to its components seed number per spike and spike number, to shoot fresh weight, root fresh and dry weights, and shoot biomass. Being a part of the shoot system the relative water content of the flag leaf was positively related to the hydration of the entire system. Leaf specific weight proved its designation as a productivity indicator (Basile, 1986), and showed a positive correlation with growth parameters.

Knowing that their abundance is a result of water deficit, roots' proline, and roots' soluble sugars were positively related. On the other hand, roots' proline showed a negative correlation to both grain yield and leaf specific weight.

In addition to the positive correlation between them, root colonization rates showed a positive correlation to growth parameters (shoot fresh and dry weights, root fresh and dry weight, and leaf area), spike number, seed number, shoot biomass and grain yield which confirms again the positive role of AMF in stimulating plant growth, improving water use efficiency and drought tolerance.

Genotype dependency

Results showed that genotype influenced root colonization rates and the environment (water stress) affected the symbiotic form of colonization (ACR). Assessing AMF inoculation response using shoot

biomass variation showed that Gtadur and Megress with a higher TCR showed a better response (202.94% and 61.88% successively) than Vitron (39.97%) with a lower TCR. The response of Gtadur and Megress was slightly higher in watered conditions than stress conditions on the opposite of Vitron where the response in stressed conditions was higher than watered conditions. These results suggest the existence of AMF-cultivar specificity, its involvement in both root colonization and inoculation response, and they are proportional. They also suggest that, as AMF-species specificity is a crucial driver to achieve a notable response (Ortaş, 2017), AMF-cultivar specificity is important too (Baum *et al.*, 2015).

Many authors supposed that specificity could be related to root traits (Newsham *et al.*, 1995; Smith and Read, 2008; Baon *et al.*, 1994; Declerck *et al.*, 1995; Schweiger *et al.*, 1995; Jakobsen *et al.*, 2005). Root systems with low root hair length and density and with relatively large diameters would display the greatest growth benefits from the symbiosis (Brundrett, 2002; Fitter, 2004; Smith and Read, 2008). However, the results of a meta-analysis study done by Maherali (2014), did not confirm this proposition. De Vita *et al.* (2018) suggested the existence of a genetic basis for this trait and identified some proteins that may be involved in this specificity.

AMF inoculation response could be variable between cultivars, it ranges from negative response to positive response (Maherali, 2014; Ellouz, 2015). Our results suggest that root colonization rate could be used as a predictor of plant growth response (Köhl *et al.*, 2016) and as a selection trait for durum wheat genotypes compatibility with AMF (Singh *et al.*, 2012). This specificity in response depends also on the environment, like water availability in our study.

Consideration should, therefore, be paid to cultivar-specific AMF receptivity and function in the development of new genotypes for the future (Thirkell *et al*, 2020), by enhancing root colonization and plant response exploiting the existing genetic variability (De vita *et al.*, 2018). Using the appropriate combination between durum wheat genotype and native and/or introduced AMF, taking into consideration environmental conditions, could improve durum wheat production and sustainability.

Conclusion

Root colonization by AMF was higher in Gtadur and Megress than Vitron, and water deficit affected the symbiotic form (ACR) of root colonization and stimulated sporulation.

AMF inoculation improved growth and water use efficiency in both conditions well-watered and stressed conditions. AMF inoculation enhanced drought tolerance and it is shown by a lower proline content, and an increase of soluble sugars content, leaf area, relative water content, leaf specific weight, higher biomass, spike fertility, and yield. AMF inoculation response varied depending on genotype which suggests the existence of cultivar specificity that may play a key role in benefiting from this symbiosis. Results showed that root colonization rate is proportional with inoculation response, therefore it could be used as a trait for selecting AMF inoculum and/or durum wheat genotypes to optimize benefits from this symbiosis. Testing inoculation in the field in different conditions, taking into consideration the native AMF, using different inoculum sources, and more durum wheat cultivars should confirm the obtained results or determine their limitations.

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