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Mycorrhizal biofertilization improves grain yield and quality of hulless Barley (*Hordeum vulgare* ssp. *nudum* L.) under water stress conditions

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ABSTRACT

Drought is one of the most important factors worldwide, which limits the crop production, especially in semi-arid areas. The use of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) may represent an eco-friendly and biological technique to increase crops yields and ensure food security. The purpose of this paper was to evaluate the effect of AMF inoculation on a promised variety of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) under three levels of water stress (well-watered, moderate drought and severe drought). Hulless barley plants were inoculated, or not, with autochthonous inoculum (AI) containing five native AMF species (*Pacispora franciscana*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Rhizophagus irregularis* and *Glomus tenebrosum*), or commercial inoculum (CI) containing *Glomus* sp. strains. Under water stress, AMF inoculation especially, with autochthonous consortium has higher mycorrhizal root colonization of hulless barley by 7-fold and 23-fold in comparison to the non-inoculated controls, under moderate drought and severe drought conditions, respectively. Water stress decreased grain yield and thousand-kernel weight of hulless barley. The reduction was less pronounced in AMF inoculated plants compared to the non-inoculated control ones. Plants with higher mycorrhizal colonization showed higher grain yield and thousand-kernel weight by approximately 90% and 68.2% with AI, and by 106% and 83% with CI, respectively than control plants with lower AMF colonization, especially under severe drought. At the same time, the amount of K, Cu, Fe, Zn and Ca in hulless barley grain increased significantly in AMF inoculated plants with AI as well as with CI. Compared to the control plants, using autochthonous AMF species led to significantly decreased Na content in grain. Fatty acids in hulless barley grain decreased with the severity of water stress. Only under well-watered condition, AMF inoculation enhance C18:0 and C18:1 contents as compared to control plants. Moreover, total polyphenol and flavonoid increased due to AMF inoculation under both medium drought and severe drought conditions. The results obtained herein indicated that inoculation with AMF can enhance the water tolerance resulting in higher hulless barley grain yield and quality. Therefore, using AMF as biofertilizers may be important in regions suffering from lack of water in order to ensure sustainable agricultural systems.

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world, ranked in the fourth position for cereal grain production. Barley species may have hulled or hulless (naked) grains without husks. Most cultivated barleys today are of the hulled form. They are mainly used for animal feed (65%), brewing malts (30%) and human consumption (3%) (Aldughpassi et al., 2016). However, hulless barley is mainly used as a human food because of ease in processing and edibility. Hulless barley (*Hordeum vulgare* ssp. *nudum* L.) has high nutritive quality as a food or feed, and has been receiving increasing attention in recent years. As compared to other cereals, hulless barley has higher soluble fibre β-glucan and protein contents, in particular essential amino acid, lysine, also fatty acids contents and polyphenols which act as

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antioxidants. It is considered as a healthy food since it has the ability to reduce cholesterol and blood sugar levels (Guo et al., 2020).

Similar to other cereals, barley crops are strongly affected by climate change that result in a considerable loss in crop productivity (Campbell et al., 2016). Tunisia is one of the most vulnerable Mediterranean countries to climate variability (USAID, 2018). Increasing temperatures coupled with varied precipitation levels could threat the availability of water resources and thus agriculture, one of the most strategic sectors in the country which contributed to 10.4% of the gross domestic product in 2018 (World Bank, 2020). Barley is considered as one of the most cereals known to be well adapted to different climatic conditions. However, increased temperatures associated with reduced water availability affect considerably its grain yield and quality (Wenzel et al., 2015).

In this context, the use of arbuscular mycorrhizal fungi (AMF) as microbial fertilizer has been described as a potential tool for sustainable agriculture and food security (Thirkell et al., 2017). AMF establish symbiotic association with the roots of over than 80% of terrestrial species. Similar to other cereal crops, barley forms association with these ubiquitous fungi, which can lead to improved plant acquisition of soil nutrients and water (Thirkell et al., 2017). There is a growing recognition that inoculation with AMF can alleviate the negative effects of abiotic stresses such as drought (Bernardo et al., 2019; Kamali and Mehraban, 2020).

However, the effect of AMF inoculation on grain yield and quality of hulless barley during drought stress has not, to our knowledge, been assessed yet.

Thus, the main objective of the present study is to evaluate, under water stress, the benefit of the mycorrhizal inoculation on the grain yield and quality of hulless barley to promote its production in semi-arid regions of Tunisia due to its great interest in the food and feed industries.

2. Materials and methods

2.1. Experimental design

The experiment consisted of a split plot design with two factors (Fig. S1). Water stress at three levels, well-watered (WW) (daily irrigated according to growth stage); medium drought stress (MD) (five days without irrigation) and severe drought stress (SD) (ten days without irrigation), were assigned as the main plot factor. AMF treatment at four levels, autochthonous mycorrhizal inoculum (AI) containing native AMF species; commercial AMF inoculum (CI) and two controls without inoculation (control AI, control CI), were the subplot factor. Three replicates per treatment were carried out making a total of 36 pots, each containing four plants.

2.2. Plant materials and growth conditions

Agricultural soil (from a depth of 0–60 cm) was collected from the experiment station of the National Agronomic Institute of Tunisia (10° 11′ N, 36° 55′ E). Soil physico-chemical characteristics are: 26.1% clay, 55% silt, 18.9% sand, 0.72% organic matter, pH (8.14), EC (0.12 dS/m), N (1 g/kg), P (0.015 g/kg), K (0.15 g/kg). Each plastic pot 20 × 26 cm (diameter × height) was filled with 5 kg of 2 mm sieved soil. The autochthonous mycorrhizal inoculum (AI) as described by Jerbi et al. (2020) composed of five native AMF species (*Pacispora franciscana*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Rhizophagus irregularis* and *Glomus tenebrosum*) and the commercial AMF inoculum (CI) (Symbivit, InoculumPlus, France) containing six *Glomus* sp. strains, were used for AMF inoculation. Mycorrhizal inoculum were applied at an average of 500 propagules per pot and were placed below the seeds at the time of sowing. The non-inoculated controls received the same amount of autoclaved inoculum. Seeds of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) population-variety named "Prophet barley" or "Moknine barley", were surface sterilized in 1% sodium hypochlorite and sown in the inoculated and non-inoculated pots. Hulless barley was sown on January 2018 and harvested on June 2018. Plants were grown under shelter and regularly irrigated to 70% of field capacity until the tillering and grain filling stages, when the plants were exposed to water stress levels. The water stress treatment was conducted by stopping water supply during five days for medium drought stress (MD) and maintained for another five days for severe drought stress (SD). For control condition, well-watered treatments (WW), soil water content was maintained at 70% of field capacity. During the water stress period, all the pots were irrigated to maintain soil water content at 70% for the control, 50% for the MD and 20% for the SD.

2.3. AMF root colonization

After plant harvest (139 days after planting), fresh roots were sampled for the determination of AMF root colonization rate. From each treatment, fine roots were cleared in 5% KOH, washed with distilled water and acidified in 2% HCl. The roots were stained with 0.05% trypan blue at 90 °C for 2 h as described by Phillips and Hayman (1970). From each sample (four plants), 45 root segments of 1 cm length were examined under microscope in order to count mycorrhizal structures (arbuscules, vesicles and hyphae) using the method of (McGonigle et al., 1990). In total, 405 observations (135 root fragments with 3 intersections per root fragment) per treatment were analyzed.

2.4. Grain yield

At harvest maturity, all plants in each treatment (3 pots of four plants each) were hand harvested. After hand threshing, the total grain yield (g/pot) and thousand-kernel weight were measured.

2.5. Grain quality

2.5.1. Mineral nutrient content

From each replicate, ground grain of barley was ashed at 450 °C during 5 h. Ashes were collected in 20 mL of nitric acid (0.1 N) and digested at 100 °C during 10 min. The digestions were filtrated, made up to 100 mL then stored at 4 °C. These extractions were used to assay potassium (K), sodium (Na), calcium (Ca), using the flame photometer, zinc (Zn), iron (Fe) and copper (Cu) with a flame atomic absorption spectrometry according to the methods described by Pauwels et al. (1992).

2.5.2. Fatty acid contents

Fatty acids were extracted from lyophilized ground grain (0.5 g) following the method described by Labidi et al. (2011). The final extracts were analyzed using a PerkinElmer Autosystem gas chromatograph (GC) equipped with a flame-ionization detector (Norwalk, CT) with hydrogen as carrier gas (40 mL/min). Fatty acids (FA) were quantified by using heptadecanoic acid methyl ester (C17:0) as an internal standard. Their identification relied on the retention times of fatty acids standards. Overall, 37 different references FA were used as standards (lipids standards: fatty acid methyl ester mixtures C4–C24:1, Sigma Aldrich).

2.5.3. Polyphenol and flavonoid contents

The grounded grain powder (1 g) was immersed in ethanol (70°) for 24 h with frequent agitation. After centrifugation, the extracts were stored at 4 °C until further use. The total phenolic content was determined for individual extracts according to the method of Folin-Ciocalteu (Singleton et al., 1999). Briefly, 1 mL of extract was mixed with 4 mL of 10% (w/v) Folin-Ciocalteu reagent. After 5 min, 5 mL of Na_2CO_3 (7.5%) was subsequently added to the mixture and incubated at obscurity for 90 min with intermittent agitation followed by the measurement of absorbance using a UV Spectrophotometer (OPTIZEN 3220UV, Daejeon, South Korea) at 760 nm against a blank (without extract). The total polyphenol contents of extracts were expressed as mg/g of gallic acid

equivalents in milligrams per gram (mg GAE/g) of dry extract. The flavonoid content of each extract was performed using the method of Dowd (Lamien-Meda et al., 2008). Briefly, 5 mL of extract solution were mixed with 5 mL of 10% (w/v) $AlCl_3$ solution in methanol and incubated at obscurity for 30 min. The absorbance was measured at 415 nm against the blank. Flavonoid contents in extract were expressed as mg/g of rutin equivalents in milligrams per gram (mg RE/g) of dry extract.

2.6. Statistical analyses

Data were tested for statistical significance applying the two-way analysis of variance (ANOVA) using the water stress treatments and mycorrhizal inoculation as independent factors. Means were compared with LSD Fisher's test and differences were considered significant at $P < 0.05$. In order to evaluate the interactions between the variables and the different applied treatments, a principal component analysis (PCA) was performed with the function "prcomp" in the R software package. All statistical analyses were conducted using the R v.3.6.0 software.

3. Results

3.1. Mycorrhizal root colonization

The statistical analysis demonstrated a significant interaction between AMF treatments and water stress levels on mycorrhizal root colonization of barley ($P < 0.001$; Table S1). The mycorrhizal rates of AMF inoculated plants were significantly higher compared to those observed in non-inoculated ones (Fig. 1). Water stress caused a decrease in root colonization of non-inoculated plants by more than 28% under the moderate drought compared to the well-watered controls (Fig. 1). For plants inoculated with the autochthonous mycorrhizal consortium (AI), total colonization increased with the increasing of water stress level, while those inoculated with the commercial inoculum (CI) and the non-inoculated (control AI) significantly decreased. Indeed, the total mycorrhizal colonization of plants inoculated with (CI) was significantly decreased by 32.8% under severe drought and more moderately by 9.8% under moderate drought as compared to well-watered plants (Fig. 1). As for plants inoculated with (AI), the total colonization rates in barley roots were higher as well under moderate drought (53.3%) and severe drought (69.6%). A similar pattern was observed for arbuscular root colonization (Table S2). Vesicular root colonization was significantly

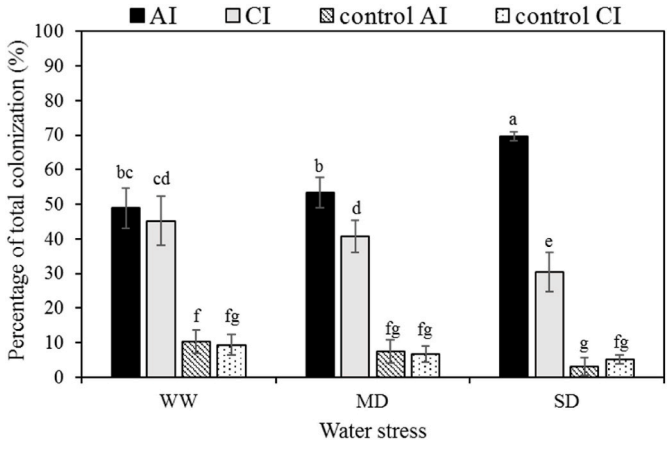

Fig. 1. Effect of arbuscular mycorrhizal fungal (AMF) treatment on mycorrhizal colonization rates of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) roots under water stress levels. WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous mycorrhizal inoculum; CI, commercial inoculum; control AI and control CI, two controls without inoculation. Bars indicate standard error of the mean ($n = 3$). Treatments with the same letter are not significantly different according to LSD test.

influenced by AMF treatment ($P < 0.001$; Table S1), by water stress level ($P < 0.01$; Table S1) and by the interaction between the two factors ($P < 0.001$; Table S1). Vesicles were not detected in non-inoculated (control AI and control CI) root plants at the different water stress treatments. The highest vesicular colonization was observed in plants inoculated with CI (28.8%) under severe drought (Table S2).

3.2. Grain yields

Grain yield and thousand-kernel weight (1000-grain weight) were significantly influenced by both AMF treatment and water stress level ($P < 0.001$; Table S3). Water stress caused a significant decrease of grain yield and thousand-kernel weight of barley (Table 1). The highest grain yield was observed in plants inoculated with AI under well-watered condition and it was 16 and 91% higher than CI and non-inoculated plants (control AI), respectively. Compared to non-inoculated plants, grain yields of those inoculated with AI and CI were 1.6 and 1.3-fold higher under moderate drought, 1.9 and 2-fold higher under severe drought, respectively (Table 1). AMF inoculation increased thousand-kernel weight under water stress conditions by approximately 1.4-fold under moderate drought and 1.7-fold under severe drought as compared to non-inoculated plants (Table 1). The most pronounced increase in thousand-kernel weight was observed with AI and CI under well-watered condition and it was by approximately 95 and 74%, respectively, in comparison to their respective controls (Table 1).

3.3. Grain quality

3.3.1. Mineral nutrient contents

The main effect of both AMF treatment and water stress level was significant on potassium (K), copper (Cu), iron (Fe) and zinc (Zn) contents in barley grain ($P < 0.001$; Table S3). However, no significant effect of the interaction between AMF treatment and water stress was noticed. When compared to plants under well-watered conditions, K, Cu, Fe and Zn contents in barley grains were significantly higher by 9, 27, 46 and 18%, respectively in plants under moderate drought level, and by 19, 49, 69 and 41%, respectively, in plants under severe drought level (Table 1). AMF inoculation increased significantly K, Cu, Fe and Zn contents, as compared to those non-inoculated (Fig. 2, Table S5). Indeed, K, Cu, Fe and Zn concentrations in barley grains were significantly higher by 33, 31, 15 and 28%, respectively in inoculated plants with AI and by 30, 63, 43 and 29% in those inoculated with CI, as compared to their respective controls (Table 1). Grain sodium (Na) and calcium (Ca) contents were significantly affected by both AMF inoculation and water stress level as well as their interaction ($P < 0.001$; Table S3). Concerning Na concentration, no significant differences were noticed between AMF inoculated plants and non-inoculated ones, under well-watered and moderate drought conditions (Table 1). However, the plants inoculated with AI had a lower Na concentration by 16% in comparison to their respective control under severe drought (Fig. 2, Table S5). The highest calcium concentration in barley grains was obtained in inoculated plants with AI under well-watered conditions (1.02 mg/g DW, Table 1) and it was about four-fold higher than in non-inoculated plants. Under moderate drought and severe drought conditions, a significant increase of Ca concentrations by 3 and 2-fold, respectively, was noticed in the grains of plants inoculated with AI as compared to the non-inoculated ones. Similarly, Ca concentrations were 2-fold higher in inoculated plants with CI, under both moderate drought and severe drought conditions, in comparison to their respective controls (Table 1, Fig. 2, Table S5).

3.3.2. Fatty acid contents

The results showed that linoleic acid (C18:2) contents in barley grains were significantly affected by the main effects of AMF treatment and water stress level whereas the interactions between these two factors were not significant. Concerning the palmitic acid (C16:0) in barley grains, only water stress level had a significant effect (Table S4). The

Table 1
Effects of arbuscular mycorrhizal fungal (AMF) treatment and water stress level (WS) as well as their interactions on grain yields and nutrient contents of hulless barley (*Hordeum vulgare* ssp. *nudum* L.).

Groups		GY (g/pot)	TKW (g)	K (mg/g DW)	Na (μg/g DW)	Ca (mg/g DW)	Cu (μg/g DW)	Fe (μg/g DW)	Zn (μg/g DW)
Water stress level (WS)									
WW		5.96 ± 1.42 a	39.72 ± 13.11 a	2.75 ± 0.40 a	42.86 ± 0.98 b	0.50 ± 0.34 a	7.19 ± 1.49 a	39.77 ± 6.23 a	38.23 ± 5.35 a
MD		4.76 ± 0.96 b	26.34 ± 4.89 b	2.52 ± 0.40 ab	43.61 ± 0.59 b	0.31 ± 0.15 b	5.68 ± 1.07 b	27.17 ± 6.31 b	32.43 ± 5.42 b
SD		3.74 ± 1.34 b	24.21 ± 7.38 b	2.31 ± 0.42 b	46.79 ± 3.49 a	0.25 ± 0.12 b	4.84 ± 1.64 b	23.50 ± 3.91 b	27.13 ± 5.85 c
AMF treatment (AMF)									
AI		6.20 ± 1.08 a	38.04 ± 10.79 a	2.91 ± 0.28 a	43.14 ± 0.74 b	0.62 ± 0.32 a	6.83 ± 1.00 a	31.91 ± 8.34 ab	38.76 ± 5.96 a
CI		5.54 ± 0.90 a	37.24 ± 11.40 a	2.84 ± 0.27 a	43.47 ± 1.51 b	0.38 ± 0.12 b	7.18 ± 1.34 a	35.84 ± 9.15 a	34.60 ± 4.73 ab
control AI		3.45 ± 0.60 b	22.26 ± 4.47 b	2.18 ± 0.20 b	46.23 ± 4.17 a	0.20 ± 0.09 c	5.20 ± 1.39 b	27.82 ± 7.55 b	30.28 ± 6.21 bc
control CI		4.09 ± 1.57 b	22.82 ± 5.71 b	2.19 ± 0.31 b	44.84 ± 2.16 ab	0.20 ± 0.06 c	4.40 ± 1.39 b	25.00 ± 7.84 b	26.76 ± 5.95 c
WS × AMF									
WW	AI	7.55 ± 0.42 a	51.52 ± 4.32 a	3.11 ± 0.15 a	42.49 ± 0.59 d	1.02 ± 0.11 a	8.03 ± 0.32 a	41.00 ± 4.40 a	44.43 ± 3.26 a
	CI	6.53 ± 0.49 b	51.39 ± 7.83 a	3.08 ± 0.18 a	42.31 ± 0.93 d	0.49 ± 0.10 b	8.53 ± 1.11 a	46.27 ± 5.95 a	39.90 ± 2.52 a
	control AI	3.95 ± 0.41 e	26.41 ± 5.52 bc	2.29 ± 0.22 a	43.17 ± 0.67 cd	0.26 ± 0.11 cd	6.70 ± 0.90 a	37.03 ± 4.66 a	36.53 ± 2.57 a
	control CI	5.80 ± 0.43 c	29.55 ± 0.73 b	2.53 ± 0.17 a	43.47 ± 1.51 cd	0.25 ± 0.08 cd	5.50 ± 1.30 a	34.77 ± 4.73 a	32.07 ± 3.23 a
MD	AI	5.75 ± 0.28 c	31.51 ± 2.66 b	2.97 ± 0.14 a	43.46 ± 0.60 cd	0.49 ± 0.11 b	6.33 ± 0.67 a	30.43 ± 4.86 a	39.50 ± 1.30 a
	CI	5.45 ± 0.31 c	29.73 ± 2.67 b	2.77 ± 0.19 a	43.01 ± 0.44 d	0.35 ± 0.12 bc	6.70 ± 0.40 a	33.93 ± 3.48 a	32.33 ± 4.14 a
	control AI	3.62 ± 0.40 e	21.88 ± 0.97 cd	2.20 ± 0.14 a	43.87 ± 0.52 cd	0.18 ± 0.10 d	5.03 ± 0.51 a	23.90 ± 3.48 a	30.70 ± 2.10 a
	control CI	4.23 ± 0.36 de	22.22 ± 1.90 cd	2.15 ± 0.19 a	44.10 ± 0.18 cd	0.20 ± 0.02 cd	4.63 ± 0.95 a	20.40 ± 1.45 a	27.17 ± 4.18 a
SD	AI	5.30 ± 0.40 c	31.08 ± 5.52 b	2.63 ± 0.29 a	43.47 ± 0.71 cd	0.35 ± 0.08 bc	6.13 ± 0.42 a	24.30 ± 4.60 a	32.33 ± 4.33 a
	CI	4.63 ± 0.40 d	30.61 ± 0.74 b	2.67 ± 0.29 a	45.08 ± 1.36 bc	0.30 ± 0.15 cd	6.30 ± 1.25 a	27.33 ± 3.18 a	31.57 ± 1.52 a
	control AI	2.79 ± 0.19 f	18.48 ± 1.07 d	2.07 ± 0.25 a	51.64 ± 1.56 a	0.17 ± 0.04 d	3.87 ± 0.78 a	22.53 ± 1.35 a	23.60 ± 4.18 a
	control CI	2.25 ± 0.20 f	16.68 ± 0.95 d	1.89 ± 0.13 a	46.95 ± 2.45 b	0.16 ± 0.04 d	3.07 ± 0.74 a	19.83 ± 2.57 a	21.03 ± 2.32 a

WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous mycorrhizal inoculum; CI, commercial inoculum; control AI and control CI, two controls without inoculation; GY, grain yield; TKW, thousand-kernel weight. Different letters in the same column indicate significant differences between water stress levels (WS), mycorrhizal inoculation (AMF) treatments, and the interaction WS × AMF. Data are represented as mean ± SD of three replicates per treatment. Treatments with the same letter are not significantly different according to the LSD test.

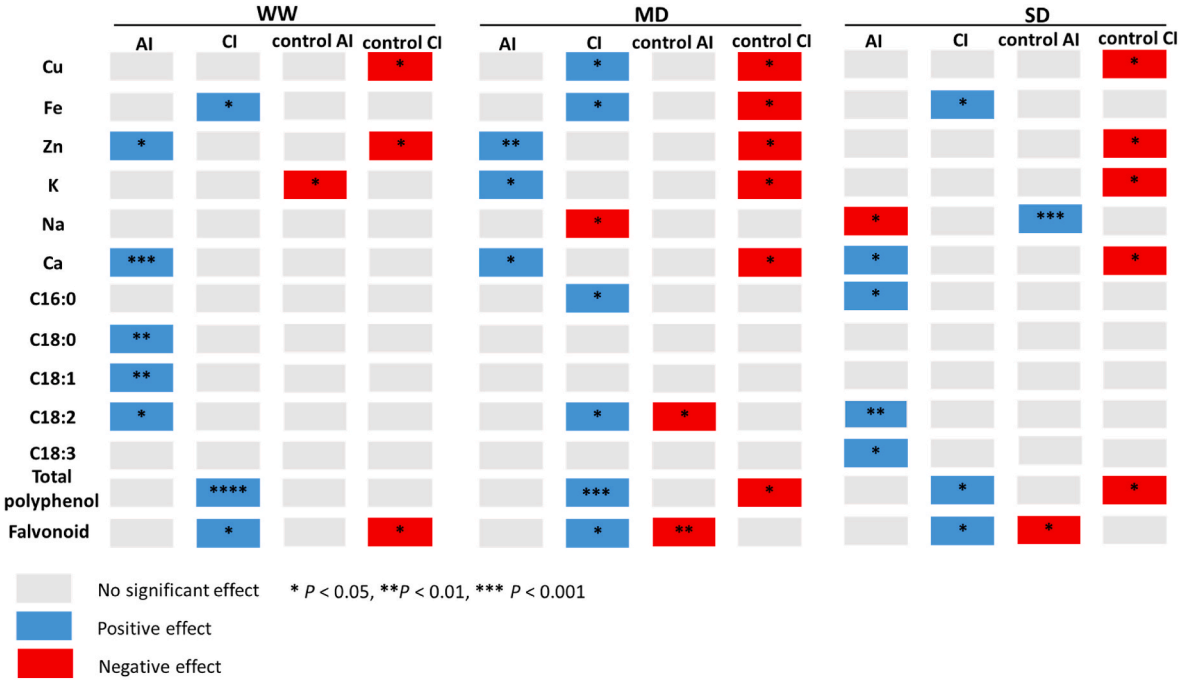

Fig. 2. General analysis of the effect of arbuscular mycorrhizal fungal (AMF) treatment on mineral nutrient, fatty acids, polyphenol and flavonoid contents of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) grain under different water stress levels. WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous mycorrhizal inoculum; CI, commercial inoculum; control AI and control CI, two controls without inoculation; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, γ linolenic acid.

linoleic acid contents were 3 and 2-fold higher in plants inoculated with AI and with CI, respectively, as compared to their respective controls (Table 2). In comparison to well-watered plants, the palmitic acid content in the grains significantly decreased by 2 and 3-fold in plants subjected to moderate drought and severe drought conditions, respectively (Table 2, Fig. 2). As well, linoleic acid contents of grains were 2-fold higher under well-watered condition than those under severe drought condition. The interaction between AMF treatment and water stress level significantly influenced stearic acid (C18:0), oleic acid (C18:1) and alpha linolenic acid (C18:3) grain contents (Table S4). Grains of

Table 2
Effect of arbuscular mycorrhizal fungal (AMF) treatment and water stress level (WS) as well as their interactions on fatty acids composition, polyphenol and flavonoid contents of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) grain.

Groups		Fatty acids (μg/g DW)					Polyphenol (mg GAE/g DW)	Flavonoid (mg RE/g DW)
		C16:0	C18:0	C18:1	C18:2	C18:3		
Water stress level (WS)								
WW		1.21 ± 0.67 a	0.38 ± 0.35 a	0.83 ± 0.81 a	1.96 ± 1.06 a	1.30 ± 0.63 a	58.82 ± 7.86 a	16.86 ± 5.14 a
MD		0.56 ± 0.41 b	0.13 ± 0.09 b	0.37 ± 0.20 b	1.68 ± 1.18 ab	0.70 ± 0.44 b	50.85 ± 7.97 b	13.04 ± 2.08 ab
SD		0.41 ± 0.20 b	0.13 ± 0.10 b	0.26 ± 0.16 b	1.10 ± 0.62 b	0.49 ± 0.20 b	38.35 ± 9.97 c	13.54 ± 5.29 b
AMF treatment (AMF)								
AI		1.04 ± 0.81 a	0.38 ± 0.40 a	0.90 ± 0.93 a	2.49 ± 0.84 a	1.13 ± 0.74 a	52.58 ± 5.14 a	17.55 ± 3.29 a
CI		0.91 ± 0.57 ab	0.23 ± 0.15 ab	0.47 ± 0.24 ab	1.89 ± 1.12 ab	0.93 ± 0.62 ab	60.83 ± 9.66 a	18.87 ± 3.33 a
control AI		0.54 ± 0.36 ab	0.06 ± 0.03 ab	0.34 ± 0.13 b	0.80 ± 0.47 bc	0.65 ± 0.30 ab	42.38 ± 10.34 b	10.37 ± 2.40 b
control CI		0.43 ± 0.25 b	0.17 ± 0.10 b	0.24 ± 0.18 b	1.14 ± 0.66 c	0.61 ± 0.38 b	41.58 ± 10.93 b	11.12 ± 1.45 b
WS × AMF								
WW	AI	1.81 ± 0.98 a	0.83 ± 0.41 a	1.94 ± 1.02 a	3.02 ± 1.29 a	1.65 ± 1.04 a	57.78 ± 0.62 c	20.88 ± 0.96 a
	CI	1.39 ± 0.49 a	0.31 ± 0.15 b	0.61 ± 0.10 b	2.08 ± 0.73 a	1.55 ± 0.58 ab	71.38 ± 0.36 a	22.35 ± 1.94 a
	control AI	0.98 ± 0.18 a	0.07 ± 0.03 bc	0.46 ± 0.08 b	0.99 ± 0.60 a	0.98 ± 0.18 abc	52.61 ± 0.75 d	12.99 ± 0.69 de
	control CI	0.66 ± 0.29 a	0.29 ± 0.05 b	0.33 ± 0.22 b	1.75 ± 0.70 a	1.00 ± 0.40 abc	53.52 ± 0.61 d	11.23 ± 0.73 ef
MD	AI	0.68 ± 0.51 a	0.18 ± 0.12 bc	0.38 ± 0.16 b	2.53 ± 0.42 a	1.05 ± 0.65 abc	53.67 ± 0.65 d	13.72 ± 1.57 cd
	CI	0.97 ± 0.42 a	0.16 ± 0.11 bc	0.54 ± 0.26 b	2.77 ± 1.10 a	0.85 ± 0.33 bc	61.89 ± 0.77 b	15.39 ± 0.56 c
	control AI	0.28 ± 0.09 a	0.09 ± 0.03 bc	0.29 ± 0.06 b	0.52 ± 0.20 a	0.58 ± 0.20 c	45.15 ± 1.45 f	10.42 ± 1.19 f
	control CI	0.33 ± 0.20 a	0.11 ± 0.09 bc	0.26 ± 0.21 b	0.88 ± 0.60 a	0.33 ± 0.21 c	42.71 ± 1.74 g	12.61 ± 0.37 de
SD	AI	0.62 ± 0.14 a	0.13 ± 0.04 bc	0.40 ± 0.13 b	1.93 ± 0.32 a	0.70 ± 0.12 c	46.30 ± 1.85 f	18.04 ± 0.90 b
	CI	0.36 ± 0.27 a	0.22 ± 0.18 bc	0.25 ± 0.19 b	0.81 ± 0.59 a	0.38 ± 0.28 c	49.21 ± 0.98 e	18.87 ± 2.00 b
	control AI	0.35 ± 0.21 a	0.03 ± 0.01 c	0.29 ± 0.17 b	0.89 ± 0.54 a	0.40 ± 0.13 c	29.37 ± 1.69 h	7.71 ± 0.35 g
	control CI	0.31 ± 0.03 a	0.12 ± 0.02 bc	0.12 ± 0.03 b	0.78 ± 0.14 a	0.50 ± 0.10 c	28.51 ± 1.52 h	9.53 ± 0.82 fg

WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous mycorrhizal inoculum; CI, commercial inoculum; control AI and control CI, two controls without inoculation; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, γ linolenic acid. Different letters in the same column indicate significant differences between water stress levels (WS), mycorrhizal inoculation (AMF) treatments, and the interaction WS × AMF. Data are represented as mean ± SD of three replicates per treatment. Treatments with the same letter are not significantly different according to the LSD test.

inoculated plants with AI under well-watered conditions had higher stearic and oleic acid levels (12 and 4-fold, respectively) than those non-inoculated, while under moderate drought and severe drought conditions, no significant differences were noticed between AMF-inoculated and non-inoculated plants (Table 2, Fig. 2, Table S5).

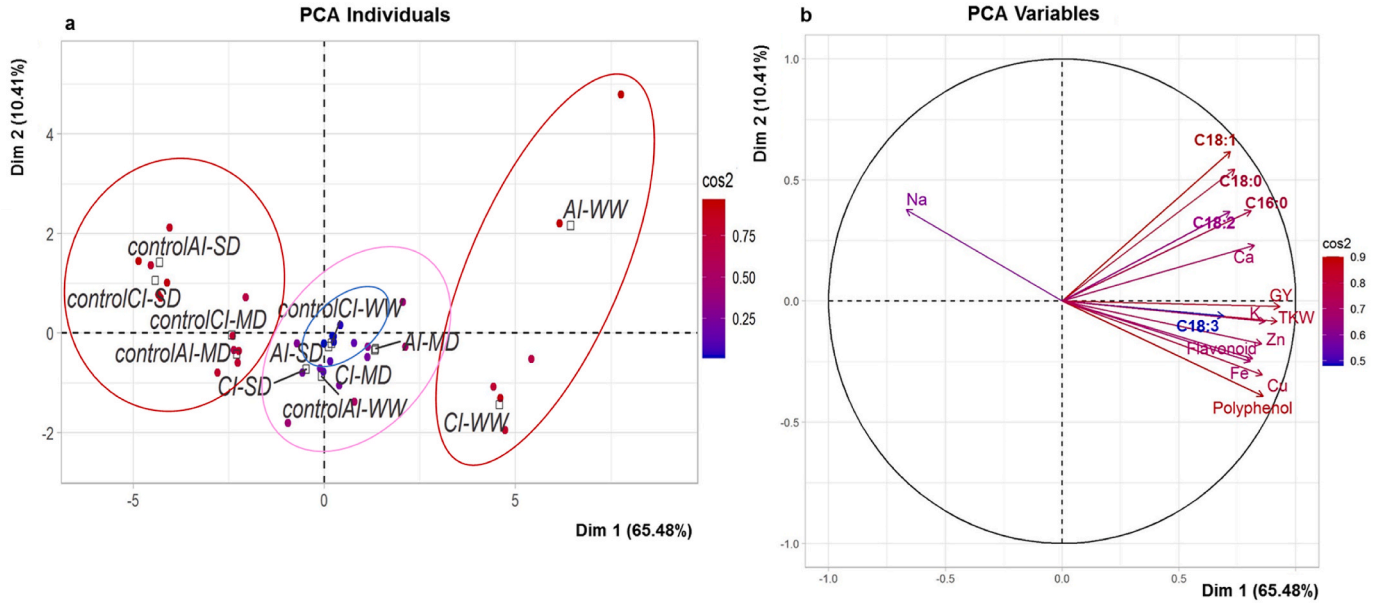

Fig. 3. Principal components analysis (PCA) of the grain yield, mineral nutrients, fatty acids, total polyphenols and flavonoid amounts in the grains of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) inoculated with AMF and non-inoculated ones cultivated under different water stress levels. Sub figures show the variation in treatments scores (a) and measured variables scores (b) along the first two PCA axes. 65.48 and 10.41% of the variation is explained by PC1 (Dim. 1) and PC2 (Dim.2), respectively. The lengths of the arrows indicate the relative importance of each variable, whereas the angles between the arrows indicate the degree to which they are correlated. WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous mycorrhizal inoculum; CI, commercial inoculum; control AI and control CI, two controls without inoculation; GY, grain yield; TKW, thousand kernel weight C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, γ linolenic acid.

3.3.3. Polyphenol and flavonoid contents

Total polyphenol and flavonoid contents were significantly affected by both AMF treatment and water stress level (Table S4). This later reduced total polyphenol contents for all the treatments (Table 2). The most pronounced decreases were by 16 and 37% in the grains of non-inoculated plants (control AI) as compared to those inoculated with AI, respectively, under moderate drought and severe drought conditions. In addition, total polyphenol contents were significantly higher by 33, 45 and 73% in the inoculated plants with CI than those in the non-inoculated (control CI), under the three water stress levels WW, MD and SD, respectively (Table 2). Flavonoid contents in grains significantly increased by 2, 1 and 2-fold in plants inoculated with AI and CI as compared to those non-inoculated, under well-watered, moderate drought and severe drought conditions, respectively (Table 2, Fig. 2, Table S5).

3.4. Principal components analysis

The principal component analysis (PCA) was conducted to examine the effect of the water stress and AMF treatments on grain quality of barley. The first two components accounted for 75.9% of the total variance (Fig. 3, Fig. S2). The first axis PC1 (Dim 1) explained most of the variation (65.5%) and mainly separated treatments according to water stress level and AMF treatment. The second axis PC2 (Dim 2) only explained 10.4% of the total variance (Fig. 3a). The PC1 showed a strong and positive correlation with the grain yield GY ($r = 0.93$; $P < 0.0001$), thousand kernel weight TKW ($r = 0.92$; $P < 0.0001$), mineral Fe ($r = 0.80$; $P < 0.0001$), Zn ($r = 0.85$; $P < 0.0001$), Cu ($r = 0.86$; $P < 0.0001$), K ($r = 0.87$; $P < 0.0001$) and Ca ($r = 0.82$; $P < 0.0001$) contents, fatty acids C16:0 ($r = 0.81$; $P < 0.0001$), C18:0 ($r = 0.74$; $P < 0.0001$), C18:1 ($r = 0.72$; $P < 0.0001$), C18:2 ($r = 0.72$; $P < 0.0001$) and C18:3 ($r = 0.61$; $P < 0.0001$) amounts, polyphenol ($r = 0.86$; $P < 0.0001$) and flavonoid ($r = 0.82$; $P < 0.0001$) contents. However, Na ($r = -0.67$; $P < 0.0001$) content was closely and negatively related to PC1 (Table S6). The results of PCA revealed that inoculated plants cultivated under well-watered condition presented the highest grain yield parameters such as GY and TKW, mineral Fe, Zn, Cu, K and Ca contents, fatty acids and polyphenol amounts (Fig. 3a and b). However, non-inoculated plants under moderate drought and severe drought showed opposite trends. The water stress level (moderate drought) was accompanied with an increase in Na content in grain as compared to the well-watered condition. Non-inoculated plants under well-watered condition and inoculated ones submitted to moderate drought and severe drought levels presented intermediate values (Fig. 3a and b).

4. Discussion

This study was carried out to evaluate the potential benefit of arbuscular mycorrhizal fungal (AMF) inoculation under water stress conditions on grain yield and quality of hulless barley for a sustainable cultivation of this crop under semi-arid and arid conditions. It is important to remind that drought is the most devastating stress that reduces crop productivity more than any other stress type. In the current study, our results showed a decrease in root mycorrhizal colonization, especially in non-inoculated plants (spontaneously colonized by the indigenous community of AMF present in the soil) and those inoculated with the commercial inoculum under stress conditions. These findings are in accordance with the results of an experiment conducted by Omirou et al. (2013) on inoculated watermelon under water stress. They found that gene copy number of AMF in roots decreased in non-inoculated plants, whereas it increased with mycorrhizal inoculation under water stress. Our results could be explained by a better ability of autochthonous AMF species (brought in the inoculum) to colonize barley roots under water stress compared to the indigenous (initially present in the soil) and commercial species. Marulanda et al. (2003) studied the effect of six AMF species on the colonization of lettuce (*Lactuca sativa*) under drought stress and they recorded that *Glomus coronatum*, *G. intraradices*, *G. claroideum* and *G. mosseae* induced the higher colonization rates compared to *G. constrictum* and *G. geosporum*. They explained their findings by the amount of external mycelium produced by each AMF, allowing the exploration of a higher soil volume and so a better contact with plant roots. The impact of soil moisture on spore germination is influenced by AMF species or genera and this could be explained by the fact that some fungal species are able to adapt to water deficit conditions more than others (Nasim, 2010). Furthermore, soil conditions like soil moisture and the cross talk with the host plants can have different effects on spore germination, hyphal growth and hyphal branching (Pérez et al., 2016).

Concerning barley grain yield, an enhancement of this parameter and the 1000-grain weight was recorded for AMF inoculated plants (with autochthonous inoculum as well with commercial one) compared to non-inoculated under well-watered conditions. It's well known that mycorrhizal inoculation enhanced plant growth which could explain the highest grain yields of mycorrhized plants. Likewise, our results demonstrated that mycorrhizal inoculation improves grain yield of hulless barley under water stress which is in accordance with the findings of Kamali and Mehraban (2020) who recorded an increase of grain yield of sorghum (*Sorghum bicolor* L.) when it was co-inoculated with *G. mosseae* and a mixture of *Azospirillum* and *Azotobacter* bacteria. They explained their results by the ability of microbial biofertilizers (AMF and bacteria) to alleviate the negative effects of water stress on plants through increasing photosynthetic activity, soluble proteins contents and osmotic regulation and decreasing electrolyte leakage. In our case, the increase in barley grain minerals: potassium (K), copper (Cu), iron (Fe), zinc (Zn) and calcium (Ca) contents in inoculated plants with the autochthonous mycorrhizal biofertilizer or the commercial one could explain the observed improvement in grain yield. Johansson et al. (2004) cited that AMF enhances plant production under drought conditions through the absorption of non-mobile nutrients such as phosphorus P, Zn and Cu. In fact, a possible translocation of minerals between the different parts of the plants is possible. Zhang et al. (2017) demonstrated a translocation of nitrogen (N) from the aboveground vegetative parts to seeds in rice inoculated with AMF. The increase in K grain content found in the current study could be explained by the key role of this element in plant water stress and its cationic solute nature, which is responsible for stomatal movement (Augé et al., 2007). Concerning micronutrients concentrations in cereal grains, many studies like those of Coccina et al. (2019) on barley (*Hordeum vulgare* L.) and bread wheat (*Triticum aestivum* L.), described an increase of Zn and Fe amounts in the grains. Similarly, in a study conducted by Colla et al. (2015), a positive effect of wheat (*Triticum durum* Desf.) inoculation with *G. intraradices* and *G. mosseae* on the mineral composition (P, K, Fe, Zn) of the grains and the grain yield was observed. Furthermore, they found that mycorrhizal plants were able to maintain a higher maximum quantum use efficiency of photosystem II (PSII) during tillering and anthesis stages. This could be due to the enhancement of micronutrients uptake, which play a fundamental role in plant growth and development (Chaudhary et al., 2020). Other components are also essential for human and animal metabolism, which are fatty acids (FAs). The fatty acids analysis of hulless barley grains showed the same composition found by Golijan et al. (2019) in maize (*Zea mays* L.), spelt (*Triticum spelta* L.) and buckwheat (*Fagopyrum esculentum* Moench) grains. Palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids were the most abundant fatty acids. In our study, the negative effect of water stress on FAs composition, especially C16:0 and C18:2 contents, was more pronounced than mycorrhizal inoculation, which only enhanced C18:0 and C18:1 contents under well-watered condition. In fact, drought could decrease saturated fatty acids and linoleic acid in canola (*Brassica napus* L.) grains. Moghadam et al. (2011) explained these results by the shorter growing season due to water stress, which reduced plant oil yield and composition. It's well-known that drought had harmful effect especially on

photosynthetic parameters, such as net photosynthetic rate, intercellular carbon concentration, stomatal conductance and transpiration leading to a decrease in grain yield and quality (Zhao et al., 2020). The Principal Component Analysis (PCA) results confirmed the positive effect of AMF inoculation on hulless barley grain quality, especially total polyphenol and flavonoid contents presenting an important source of antioxidants for enhancing human health and decreasing disease risk (Deng et al., 2012).

5. Conclusion

The current study revealed that, in a semi-controlled conditions, AMF can alleviate the negative effect of water stress which, in turn, led to a better grain yield and quality (higher nutrient contents such as K, Cu, Fe, Zn and Ca, lower Na content) and increased total polyphenol and flavonoid amounts of hulless barley. Inoculated plants especially with autochthonous AMF species have positive impact on all the response variables as compared to non-inoculated control plants. Using native inoculum which contains several AMF species can be one of the best approaches to enhance plant performance. Further research, should evaluate the potential of native inoculum under field conditions. The obtained results may be relevant under future climate change scenarios, especially in semi-arid areas where the yield of crops is mainly threatened by drought. On the other hand, this study suggested that hulless barley had a huge potential that needs to be explored further as an important component of healthy food and feed in industrial applications.

CRediT authorship contribution statement

Maroua Jerbi: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing - Original Draft, Writing - Review & Editing. Sonia Labidi: Conceptualization, Methodology, Visualization, Supervision, Writing - Original Draft, Writing - Review & Editing. Frédéric Laruelle: Analysis. Benoit Tisserant: Analysis. Faysal Ben Jeddi: Conceptualization, Methodology, Resources, Supervision, Writing - Review & Editing. Anissa Lounès-Hadj Sahraoui: Conceptualization, Methodology, Resources, Supervision, Writing - Review & Editing.

Declaration of competing interest

We have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2022.103436.

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