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

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16

17 **Abstract**

18 Drought is one of the most important factors worldwide, which limits the crop
19 production, especially in semi-arid areas. The use of beneficial microorganisms such as
20 arbuscular mycorrhizal fungi (AMF) may represent an eco-friendly and biological technique
21 to increase crops yields and ensure food security. The purpose of this paper was to evaluate
22 the effect of arbuscular mycorrhizal fungi (AMF) on a promised variety of hulless barley
23 (*Hordeum vulgare* ssp. *nudum* L.) under three levels of water stress (well-watered, moderate
24 drought and severe drought). Hulless barley plants were inoculated, or not, with
25 autochthonous inoculum (AI) containing five native AMF species (*Pacispora franciscana*,
26 *Funneliformis mosseae*, *Funneliformis geosporum*, *Rhizophagus irregularis* and *Glomus*
27 *tenebrosum*), or commercial inoculum (CI) containing *Glomus* sp. strains. Under water stress,
28 AMF inoculation especially, with autochthonous consortium has higher mycorrhizal root

29 colonization of hulless barley by 7-fold and 23-fold in comparison to the non-inoculated
30 controls, under moderate drought and severe drought conditions, respectively. Water stress
31 decreased grain yield and thousand-kernel weight of hulless barley. The reduction was less
32 pronounced in AMF inoculated plants compared to the non-inoculated control ones. Plants
33 with higher mycorrhizal colonization showed higher grain yield and thousand-kernel weight
34 by approximately 90% and 68.2% with AI, and by 106% and 83% with CI, respectively than
35 control plants with lower AMF colonization, especially under severe drought. At the same
36 time, the amount of K, Cu, Fe, Zn and Ca in hulless barley grain increased significantly in
37 AMF inoculated plants with AI as well with CI. Compared with the control plants, using
38 autochthonous AMF species led to significantly decreased Na content in grain. Fatty acids in
39 hulless barley grain decreased with the severity of water stress. Only under well-watered
40 condition, AMF inoculation enhance C18:0 and C18:1 contents as compared to control plants.
41 Moreover, total polyphenol and flavonoid increased due to AMF inoculation under both
42 medium drought and severe drought conditions. The results obtained herein indicated that
43 inoculation with AMF can enhance the water tolerance resulting in higher hulless barley grain
44 yield and quality. Therefore, using AMF as biofertilizers may be important in regions
45 suffering from lack of water in order to ensure sustainable agricultural systems.

46 **Keywords:** Arbuscular mycorrhizal fungus, Drought, Hulless barley, Grain yield.

47

48

49 **1. Introduction**

50 Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world,
51 ranked in the fourth position for cereal grain production. Barley species may have hulled or
52 hulless (naked) grains without husks. Most cultivated barleys today are of the hulled form.
53 They are mainly used for animal feed (65%), brewing malts (30%) and human consumption

54 (3%) (Aldughpassi et al., 2016). However, hulless barley is mainly used as a human food
55 because of ease in processing and edibility. Hulless barley (*Hordeum vulgare* ssp. *nudum* L.)
56 has high nutritive quality as a food or feed, and has been receiving increasing attention in
57 recent years. As compared to other cereals, hulless barley has higher soluble fibre β -glucan
58 and protein contents, in particular essential amino acid, lysine, also fatty acids contents and
59 polyphenols which act as antioxidants. It is considered as a healthy food since it has the
60 ability to reduce cholesterol and blood sugar levels (Guo et al., 2020).

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

70 In this context, the use of arbuscular mycorrhizal fungi (AMF) as microbial fertilizer
71 has been described as a potential tool for sustainable agriculture and food security (Thirkell et
72 al., 2017). AMF establish symbiotic association with the roots of over than 80% of terrestrial
73 species. Similar to other cereal crops, barley forms association with these ubiquitous fungi,
74 which can lead to improved plant acquisition of soil nutrients and water (Thirkell et al., 2017).
75 There is a growing recognition that inoculation with AMF can alleviate the negative effects of
76 abiotic stresses such as drought (Bernardo et al., 2019; Kamali and Mehraban, 2020).
77 However, the effect of AMF inoculation on grain yield and quality of hulless barley during
78 drought stress has not, to our knowledge, been assessed yet.

79 The main objective of the present study is to evaluate, under water stress, the benefit
80 of the mycorrhizal inoculation on the grain yield and quality of hulless barley to promote its
81 production in semi-arid regions of Tunisia due to its potential benefits in the food and feed
82 industries.

83

84 **2. Materials and methods**

85 *2.1. Experimental design*

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

94 *2.2. Plant materials and growth conditions*

95 Agricultural soil (from a depth of 0-60 cm) was collected from the experiment station
96 of the National Agronomic Institute of Tunisia (10° 11' N, 36° 55' E). Soil physico-chemical
97 characteristics are: 26.1% clay, 55% silt, 18.9% sand, 0.72% organic matter, pH (8.14), EC
98 (0.12 dS/m), N (1g/kg), P (0.015 g/kg), K (0.15 g/kg). Each plastic pot 20 × 26 cm (diameter
99 × height) was filled with 5 kg of 2 mm sieved soil. The autochthonous mycorrhizal inoculum
100 (AI) as described by Jerbi et al. (2020) composed of five native AMF species (*Pacispora*
101 *franciscana*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Rhizophagus irregularis* and
102 *Glomus tenebrosum*) and the commercial AMF inoculum (CI) (Symbivit, InoculumPlus,
103 France) containing six *Glomus* sp. strains, were used for AMF inoculation. Mycorrhizal

104 inoculum were applied at an average of 500 propagules per pot and were placed below the
105 seeds at the time of sowing. The non-inoculated controls received the same amount of
106 autoclaved inoculum. Seeds of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) population-
107 variety named “Prophet barley” or “Moknine barley”, were surface sterilized in 1% sodium
108 hypochlorite and sown in the inoculated and non-inoculated pots. Hulless barley was sown on
109 January 2018 and harvested on June 2018. Plants were grown under shelter and regularly
110 irrigated to 70% of field capacity until the tillering and grain filling stages, when the plants
111 were exposed to water stress levels. The water stress treatment was conducted by stopping
112 water supply during five days for medium drought stress (MD) and maintained for another
113 five days for severe drought stress (SD). For control condition, well-watered treatments
114 (WW), soil water content was maintained at 70% of field capacity. During the water stress
115 period, all the pots were irrigated to maintain soil water content at 70% for the control, 50%
116 for the MD and 20% for the SD.

117 *2.3. AMF root colonization*

118 After plant harvest (139 days after planting), fresh roots were sampled for the
119 determination of AMF root colonization rate. From each treatment, fine roots were cleared in
120 5% KOH, washed with distilled water and acidified in 2% HCl. The roots were stained with
121 0.05% trypan blue at 90 °C for 2 h as described by Phillips and Hayman (1970). From each
122 sample (four plants), 45 root segments of 1 cm length were examined under microscope in
123 order to count mycorrhizal structures (arbuscules, vesicles and hyphae) using the method of
124 (McGonigle et al., 1990). In total, 405 observations (135 root fragments with 3 intersections
125 per root fragment) per treatment were analyzed.

126

127 *2.4. Grain yield*

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

131 *2.5. Grain quality*

132 *2.5.1. Mineral nutrient content*

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

139 *2.5.2. Fatty acid contents*

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

147 *2.5.3. Polyphenol and flavonoid contents*

148 The grounded grain powder (1 g) was immersed in ethanol (70°) for 24 h with
149 frequent agitation. After centrifugation, the extracts were stored at 4 °C until further use. The
150 total phenolic content was determined for individual extracts according to the method of
151 Folin-Ciocalteu (Singleton et al., 1999). Briefly, 1 mL of extract was mixed with 4 mL of

152 10% (w/v) Folin-Ciocalteu reagent. After 5 min, 5 mL of Na₂CO₃ (7.5%) was subsequently
153 added to the mixture and incubated at obscurity for 90 min with intermittent agitation
154 followed by the measurement of absorbance using a UV Spectrophotometer (OPTIZEN
155 3220UV, Daejeon, South Korea) at 760 nm against a blank (without extract). The total
156 polyphenol contents of extracts were expressed as mg/g of gallic acid equivalents in
157 milligrams per gram (mg GAE/g) of dry extract. The flavonoid content of each extract was
158 performed using the method of Dowd (Lamien-Meda et al., 2008). Briefly, 5 mL of extract
159 solution were mixed with 5 mL of 10% (w/v) AlCl₃ solution in methanol and incubated at
160 obscurity for 30 min. The absorbance was measured at 415 nm against the blank. Flavonoid
161 contents in extract were expressed as mg/g of rutin equivalents in milligrams per gram (mg
162 RE/g) of dry extract.

163 *2.6. Statistical analyses*

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

171 **3. Results**

172 *3.1. Mycorrhizal root colonization*

173 The statistical analysis demonstrated a significant interaction between AMF treatments
174 and water stress levels on mycorrhizal root colonization of barley ($P < 0.001$; Table S1). The
175 mycorrhizal rates of AMF inoculated plants were significantly higher compared to those

176 observed in non-inoculated ones (Fig. 1). Water stress caused a decrease in root colonization
177 of non-inoculated plants by more than 28% under the moderate drought compared to the well-
178 watered controls (Fig. 1). For plants inoculated with the autochthonous mycorrhizal
179 consortium (AI), total colonization increased with the increasing of water stress level, while
180 those inoculated with the commercial inoculum (CI) and the non-inoculated (control AI)
181 significantly decreased. Indeed, the total mycorrhizal colonization of plants inoculated with
182 (CI) was significantly decreased by 32.8% under severe drought and more moderately by
183 9.8% under moderate drought as compared to well-watered plants (Fig. 1). As for plants
184 inoculated with (AI), the total colonization rates in barley roots were higher as well under
185 moderate drought (53.3%) and severe drought (69.6%). A similar pattern was observed for
186 arbuscular root colonization (Table S2). Vesicular root colonization was significantly
187 influenced by AMF treatment ($P < 0.001$; Table S1), by water stress level ($P < 0.01$; Table
188 S1) and by the interaction between the two factors ($P < 0.001$; Table S1). Vesicles were not
189 detected in non-inoculated (control AI and control CI) root plants at the different water stress
190 treatments. The highest vesicular colonization was observed in plants inoculated with CI
191 (28.8%) under severe drought (Table S2).

192 *3.2. Grain yields*

193 Grain yield and thousand-kernel weight (1000-grain weight) were significantly
194 influenced by both AMF treatment and water stress level ($P < 0.001$; Table S3). Water stress
195 caused a significant decrease of grain yield and thousand-kernel weight of barley (Table 1).
196 The highest grain yield was observed in plants inoculated with AI under well-watered
197 condition and it was 16 and 91% higher than CI and non-inoculated plants (control AI),
198 respectively. Compared to non-inoculated plants, grain yields of those inoculated with AI and
199 CI were 1.6 and 1.3-fold higher under moderate drought, 1.9 and 2-fold higher under severe
200 drought, respectively (Table 1). AMF inoculation increased thousand-kernel weight under

201 water stress conditions by approximately 1.4-fold under moderate drought and 1.7-fold under
202 severe drought as compared to non-inoculated plants (Table 1). The most pronounced
203 increase in thousand-kernel weight was observed with AI and CI under well-watered
204 condition and it was by approximately 95 and 74%, respectively, in comparison to their
205 respective controls (Table 1).

206 3.3. Grain quality

207 3.3.1. Mineral nutrient contents

208 A significant effect of both AMF treatment and water stress level, separately, on
209 potassium (K), copper (Cu), iron (Fe) and zinc (Zn) contents in barley grain was observed (P
210 < 0.001 ; Table S3). However, no significant effect of the interaction between AMF treatment
211 and water stress was noticed. When compared to plants under well-watered conditions, K, Cu,
212 Fe and Zn contents in barley grains were significantly higher by 9, 27, 46 and 18%,
213 respectively in plants under moderate drought level, and by 19, 49, 69 and 41%, respectively,
214 in plants under severe drought level (Table 1). AMF inoculation increased significantly K,
215 Cu, Fe and Zn contents, as compared to those non-inoculated (Fig. 2, Table S5). Indeed, K,
216 Cu, Fe and Zn concentrations in barley grains were significantly higher by 33, 31, 15 and
217 28%, respectively in inoculated plants with AI and by 30, 63, 43 and 29% in those inoculated
218 with CI, as compared to their respective controls (Table 1). Grain sodium (Na) and calcium
219 (Ca) contents were significantly affected by both AMF inoculation and water stress level as
220 well as their interaction ($P < 0.001$; Table S3). Concerning Na concentration, no significant
221 differences were noticed between AMF inoculated plants and non-inoculated ones, under
222 well-watered and moderate drought conditions (Table 1). However, the plants inoculated with
223 AI had a lower Na concentration by 16% in comparison to their respective control under
224 severe drought (Fig. 2, Table S5). The highest calcium concentration in barley grains was
225 obtained in inoculated plants with AI under well-watered conditions (1.02 mg/g DW, Table 1)

226 and it was about four-fold higher than non-inoculated plants. Under moderate drought and
227 severe drought conditions, a significant increase of Ca concentrations by 3 and 2-fold,
228 respectively, was noticed in the grains of plants inoculated with AI as compared to the non-
229 inoculated ones. Similarly, Ca concentrations were 2-fold higher in inoculated plants with CI,
230 under both moderate drought and severe drought conditions, in comparison to their respective
231 controls (Table 1, Fig. 2, Table S5).

232 *3.3.2. Fatty acid contents*

233 The results showed that linoleic acid (C18:2) contents in barley grains were
234 significantly affected by AMF treatment and water stress level separately whereas the
235 interactions between these two factors were not significant. Concerning the palmitic acid
236 (C16:0) in barley grains, only water stress level had a significant effect (Table S4). The
237 linoleic acid contents were 3 and 2-fold higher in plants inoculated with AI and with CI,
238 respectively, as compared to their respective controls (Table 2). In comparison to well-
239 watered plants, the palmitic acid content in the grains significantly decreased by 2 and 3-fold
240 in plants subjected to moderate drought and severe drought conditions, respectively (Table 2,
241 Fig. 2). As well, linoleic acid contents of grains were 2-fold higher under well-watered
242 condition than those under severe drought condition. The interaction between AMF treatment
243 and water stress level influenced significantly stearic acid (C18:0), oleic acid (C18:1) and
244 alpha linolenic acid (C18:3) grain contents (Table S4). Grains of inoculated plants with AI
245 under well-watered conditions had higher stearic and oleic acid levels (12 and 4-fold,
246 respectively) than those non-inoculated, while under moderate drought and severe drought
247 conditions, no significant differences were noticed between AMF inoculated and non-
248 inoculated plants (Table 2, Fig. 2, Table S5).

249 *3.3.3. Polyphenol and flavonoid contents*

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

261

262 *3.4. Principal components analysis*

263 Principal component analysis (PCA) was conducted to examine the effect of the water
264 stress and AMF treatment on grain quality of barley. The first two components accounted for
265 75.9% of the total variance (Fig. 3, Fig. S2). The first axis PC1 (Dim 1) explained most of the
266 variation (65.5%) and mainly separated treatments according to water stress level and AMF
267 treatment. The second axis PC2 (Dim 2) only explained 10.4% of the total variance (Fig. 3a).
268 The PC1 showed a strong and positive correlation with the grain yield GY ($r = 0.93$; $P <$
269 0.0001), thousand kernel weight TKW ($r = 0.92$; $P < 0.0001$), mineral Fe ($r = 0.80$; $P <$
270 0.0001), Zn ($r = 0.85$; $P < 0.0001$), Cu ($r = 0.86$; $P < 0.0001$), K ($r = 0.87$; $P < 0.0001$) and
271 Ca ($r = 0.82$; $P < 0.0001$) contents, fatty acids C16:0 ($r = 0.81$; $P < 0.0001$), C18:0 ($r = 0.74$;
272 $P < 0.0001$), C18:1 ($r = 0.72$; $P < 0.0001$), C18:2 ($r = 0.72$; $P < 0.0001$) and C18:3 ($r = 0.61$;
273 $P < 0.0001$) amounts, polyphenol ($r = 0.86$; $P < 0.0001$) and flavonoid ($r = 0.82$; $P < 0.0001$)
274 contents. However, the nutrient Na ($r = -0.67$; $P < 0.0001$) content was closely and negatively

275 related to PC1 (Table S6). The results of PCA revealed that inoculated plants cultivated under
276 well-watered condition presented the highest grain yield parameters such as GY and TKW,
277 mineral Fe, Zn, Cu, K and Ca contents, fatty acids and polyphenol amounts (Fig. 3a and b).
278 However, non-inoculated plants under moderate drought and severe drought showed opposite
279 trends. The water stress level (moderate drought) was accompanied with an increase in Na
280 content in grain as compared to the well-watered condition. Non-inoculated plants under well-
281 watered condition and inoculated ones submitted to moderate drought and severe drought
282 levels presented intermediate values (Fig. 3a and b).

283 **4. Discussion**

284 This study was carried out to evaluate the potential benefit of arbuscular mycorrhizal
285 fungal (AMF) inoculation under water stress conditions on grain yield and quality of hulless
286 barley for a sustainable cultivation of this crop under semi-arid and arid conditions. It is
287 important to remind that drought is the most devastating stress that reduces crop productivity
288 more than any other stress type. In the current study, our results showed a decrease in root
289 mycorrhizal colonization, especially in non-inoculated plants (spontaneously colonized by the
290 indigenous community of AMF present in the soil) and those inoculated with the commercial
291 inoculum under stress conditions. These findings are in accordance with the results of an
292 experiment conducted by Omirou et al. (2013) on inoculated watermelon under water stress.
293 They found that gene copy number of AMF in roots decreased in non-inoculated plants,
294 where as it increased with mycorrhizal inoculation under water stress. Our results could be
295 explained by a better ability of autochthonous AMF species (brought in the inoculum) to
296 colonize barley roots under water stress compared to the indigenous (initially present in the
297 soil) and commercial species. Marulanda et al. (2003) studied the effect of six AMF species
298 on the colonization of lettuce (*Lactuca sativa*) under drought stress and they recorded that
299 *Glomus coronatum*, *G. intraradices*, *G. claroideum* and *G. mosseae* induced the higher

300 colonization rates compared to *G. constrictum* and *G. geosporum*. They explained their
301 findings by the amount of external mycelium produced by each AMF, allowing the
302 exploration of a higher soil volume and so a better contact with plant roots. The impact of soil
303 moisture on spore germination is influenced by AMF species or genera and this could be
304 explained by the fact that some fungal species are able to adapt to water deficit conditions
305 more than others (Nasim, 2010). Furthermore, soil conditions like soil moisture and the cross
306 talk with the host plants can have different effects on spore germination, hyphal growth and
307 hyphal branching (Pérez et al., 2016).

308 Concerning barley grain yield, an enhancement of this parameter and the 1000-grain
309 weight was recorded for AMF inoculated plants (with autochthonous inoculum as well with
310 commercial one) compared to non-inoculated under well-watered conditions.
311 It's well known that mycorrhizal inoculation enhanced plant growth which could explain the
312 highest grain yields of mycorrhized plants. Likewise, our results demonstrated that
313 mycorrhizal inoculation improves grain yield of hulless barley under water stress which is in
314 accordance with the findings of Kamali and Mehraban (2020) who recorded an increase of
315 grain yield of sorghum (*Sorghum bicolor* L.) when it was co-inoculated with *G. mosseae* and
316 a mixture of *Azospirillum* and *Azotobacter bacteria*. They explained their results by the ability
317 of microbial biofertilizers (AMF and bacteria) to alleviate the negative effects of water stress
318 on plants through increasing photosynthetic activity, soluble proteins contents and osmotic
319 regulation and decreasing electrolyte leakage. In our case, the increase in barley grain
320 minerals potassium (K), copper (Cu), iron (Fe), zinc (Zn) and calcium (Ca) contents in
321 inoculated plants with the autochthonous mycorrhizal biofertilizer or the commercial one
322 could explain the observed improvement in grain yield. Johansson et al. (2004) cited that
323 AMF enhances plant production under drought conditions through the absorption of non-
324 mobile nutrients such as phosphorus (P), Zn and Cu. In fact, a possible translocation of

325 minerals between the different parts of the plants is possible. Zhang et al. (2017)
326 demonstrated a translocation of nitrogen (N) from the aboveground vegetative parts to seeds
327 in rice inoculated with AMF. The increase in K grain content found in the current study could
328 be explained by the key role of this element in plant water stress and its cationic solute nature,
329 which is responsible for stomatal movement (Augé et al., 2007). Concerning micronutrients
330 concentrations in cereal grains, many studies like those of Coccina et al. (2019) on barley
331 (*Hordeum vulgare* L.) and bread wheat (*Triticum aestivum* L.), described an increase of Zn
332 and Fe amounts in the grains. Similarly, in a study conducted by Colla et al. (2015), a positive
333 effect of wheat (*Triticum durum* Desf.) inoculation with *G. intraradices* and *G. mosseae* on
334 the mineral composition (P, K, Fe, Zn) of the grains and the grain yield was observed.
335 Furthermore, they found that mycorrhizal plants were able to maintain a higher maximum
336 quantum use efficiency of photosystem II (PSII) during tillering and anthesis stages. This
337 could be due to the enhancement of micronutrients uptake, which play a fundamental role in
338 plant growth and development (Chaudhary et al., 2020). Other components are also essential
339 for human and animal metabolism, which are fatty acids (FAs). The fatty acids analysis of
340 hullless barley grains showed the same composition found by Golijan et al. (2019) in maize
341 (*Zea mays* L.), spelt (*Triticum spelta* L.) and buckwheat (*Fagopyrum esculentum* Moench)
342 grains. Palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2),
343 and linolenic (C18:3) acids were the most abundant fatty acids. In our study, the negative
344 effect of water stress on FAs composition, especially C16:0 and C18:2 contents, was more
345 pronounced than mycorrhizal inoculation, which only enhanced C18:0 and C18:1 contents
346 under well-watered condition. In fact, drought could decrease saturated fatty acids and
347 linoleic acid in canola (*Brassica napus* L.) grains. Moghadam et al. (2011) explained these
348 results by the shorter growing season due to water stress, which reduced plant oil yield and
349 composition. It's well-known that drought had harmful effect especially on photosynthetic

350 parameters, such as net photosynthetic rate, intercellular carbon concentration, stomatal
351 conductance and transpiration leading to a decrease in grain yield and quality (Zhao et al.,
352 2020). The Principal Component Analysis (PCA) results confirmed the positive effect of
353 AMF inoculation on hulless barley grain quality, especially total polyphenol and flavonoid
354 contents presenting an important source of antioxidants for enhancing human health and
355 decreasing disease risk (Deng et al., 2012).

356 **5. Conclusion**

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

369 **Acknowledgment**

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510

511 **Figures**

512 **Figure 1.** Effect of arbuscular mycorrhizal fungal (AMF) treatment on mycorrhizal
513 colonization rates of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) roots under water stress
514 levels. WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous
515 mycorrhizal inoculum; CI, commercial inoculum; control AI and control CI, two controls

516 without inoculation. Bars indicate standard error of the mean ($n = 3$). Treatments with the
517 same letter are not significantly different according to LSD test.

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

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

537 **Tables**

538 **Table 1.** Effects of arbuscular mycorrhizal fungal (AMF) treatment and water stress level (WS) as well as their interactions on grain yields and
 539 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

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

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

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

548 WW, well-watered; MD, medium drought; SD, severe drought; AI, autochthonous mycorrhizal inoculum; CI, commercial inoculum; control AI
549 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
550 linolenic acid. Different letters in the same column indicate significant differences between water stress levels (WS), mycorrhizal inoculation
551 (AMF) treatments, and the interaction WS \times AMF. Data are represented as mean \pm SD of three replicates per treatment. Treatments with the
552 same letter are not significantly different according to the LSD test.
553

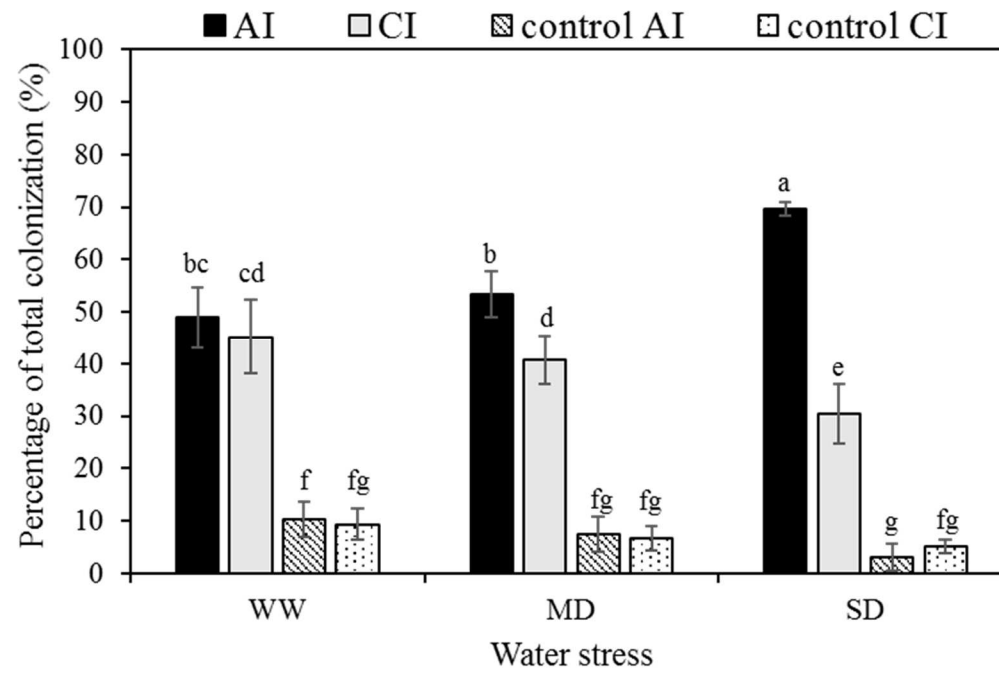


Figure 1.

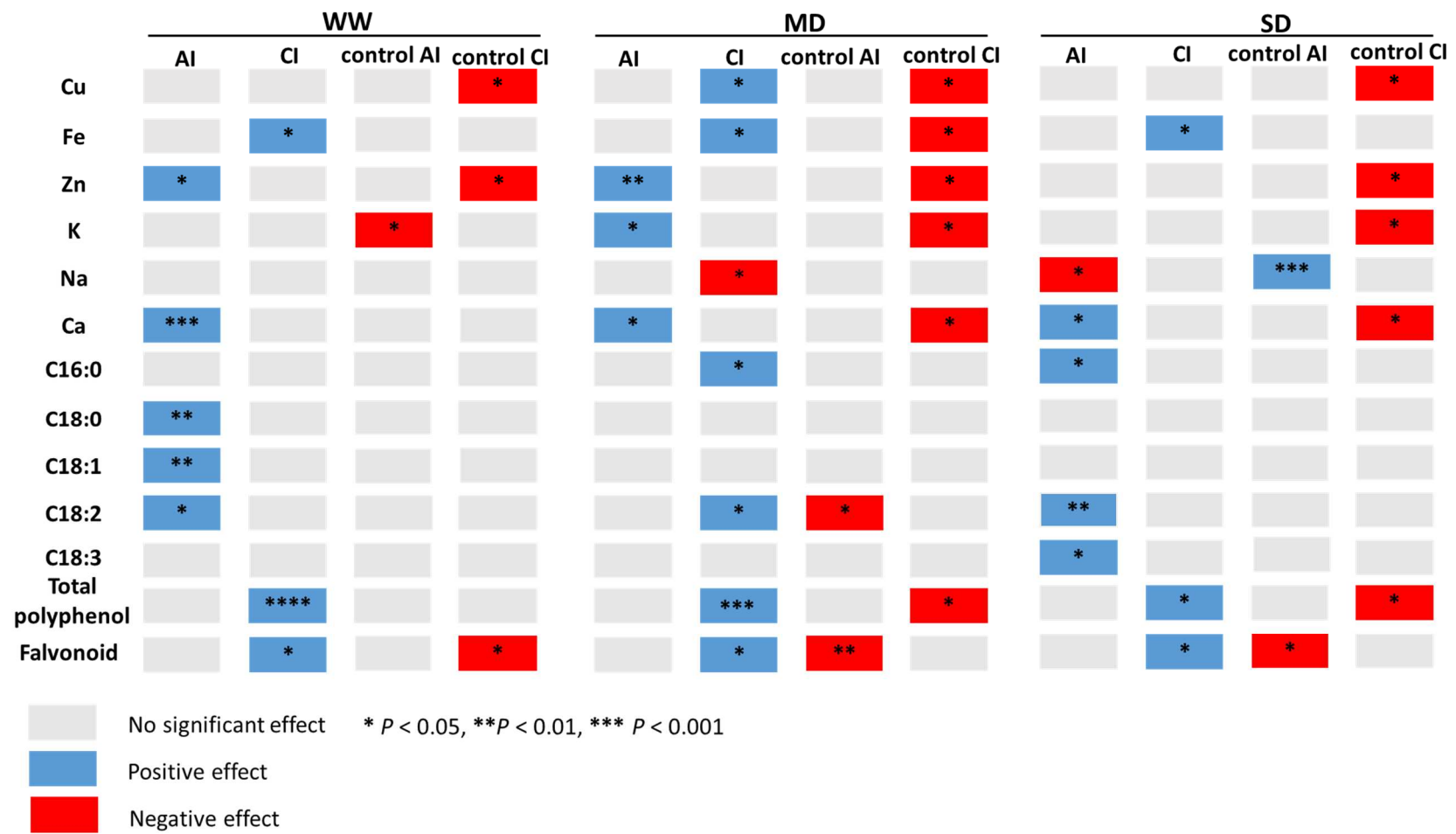


Figure 2.

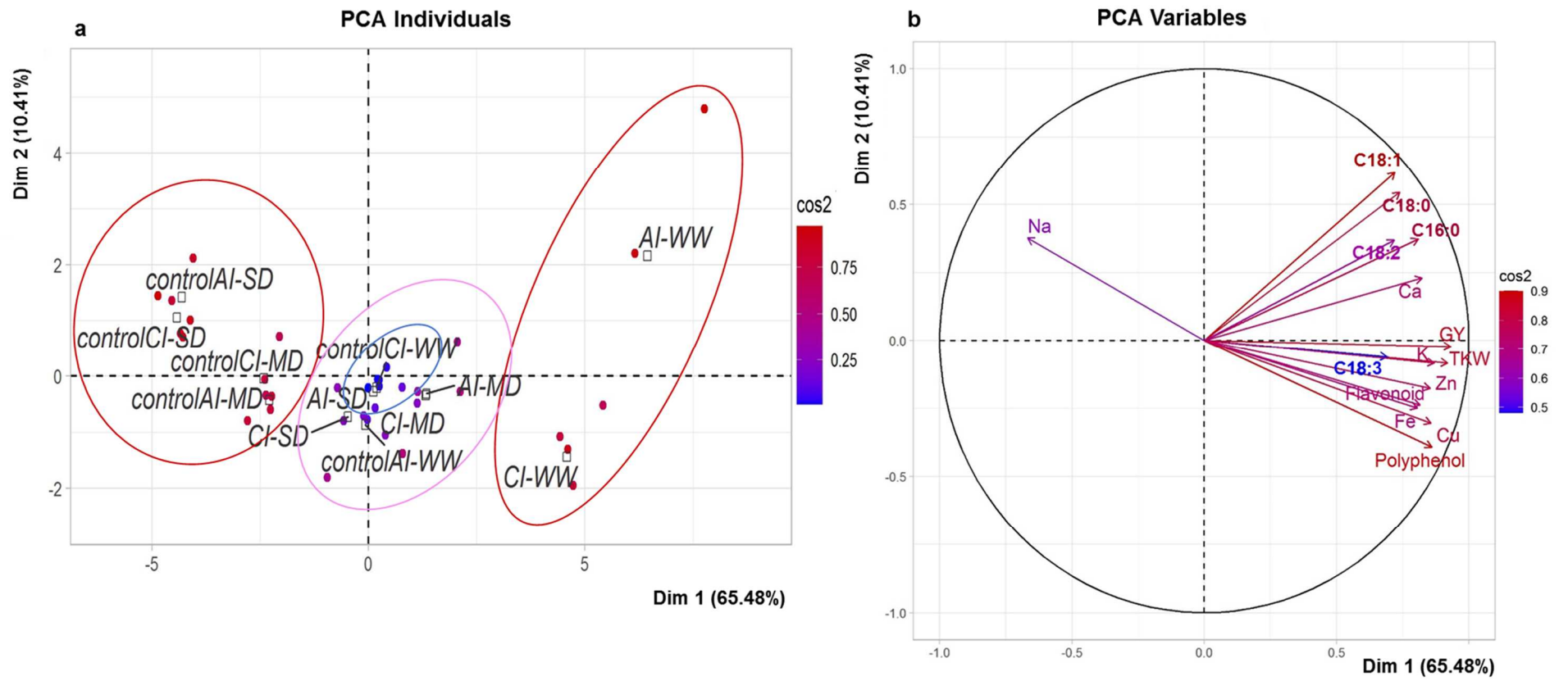


Figure 3.

