1 **TO WHAT EXTENT ARBUSCULAR MYCORRHIZA CAN PROTECT CHICORY**

2 **(*Cichorium intybus* L.) AGAINST DROUGHT STRESS**

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ABSTRACT

Water deficiency is one of the most significant limiting factors resulting in severe crop yield reduction. This study hypothesizes that the inoculation of arbuscular mycorrhiza (AM) minimize the detrimental effects of drought in chicory (*Cichorium intybus* L.). The objective was to evaluate how AM inoculation affects agro-biochemical traits of chicory under different irrigation rates. Field experiments were conducted in 2017 and 2018 in north of Iran and designed as a factorial combination of three irrigation rates [40, 65 and 90% of maximum allowable depletion of available soil water (IR^1, IR^2 and IR^3, respectively)], two AM inoculations [inoculated and not inoculated (AM^+ and AM^-, respectively)] and two chicory ecotypes [Sefid Isfahan and Siyah Shiraz (E^1 and E^2, respectively)]. Crop growth, pigments and minerals content, carbon exchange rate, transpiration rate and stomatal conductance were evaluated. The data showed that the mycorrhizal colonization increased chicory growth performance by 12.4%, 16.1% and 21.0%, under I^1, I^2, and I^3 water regime, respectively. The mineral content and photosynthesis parameters decreased as irrigation water decreased, irrespective of whether plants were inoculated or not. Similarly, AM^+ plants had higher inulin percentage and the mean inulin degree of polymerisation than AM^- plant under all of irrigation levels. Furthermore, inoculated chicory plants under drought stress showed an enhanced activity of the antioxidant system, such as superoxide dismutase, peroxidase, ascorbic acid and glutathione, while the accumulation of hydrogen peroxide and the oxidative damage were reduced. The two ecotypes tended to respond similarly to irrigation and AM treatments for all growth and quality parameters measured in the experiment, even if ecotype Siyah Shiraz performed better than ecotype Sefid Isfahan. The improved plant performance and inulin content in the inoculated plants demonstrate that AM has the potential to minimize the detrimental effects of drought stress on chicory under semi-arid conditions.

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40 **KEYWORDS***:* Arbuscular mycorrhiza; *Cichorium intybus* L.; Growth parameters; Antioxidant

41 activity; Inulin; Irrigation water.

42

1.   INTRODUCTION

The productivity of the agricultural sector is strongly related to water availability, especially in arid and semi-arid regions (FAO, 2011). Scarce water resources are associated to very low annual rainfall between 250 and 500 mm usually concentrated from October untill May (Ghanaatiyan and Sadeghi, 2017). Thus, given that rainfalls vary highly in time and amount in these areas of the world indicating that water represents an important limiting factor (Modarres and de Paulo Rodrigues da Silva, 2007). In warmer climates, effects of water scarcity will become increasingly important (IPCC, 2007). Long periods of water deficit are a serious constraint to the survival of plant and their productivity (Chaves *et al*., 2003). In fact, reduced availability of water imposes changes to plant morphological traits like canopy structure (height and leaf area) and biomass production (Sapeta *et al*., 2013), even if plants have developed several morpho-physiological response for improving the drought tolerance (Blum, 1996). Consequently, alternative strategies should be investigated to ensure sustainable agricultural production for the increasing world population (Atzori *et al*., 2019).

Chicory (*Cichorium intybus* L.), is an *Asteraceae* native of a wide area that included the Mediterranean region and middle Asia (Mosaddegh *et al*., 2012). Historically, Egyptians cultivated chicory for its characteristics such as medicinal plants and vegetable crops (Petropoulos *et al*., 2017; Somasiri *et al*., 2015). Today, chicory is a cosmopolitan crop that has tolerance to a diverse range of climatic and soil conditions and it is widely cultivated for many commercial purposes in Europe, North America and parts of Asia (Wang *et al*., 2011). Several studies have reported the chemical composition and health benefits of chicory. In fact, it can be used as antioxidant, antidiabetic, and for wound healing (Carazzone *et al*., 2013; Spina *et al*., 2008). However, little is known about the interactive effects of water stress and AM fungi on the physiological and morphological characteristics of chicory plants.

66 Arbuscular mycorrhizal (AM) fungi are prevalent in various ecosystems and are well-known for their

67 ability to establish synergistic and complementary effects with roots of the most vascular plant

68 species (Hodge, 2000). This symbiosis is known as a plant strategy that allowed the gradual land

69 colonization by plants due to an enhanced soil nutrient acquisition despite environmental related

70 stress (Brachmann and Parniske, 2006). The proliferation of the plant root cortex by intra-radical

71 mycelium and the spread of extra-radical hyphae of AM around the roots promotes an improved

72 surface area by which additional nutrients for both AM and the host plant can be adsorbed (Ferrol *et*

73 *al.*, 2018). Furthermore, physical and hydraulic soil properties, such as water retention and soil

74 hydraulic conductivity, could be affected by AM. Nowadays, it is widely recognized that the

75 presence of AM could serve as a protection for host plants against drought stress (Asrar and Elhindi,

76 2011; Wu *et al.*, 2006), partly as a result of improved soil water uptake (Duc *et al.*, 2018); improved

77 turgor maintenance associated with osmotic adjustment (Augé, 2001); improved hydraulic

78 conductivity of the root (Borowicz, 2010) both outside the rooted zone (Bitterlich *et al.*, 2018a) and

79 in rooted substrates (Bitterlich *et al.*, 2018b). Moreover, the improved root hydraulic conductivity

80 associate with higher root cell water permeability could determine different root to shoot ABA

81 signaling patterns in drying incidents that affect stomatal regulation or reduced transpiration rate

82 (Duan *et al.*, 1996; Ruíz-Sánchez *et al.*, 2011) that may lead to higher photysynthesis and enhanced

83 carbohydrate metabolism of plants (Boldt et al., 2011), essential in nutrition of an AM inoculated

84 plant (Abdel-Salam *et al.*, 2017). Understanding the differential response of specific mycorrhyzal

85 fungus among the cultivars of host plants is a growing area of research with high economic potential.

86 The mycorrhizal responsiveness of a fungus among field crops has been studied in wheat (Al-Karaki

87 *et al.*, 2004) and medicinal and aromatic plants (Farahani *et al.*, 2013).

This study hypothesizes that the inoculation of AM could be an effective agricultural practice to adopt for the chicory crop to reduce the detrimental impacts of drought and to maintain crop productivity in terms of yield and quality. Therefore, the main objective was to examine how arbuscular mycorrhizal fungus (*Glomus intraradices*) affects growth parameters, agronomical and biochemical traits of field-grown chicory when treated with different irrigation rates. Therefore the main objectives were: (1) to investigate the range effect of arbuscular myccorrhizal and irrigation rates on chicory growth traits and nutrient contents under a semi-arid environment of Iran; (2) to recommend a best combination of arbuscular mycorrhiza and irrigation rate to ensure chicory yield and quality parameters.

2.   MATERIALS AND METHODS

2.1.   *Production of inoculum*

The AM fungal inoculants adopted for chicory roots in this experiment was *Rhizophagus irregularis* (known also as *Glomus intraradices* Schenck & Smith, Campagnac *et al*., 2010), provided by Royan Co. (Karaj, Iran). Pot cultures of *Rhizophagus irregularis* were initiated, in greenhouse conditions, on corn (*Zea mays* L.) within the period January to March, in both 2017 and 2018. The soil used for mycorrhizal inoculum production in the pot cultures was previously collected up to 30 cm of soil depth in the field where the study was conducted. Collected soil was oven dried and sieved via a 2 mm mesh, then added with sand using 2:1 (V:V) proportion. Soil was steam-sterilized and put in pots of 5 l size meant for chicory growth. Just prior to inoculation corn roots were harvested, slice into 1 cm pieces, then mixed with the soil.

2.2.   *Cultural practices and experimental treatments*

Field trials were carried out on chicory plant in 2017 and 2018 at the Seed Research Station of the Agriculture Department, Payame Noor University (lat. 36°53'N, long. 54°24' E) in Gorgan (Golestan province of Iran). The experimental field soil was silty loam with an average based on the characteristics in 0-30 cm top-soil depth: pH of 7.1 (1:2.5 H_2O), salinity of 0.07 dS m^{-1}, organic matter content of 0.20 %, total nitrogen of 0.08 %, available phosphorous of 0.25 mg kg^{-1} and available potassium of 424 mg kg^{-1}.

A randomized complete block design made up of a factorial combination of AM, chicory ecotype and irrigation rate ($2 \times 2 \times 3$, respectively) was adopted. Each field experiment consisted in: (i) two AM treatments [with the addition of AM and without the addition of AM (denominated AM^+ and AM^-, respectively)], (b) two chicory ecotypes [Sefid Isfahan and Siyah Shiraz (denominated E^1 and E^2, respectively)], and (c) three level of irrigation rate [40%, 65% and 90% of total irrigation water (denominated IR^1, IR^2 and IR^3, respectively)] on the basis of a determined level of maximum allowable depletion (MAD) of the available soil water (ASW) threshold. The treatments were placed in four replicates resulting to 48 plots, of 20 m^2 (5 m × 4 m) per plot size.

Field experiments were conducted in sites kept fallow the previous year to minimize native mycorrhizal fungi, to allow decomposition of fragments of the previous crop roots and to allow degradation of propagules. At the beginning of each growing season (last week of March), the experimental area was ploughed at a depth of 30 cm using a moldboard plough, then it prepared by disking. Soil samples collected to a depth of 30 cm were evaluated for specified soil properties and native AM spores. The field was not fumigated because of the observed minute level (1–2 per kg) of the native fungi.

All plots were treated with the recommended fertilizer rates of the area, and these included 45 kg ha^{-1} of P_2O_5 applied as single superphosphate and 50 kg ha^{-1} of K_2O applied as muriate of potash at

seedbed preparation as well as 100 kg ha⁻¹ of N applied in two splits. The first split (60% of total N

rate) was basally added to the soil the day of chicory sowing, and the next split (40% of total N rate)

was supplied 45 days after sowing. To prevent water loss, the plots were bounded by dykes couple

with a wide strip (1.5 m wide) separating adjacent plots. Before sowing of chicory, furrows of a

depth of about 10 cm were opened and AM inoculum was equally placed along the bottom of the

furrows of each plot. The inoculum which was lightly covered with soil was placed below chicory

seedlings in the furrow. The inoculum was mainly composed of pieces of AM-colonized root,

hyphae, and spores were minor component (150 ± 5 spores per kg soil for *Rhizophagus irregularis*)

mixed with soil and applied at the rate of 3 kg per row. The same quantity of soil without AM

inoculums was applied to AM⁻ plots.. Chicory ecotypes were sown on 30 March 2017 and 5 April

2018, respectively, placing the seedlings in rows 0.5 m apart with a density of 10 plants m⁻². In all

plots, weeds were controlled when started to emerge by hand-weeding to avoid interference with the

crop growth.

Chicory crop was irrigated by using drip irrigation tape installed on soil surface near about 5 cm to

plant rows. Irrigation rates were established on the basis of water depletion (expressed ad percentage

of soil moisture) in the rhizosphere and uniformly distributed. Soil moisture was determined by

gravimetric method by taking soil samples, weighing, ovendrying at 105 °C for 48 h, and

reweighing. The ASW was calculated as moisture storage of the root zone at field capacity minus

moisture at permanent wilting point. The effective root zone was assumed to be 0 – 25 cm in the 40

days after sowing and 0 – 50 cm until crop maturity according to Atzori *et al*. (2019). The quantity of

water supplied following an achievement of predefined MAD was determined as described by Panda

et al. (2004):

$$Vd = \frac{MAD(\%)(FC-WP)RzA}{100}$$
(Eq. 1)

158   where Vd was the quantity of irrigated water, Rz was the effective depth of chicory rooting, A was

159   the plot surface, FC and WP were the amount of water stored in the rhizosphere at field capacity and

160   wilting point, correspondingly. The total amount of irrigation water in each IR treatment applied

161   during chicory cultivation are reported in Table 1. After sowing, the same amount of irrigation was

162   applied in all irrigation levels to ensure uniform crop establishment. Drought stress was imposed by

163   rationing irrigation (according to three irrigation rates) starting from 20 days after seedling

164   emergence. The weather data, including temperatures and rainfall, were obtained from a

165   meteorological station located near the experimental field (Table 1).

166

167   *2.3.   Data collection*

168   *2.3.1. Photosynthesis measurement*

169   An infrared gas analyser (Li-6400; LI-COR, Lincoln, NE, USA) was used to measure carbon

170   exchange rate (CER), stomatal conductance (gs) and transpiration rate (E). The leaf gas exchange

171   data were collected from fully expanded leaves (two replicates for each leaf) up to 11.30 h to avoid

172   midday depressive effect on CER at a sunny day before harvest (the third week of July in both years,

173   flowering stage). At the time of measuring, photosynthetic photon flux density was maintained at

174   750 μmol m^{-2} s^{-1} using internal red/ blue LED light source and CO_2 concentration was set at 400

175   μmol mol^{-1} by mixing external air with CO_2 from a source attached to the unit. The leaf temperature

176   was recorded as 25–26 °C with the sample relative humidity higher than 65%. Measurements were

177   recorded when the total coefficient of variation was less than 0.5%.

178

179   *2.3.2. Growth measurements and Physiological-biochemical analysis*

9

180 Leaf area index (LAI) was measured evaluating the area covered by green leaves by utilizing an area

181 meter, Li-cor 3100. Moreover, a line sensor [Technical and Physical Engineering Research Service

182 (TFDL-DLO), Wageningen, Netherlands] was utilized to measure the interception of photosynthetic

183 active radiation (PAR) by chicory canopy. The fraction interception of PAR was also extrapolated

184 from the LAI, according to Beer's law:

185 $F_i = 1 - e^{-kl}$ (Eq. 2)

186

187 Where l was the LAI and k was the extinction factor. The value of k adopted was 0.7 as observed by

188 Meijer and Mathijssen (1992). The F_i was considered constant when LAI values increased to 4.

189 In each experimental year, at the beginning of the flowering stage, the same ten chicory plants which

190 were subject to gas exchange assessment were used to measure the following parameters: shoot and

191 root dry mass, leaf count, mycorrhizal root colonization (percentage). Moreover, the mycorrhizal

192 dependency (MD) expressed as the change in plant growth due to mycorrhizal colonization was

193 calculated utilizing the following formula by Menge *et al*. (1978):

194 $\mathrm{MD} = \dfrac{\text{with AM plant} - \text{without AM plant}}{\text{with AM plant}} \times 100$ (Eq. 3)

195

196 Roots samples were rinsed free of soil, cut into 1–2 cm fragments and thoroughly mixed, and

197 subsamples (1 g) saved for determination of root colonization with AM fungi. These samples were

198 cleared with 10% (w/v) KOH and stained with 0.03% (v/v) trypan blue in lactoglycerol according to

199 the method of Phillips and Hayman (1970), and microscopically examined for colonization by

200 determining percentage root segments containing arbuscules and vesicles using a gridline intercept

201 method (Giovannetti and Mosse 1980).

202   At the same time, chicory shoots were oven-dried at 70 °C for 48 h, finely grounded and sieved for

203   nutrients and bioactive compounds. Nutrients estimation were performed using 5 g of grounded

204   shoots chemically digested and analyzed with a colorimetric method (Jackson, 1973) for the total

205   phosphorus (P) and flame photometer (Corning 400, UK) for potassium (K). The content of

206   magnesium (Mg) was measured using atomic absorption system (Perkin Elmer, Model 2380, USA)

207   and the amount of total nitrogen (N) was determined with Micro-Kjeldahl template described by

208   Nelson and Sommers (1973).

209   Relative water content (RWC) was determined in the fully expanded fifth green leaf from the top at

210   the flowering stage according to Ünyayar *et al*. (2004).

211   The total chlorophyll was assessed with a spectrophotometer at 646.6, 663.6 and 470 nm according

212   to the method of Porra (2002). The pigment concentrations were calculated according to the equation

213   proposed by Lichtenthaler (1987);

214   $mg\ totla\ \frac{chl}{g\ tissue} = [(17.76 \times A646.6) + (7.34 \times A663.6)] \times [V/(1000 \times W)]$ (Eq. 4)

215

216   $mg\ totla\ \frac{carotenoid}{g\ tissue} = [1000A470 - 3.27chl\ a - 104chl\ b]/227$ (Eq. 5)

217

218   *2.3.3. Abscisic acid (ABA) measurement in leaf*

219   Leaf tissue was finely grounded and extracted with 2-propanol/H_2O/HCl 37% in a proportion of

220   2:1:0.002 (v:v:v). Samples were mixed with dichloromethane then shaken and centrifuged.

221   Afterward, the solvent was transferred into a screw-cap vial which was concentrated utilizing

222   nitrogen flow-based evaporator. The quantification of abscisic acid (ABA) was conducted by using

223   Phytodetek® ABA Test Kit (Agdia Biofords, Evry, France) as described by Quarrie *et al*., (1988).

224

225 *2.3.4. Ethylene measurement in leaf*

226 The ethylene concentration was determined in five uppermost fully expanded leaves using a flame

227 ionization detector gas chromatograph equipped with a flame ionization detector (Hitachi 063) (Ishii

228 *et al*. 1982).

229

230 *2.3.5. Activities of antioxidant enzymes in leaf*

231 A unit (U) of superoxide dismutase (SOD) activity measured the amount of enzyme associated with

232 half of the highest inhibition of nitroblue tetrazolium (Ug^{-1} FW). Peroxidase (POD) activity was

233 determined by differences at 470 nm of absorbance (Omran, 1980).

234

235 *2.3.6. Malondialdehyde (MDA) and H_2O_2 determination in leaf*

236 Leaf sample was homogenized with trichloroacetic acid and centrifuged for determining

237 Malondialdehyde (MDA) contents. Then 2-thiobarbituric acid (TBA) was added and heated. After

238 cooling, sample absorbance was calculated at 532 nm for MDA determination (Janero, 1990). The

239 Velikova *et al*. (2000) method was adopted for preparing the leaf samples for H_2O_2 concentration

240 and the absorbance data was taken at 390 nm.

241

242 *2.3.7. Proline determination, glutathione and ascorbate contents in leaf*

243 The concentration of the free portion of proline was assayed using the acid-ninhydrin approach

244 proposed by Bates *et al*. (1973). Glutathione was assayed as demonstrated by Anderson (1985),

245 while Ascorbate was assayed by the decreasing of 2,6-dichlorophenolindophenol (DCPIP) using

246 photometric as described by Leipner *et al*. (1997).

247

248 *2.3.8. Sugar concentration in root*

249 The determination of the sugar concentration was performed by using grounded roots incubated in 37

250 %HCl. The sample was neutralized with NaOH after cooling to 25°C. The concentration of sugar

251 (glucose and fructose) was measured utilizing an HPLC (Varian Carbohydrates, CA, USA) at a

252 temperature of 85°C. The pump flow, the eluent and the injection volume were 0.5 mL min^{-1}, 50 mg

253 L^{-1} Ca-EDTA and 20 μL, respectively, with an analysis period of 15 min per sample. The inulin

254 percentage (IP) and the average polymerization degree (DP) were determined as the concentration of

255 glucose and fructose (Waes *et al.*, 1998):

256 IP = (% glucose + % fructose)/x   (Eq. 6)

257 DP = (% glucose + % fructose) + 1   (Eq. 7)

258 where x was the inulin synthesis factor. This factor represented the loss of a molecule of water from

259 the added fructose to the chain of inulin (Baert, 1997):

260 $X = \dfrac{180 \times n}{(180 \times n) - [(n-1) \times 18]}$, with n = DP   (Eq. 8)

261

262 *2.4. Statistical analysis*

263 Data were subjected to analysis of variance (ANOVA) utilizing JMP statistical software package

264 version 4.0 (SAS, 1996). Before the analysis, the normal distributions of the data were analyzed

265 adopting a Shapiro–Wilk test. The total soluble sugars content was log transformed [y = log (x)] to

266 ensure homogenous the variance (Gomez and Gomez, 1984). Meanwhile, the reported data in the

267 table were back transformed. A three-way factorial analysis was performed for the 2-year period for

268 measured characteristics where the Arbuscular mycorrhizal application, irrigation rates and chicory

269 ecotype were the factors, and the growing season was considered as repeated measure (Gomez and

270 Gomez, 1984). Averages were compared with Fisher's protected least significant difference (LSD) at

271 5% significant level.

272

273 **3.   RESULTS**

274 *3.1.   Mycorrhizal colonisation and dependency*

275 At the flowering stage of the chicory crop, in AM inoculated treatments (AM$^+$), the intensity of

276 mycorrhizal colonization was the highest in chicory plants grown under IR^1 irrigation rate regardless

277 of the chicory ecotypes (Fig. 1). The data regarding the intensity of mycorrhizal colonization in IR^2

278 and IR^3 irrigation rates showed that the drought-tolerant ecotype Siyah Shiraz (E^2) generally had

279 higher root AM colonization than the drought-sensitive ecotype Sefid Isfahan (E^1, Fig. 1).

280 Mycorrhizal dependency values of the chicory plants in response to AM fungal inoculation were

281 significantly higher under IR^3 followed by IR^2 and IR^1 water irrigation rate (Fig. 1). The E^1 chicory

282 ecotype showed generally the highest values of mycorrhizal dependency than E^2 chicory ecotype,

283 except in IR^1 irrigation rate where an opposite trend was observed (Fig.1).

284

285 *3.2.   Growth parameters and nutritional status*

286 Growth parameters were always significant for irrigation levels and AM inoculation (P $\leq$ 0.05),

287 while only root diameter and root length were significant regarding the ecotypes. All interactions

288 among the main effects were always significant (P $\leq$ 0.05, Table 2). Inoculation of arbuscular

289 mycorrhiza (AM$^+$) compared with AM$^-$ increased all growth parameters measured, such as root

290 diameter (on average 2.4 *vs*. 2.0 cm, respectively), number of leaves (on average 31 *vs*. 27 no. plant$^-$

291 ^{1}, respectively), shoot dry weight (on average 3601.1 *vs*. 3241.9 kg ha^{-1}, respectively), root dry

292 weight (on average 862.4 *vs*. 750.4 kg ha^{-1}, respectively) and root length (on average 23.8 *vs*. 21.9

14

293   cm, respectively) of both ecotypes regardless of irrigation rate (Table 2). However, ecotype Siyah

294   Shiraz (E^2) had generally higher growth parameters than ecotype Sefid Isfahan (E^1), especially under

295   both IR^2 and IR^3 (Table 2).

296   As expected, the fraction of intercepted radiation (FINT) decreased gradually from IR^1 to IR^3 and it

297   was significantly greater in AM^+ treatments (Fig. 2). The FINT values were similar for two ecotypes

298   grown under IR^1 treatment, while, under IR^2 and IR^3 treatments, it was generally higher in E^2

299   compared with E^1 (data not shown). The N, K, Mg and P content in the chicory plants were affected

300   by irrigation level and AM inoculation as main effect ($P \leq 0.05$), while ecotype affected only the P

301   content. All interactions among the main effects were always significant ($P \leq 0.05$, Table 3). The N,

302   K, Mg and P content measured in the shoots of chicory plants were higher in AM^+ (on average 2.48,

303   0.158, 0.167 and 1.97 g of N, P, Mg and K per plant, respectively) compared with the AM^- plants (on

304   average 2.21, 0.128, 0.127 and 1.44 g of N, P, Mg and K per plant, respectively). The N, K, Mg and

305   P content tended to decrease from IR^1 to IR^3 irrigation rate (Table 3). Regardless of AM treatment,

306   few differences were observed on mineral contents regarding the E^1 and E^2 ecotypes in IR^1 irrigation

307   rate, while under IR^2 and IR^3 treatments E^2 tended to show high values of nutrient content than E^1

308   ecotype (Table 4).

309

310   *3.3.   Photosynthesis parameters, pigments and relative water content (RWC)*

311   The photosynthesis parameters measured in the chicory leaves were affected by irrigation level and

312   AM inoculation, while ecotypes did not affect photosynthesis paramenters ($P > 0.05$). All

313   interactions among irrigation rate, arbuscular mycorrhizal, and ecotypes were significant (Table 4).

314   Generally, IR^1 and IR^2 irrigation rate similar values on carbon exchange rate (on average 15.43

315   μmol CO_2 m^{-2} s^{-1}), transpiration rate (on average 1.51 mol H_2O m^{-2} s^{-1}), stomatal conductance (on

316　average 0.152 mol H_2O m^{-2} s^{-1}), chlorophyll content (on average 1636 mg g^{-1} plant^{-1}) and carotenoids

317　content (on average 311 mg g^{-1} plant), while a strong reduction in theses parameter were noticed

318　when irrigation was scheduled at MAD of 90% (IR^3, Table 4). All photosynthesis parameters showed

319　higher values in AM$^+$ compared with AM$^-$ treatments regardless the irrigation rate, while E^2 ecotype

320　tended to show higher values of photosynthesis parameters compared with E^1 ecotype, especially

321　under IR^2 and IR^3 irrigation rate (Table 4).

322　Relative water content, hydrogen peroxide content and malondialdeyhde in leaves of chicory crop at

323　harvesting were affected by irrigation level and AM inoculation, while no effect were observed on

324　ecotypes main effect (P > 0.05). All interactions among the main effects were always significant (P $\leq$

325　0.05, Table 5). The relative water content (RWC) measured in the chicory leaves of both ecotypes

326　was similar in IR^1 irrigation rate regardless of the AM treatments (on average of 92.9 %), while it

327　was higher in AM$^+$ compared with AM$^-$ treatments under IR^2 (on average of 85.2 vs. 77.0 %,

328　respectively) and IR^3 (on average of 76.4 vs. 65.8 %, respectively) irrigation rate (Table 5).

329

330　*3.4.　Malondialdehyde (MDA) and H_2O_2*

331　The measurements of H_2O_2 and MDA content were higher in AM$^-$ (on average of 21.4 and 150.6

332　nmol g^{-1} FW, respectively) compared with AM$^+$ (on average of 16.0 and 114.6 nmol g^{-1} FW,

333　respectively) irrespective of the irrigation rate (Table 5). Regarding the chicory ecotypes no

334　differences were observed under IR^1 and IR^2 irrigation rates regardless the AM inoculation

335　treatment, while the differences were observed in IR^3 under AM$^-$ treatments (Table 5).

336

337　*3.5.　Antioxidant enzymes activities*

In general irrigation level and AM inoculation significantly affected the antioxidant activities, accumulation of antioxidant compounds and proline content of chicory crop at harvesting ($P < 0.05$), except POD which was not significant for all main effects and their interaction ($P > 0.05$). Ecotypes affected only the proline content (Table 6). The SOD activity was increased in AM+ compare to AM- treatments (on average of 14.2 vs. 12.1 U g^{-1} FW, Table 6) and it tended to be higher in IR^3, intermediate in IR^2 and low in IR^1 irrigation rate (on average of 15.1, 12.9 and 11.4 U g^{-1} FW, respectively, Table 6). Among the chicory ecotypes significant differences were observed in the interaction between AM^- and IR^3, and interaction between AM^+ and IR^3, where the E^2 had higher values of SOD activity than E^1 (Table 6). A similar trend was observed for POD (Table 6).

3.6.   Accumulation of antioxidant compounds and proline content

The amount of glutathione and ascorbate accumulated in chicory plants was significantly higher in AM^+ than in AM^- treatments (on average of 56.0 and 33.5 vs. 27.8 and 18.1 nmol g^{-1} DW, respectively, Table 6). This effect was observed in all irrigation rate, as they tended to increase from IR^1 to IR^3 irrigation rate (Table 6). Few differences were observed among the chicory ecotypes (Table 6).

The proline content values showed a similar trend to that observed for glutathione and ascorbate (Table 6). However, tendency of high proline content was observed in ecotype Siyah Shiraz than ecotype Sefid Isfahan especially in IR^2 and IR^3 irrigation rate in both AM treatments.

3.7.   Inulin percentage (IP) and degree of polymerization (DP)

The percent of inulin and mean inulin degree of polymerization were affected by all main effects, except ecotypes ($P > 0.05$), and their interation (Table 7). The IP measurements revealed that the

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361 highest values were attained when irrigation rate was scheduled at 65% MAD (IR^2), even if it was

362 higher in AM^+ than AM^- (on average of 73.0 *vs*. 68.3 % respectively, Table 7). Similarly, the IP was

363 high in IR^1 and IR^2 irrigation rate, while it decreased drastically in IR^3 treatments. There were few

364 differences between the two ecotypes for these traits (Table 7).

365

366 *3.8.   Leaf ABA and Ethylene*

367 The leaf ABA accumulation and ethylene were affected by all main effects, except ecotypes (P >

368 0.05), and their interation (Table 7). Leaf ABA accumulation in chicory leaves at harvesting was

369 higher in AM^- than AM^+ treatments (on average 95.0 *vs*. 70.4 ng g FW^{-1}, respectively, Table 7), even

370 if it tended to be higher in IR^3 compared with IR^2 and IR^1 irrigation rates (on average of 112.1 *vs*.

371 68.0 ng g FW^{-1}, respectively). Conversely, the ethylene accumulation was always the highest in E^2

372 under IR^3 in both AM^- (1.6 μmol g^{-1} FW h^{-1}) and AM^+ (1.3 μmol g^{-1} FW h^{-1}) and was similar in IR^1

373 irrigation rate regardless of the AM treatments (on average of 0.49 μmol g^{-1} FW h^{-1}), while it was

374 higher in AM^- than AM^+ in IR^2 and IR^3 irrigation rates (on average 1.12 *vs*. 0.95 μmol g^{-1} FW h^{-1},

375 respectively, Table 7). The ABA and ethylene accumulation in ecotype E^2 was significantly higher

376 than in the E^1 ecotype, especially at 90% MAD (IR^3, Table 7).

377

378 **4.   DISCUSSION**

379 Inoculation of AM enhanced the colonization level in both chicory ecotypes. The level of

380 colonization was higher under IR^1 compared with IR^2 and IR^3 irrigation rate treatments. These data

381 are in agreement with the findings of Al-Karaki *et al*. (2004) where the intensity of AM fungal

382 inoculation was lower under water deficit condition than well watered condition. Drought stress, due

383 to reduced water availability for plant growth, could affects mycorrhizal development, thus hindering

384 AM colonization, germination of spore and fungal hyphae growth after initiation of colonization

385 (Kumar *et al*., 2015).

386 Nowadays, it is agreed that mycorrhizal based symbiosis reduce the negative impacts of water

387 deficiency in the host plants and could contribute to improving drought tolerance in the plant due to

388 combined effects of improved nutritional, physical and cellular status (Ruiz-Lozano, 2003; Al-

389 Karaki *et al*., 2004). Similarly, the results of this study suggest that drought tolerance of chicory

390 plants was improved by AM inoculation and this was attributed to enhanced host plant nutritional

391 status and water uptake, particularly in chicory plant grown under IR^2 and IR^3 irrigation rate

392 treatments. Wu *et al*. (2008) suggested that the drought tolerance observed in the host plant caused

393 by AM inoculation could be due to a dense root growth with a greater absorption root surface area

394 that affects hydraulic characteristics of a root system. Similarly, the chicory plant inoculated with

395 mycorrhizal fungi showed a higher root development, in terms of length and diameter, compared

396 with AM^- chicory plant, thus boosting the productivity of the whole root system. Consequently, these

397 processes will impact shoot growth such as more, larger and fully expanded leaves of the chicory

398 plants, as observed through the LAI data that were significantly greater in AM^+ plants (Liu *et al*.,

399 2016). Moreover, the high FINT values observed in all AM^+ treatments regardless of the irrigation

400 rates, probably demonstrated more efficient photosynthetic production of chicory leaves (Mandal *et*

401 *al*., 2006). Furthermore, chicory plants inoculated with AM maintain increased leaf RWC in all

402 levels of irrigation rates. The improved leaf water condition of AM^+ chicory plants could be

403 associated with improved nutrition in the plant which could have provided increase ability for soil

404 moisture extraction compared with non-inoculated chicory plants (Sun *et al*., 2017). Similarly,

405 Subramanian *et al*. (2006) showed that greater P nutrition promotes positive leaf water potential in an

406 AM^+ plants, regardless of increasing negative soil water potential. In addition, the P content was

407 consistently higher in the inoculated chicory plants (AM$^+$) than non-inoculated plants (AM$^-$).

408 Similarly, AM$^+$ had high N content in shoots in all irrigation rates. As a result of improved

409 nutritional and water status, inoculated chicory produced relatively higher biomass. However, the

410 drought-tolerant ecotype, Siyah Shiraz generally showed higher growth and root AM colonization

411 compared with the drought-sensitive ecotype Sefid Isfahan; as expected differences were more

412 pronounced in IR^2 and IR^3 irrigation rates. Al-Karaki *et al*. (2004) indicated that mycorrhiza

413 comparably has more impacts on plant growth in drought condition than in well-watered condition.

414 The inoculated chicory plants showed better performance of photosynthesis parameters measured

415 (CER, E and gs), chlorophyll content and carotenoids content when subjected to reduced irrigation

416 rates. The enhanced photosynthetic efficiency in AM$^+$ plants indicated that plants were less subjected

417 to drought effects. Furthermore, the high transpiration rate observed in an inoculated plant leaf is

418 expected consistent with the increased stomatal conductance rate that usually happens with

419 mycorrhizal symbiosis necessary to meet carbon needs of a symbiont (Augé, 2001). Similarly, the

420 high P content in chicory tissues, probably influenced by hyphae activity, has also been recognized

421 as a way where the AM symbiosis influences the stomatal behaviour (Abdel-Salam *et al*., 2017). The

422 capacity of AM fungal to influence leaf area, leaf number, and root mass had been reported by

423 (Abdel-Fattah *et al*., 2013). In this study, the accumulation of H_2O_2 increased in treatments subjected

424 to reduced water availability, especially in AM$^-$ plants irrigated with 65% and 90% MAD. The

425 oxidation of membrane lipids (MDA) is an indicator of uncontrolled free radical production, and

426 oxidative stress (Noctor and Foyer, 1998). The quantity of the oxidation of membrane lipids (MDA)

427 measured in the shoots of chicory plants tended to be accumulated in the AM$^-$ plants, while it was

428 generally similar among the AM$^+$ treatments. Moreover, the higher amount of carotenoid contents in

429 the inoculated plants suggested that could be considered a part of antioxidant defence system

430 operated by mycorrhizal for alleviating adverse effects of reduced water availability (Mittler, 2002).

431 In agreement with the findings of Baslam and Goicoechea (2012), the improvement of the tolerance

432 mechanisms operated by AM fungal is frequently associated with the amelioration of plant

433 antioxidant activities by means of antioxidant enzymes that increased when plants are subjected to

434 stress conditions with an additional important role in ROS catabolism (Mittler, 2002). Chicory plants

435 that were grown under IR^2 and IR^3 irrigation rates had an increase in SOD and POD activity which

436 can be indirectly considered as a sign of increased amounts of free radicals in drought stress,

437 especially in AM^+ enhancing plant growth under water deficit stress. The enhanced SOD and POD

438 activity may crucial a key role in drought tolerance of plants, especially by avoiding the occurrence

439 of oxidative damage. In addition, the reduced chlorophyll content associated with drought stress

440 could be due to reduced K and Mg concentrations (Augé, 2001). In this study, the amount of those

441 elements is higher in AM^+ than in AM^- plants. The ascorbate, a key non-enzymatic antioxidant

442 compound that removes H_2O_2 and in the ascorbate–glutathione cycle, is closely related to glutathione

443 (Noctor and Foyer, 1998). A decrease in the oxidative destruction to biomolecules and protection of

444 inoculated soybean plants against drought associated with AM symbiosis was attributed to an

445 improved activity of glutathione reductance in roots (Porcel *et al*., 2003). In this current study, the

446 content of ascorbate in an AM^+ plant was higher than in AM^- plants, thus, the higher contents of

447 glutathione observed in an AM^+ plant could have contributed to the protection of chicory against the

448 oxidation caused by drought.

449 Generally, the accumulation of proline in the chicory plants tended to increase when the irrigation

450 water was reduced. The quantity of proline was mostly greater in AM^+ than in AM^- plants. Similarly,

451 Ruíz-Sánchez *et al*. (2011) observed high proline accumulation in inoculated plants of *Oryza sativa*

452 under drought stress. Cellular osmotic potential of many plants species is decreased by accumulated

453 osmolytes including proline, which are subsequently involved in osmotic adjustment, thereby

454 promoting the tolerance to drought (Yoshiba *et al*., 1997). The ABA and ethylene content are

455 involved in many morphophysiological responses of a plant under different plant stress (Asensio *et*

456 *al*., 2012; Cruz *et al*., 2000). Our results showed that both ABA and ethylene levels were lower in

457 AM$^+$ chicory plants than in AM$^-$ chicory plants, suggesting that AMF would postpone senescence of

458 the host plants as observed by (Cruz *et al*., 2000) in papaya crop under drought stress conditions.

459 The findings from various studies suggest that antioxidant enzymes can be used in chicory to reduce

460 oxidative stress caused by abiotic process, thereby preventing oxidative damage in plant cells

461 (Ghanaatiyan and Sadeghi, 2017). Moreover, the study demonstrates that this severe drought stress

462 (i.e. IR^3) has a devastating effect on the IP and DP from root chicory, in agreement with (Vandoorne

463 *et al*., 2012). However, chicory root growth decreased by drought stress, but not biosynthesis of

464 inulin (Vandoorne *et al*., 2012). This influence may be explained by the micro and macro nutrients

465 supplied by AM during the symbiosis, and these nutrients are required in the key metabolic processes

466 that include but not limited to photosynthesis (Rapparini and Peñuelas, 2014).

467

468 **5.   CONCLUSION**

469 Arbuscular mycorrhiza could be positively employed in various protective mechanisms to counteract

470 drought stress. In this study, inoculated chicory plants develop the root morphology and its activity

471 in response to drought stress. Moreover, AM fungal enhanced tolerance of chicory plants to drought

472 stress as indicated nutrient uptake and bio-chemical evens such as osmotic adjustment, hormonal

473 activities and antioxidant systems. The AM fungal inoculation in chicory plant could contribute to

474 reducing the negative effects of drought stress, especially under moderate dry condition (IR^2).

475 Ecotypes mostly responded equally to irrigation and AM inoculation for growth and biochemical

476 parameters, but the results show Siyah Shiraz was better equipped with free radical quenching

477 system that is more efficient in plant protection against oxidative stress. Based on the results of this

478 study, it can be concluded that AM fungal colonization mitigates the deleterious effects of drought

479 stress by enhancing different tolerance mechanisms; thus, it could be effectively adopted for chicory

480 cultivation in an arid and semi-arid area.

481

482 **ACKNOWLEDGMENTS**

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658

FIGURE CAPTIONS

Figure 1. Intensity of mycorrhizal colonization and mycorrhizal dependency of chicory ecotypes
under different irrigation rates. Means without common letters are significantly different at the 5%
level according to LSD test. (data averaged over 2 years, 2017 and 2018). Bars represent the standard
error (n = 4).

Irrigation rate, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of maximum allowable depletion,
respectively; E^1 and E^2 are Sefid Isfahan and Siyah Shiraz ecotypes, respectively.

Figure 2. Leaf area index (LAI) and fraction of intercepted radiation (FINT) of mycorrhizal (AM^+)
and non-mycorrhizal (AM^-) chicory plants grown under different irrigation rates during growing
seasons. (data averaged over 2 years, 2017 and 2018). Bars represent the standard error (n = 4).

Irrigation, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of maximum allowable depletion, respectively;
AM^+ and AM^- are chicory plant inoculated with arbuscular mycorrhizal, without inoculation,
respectively.
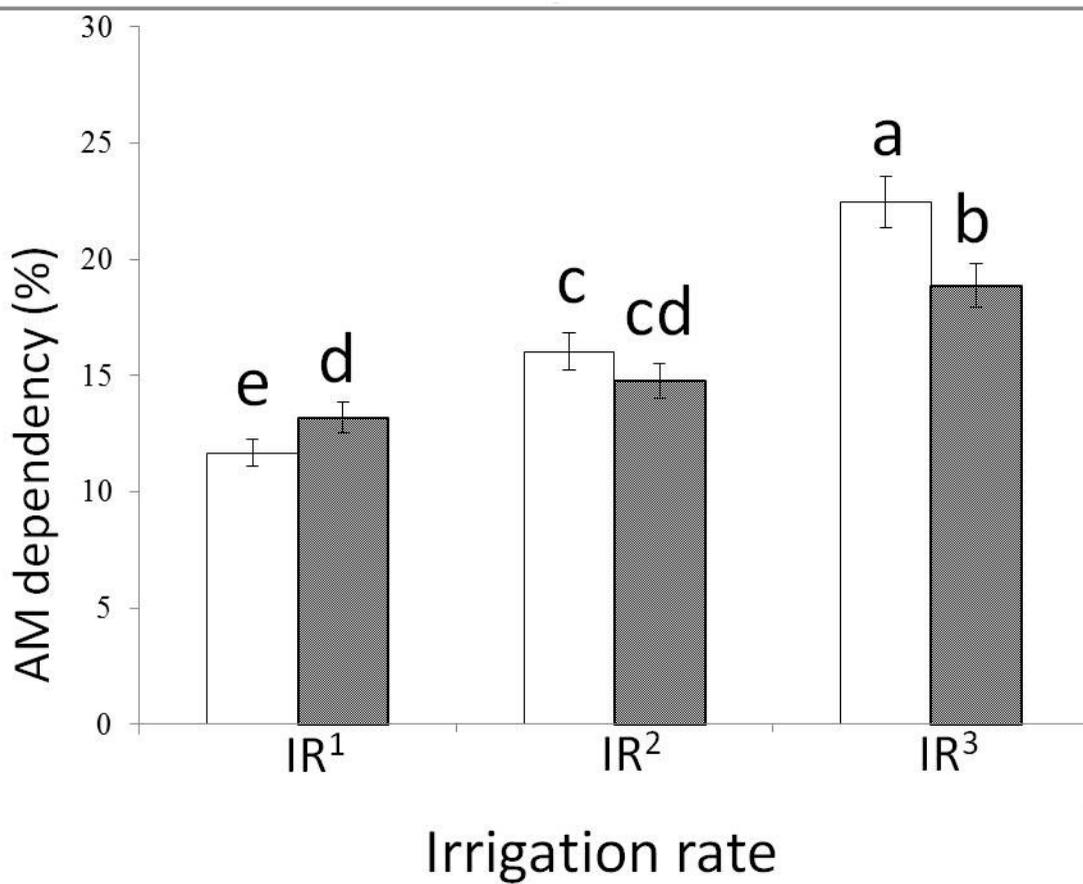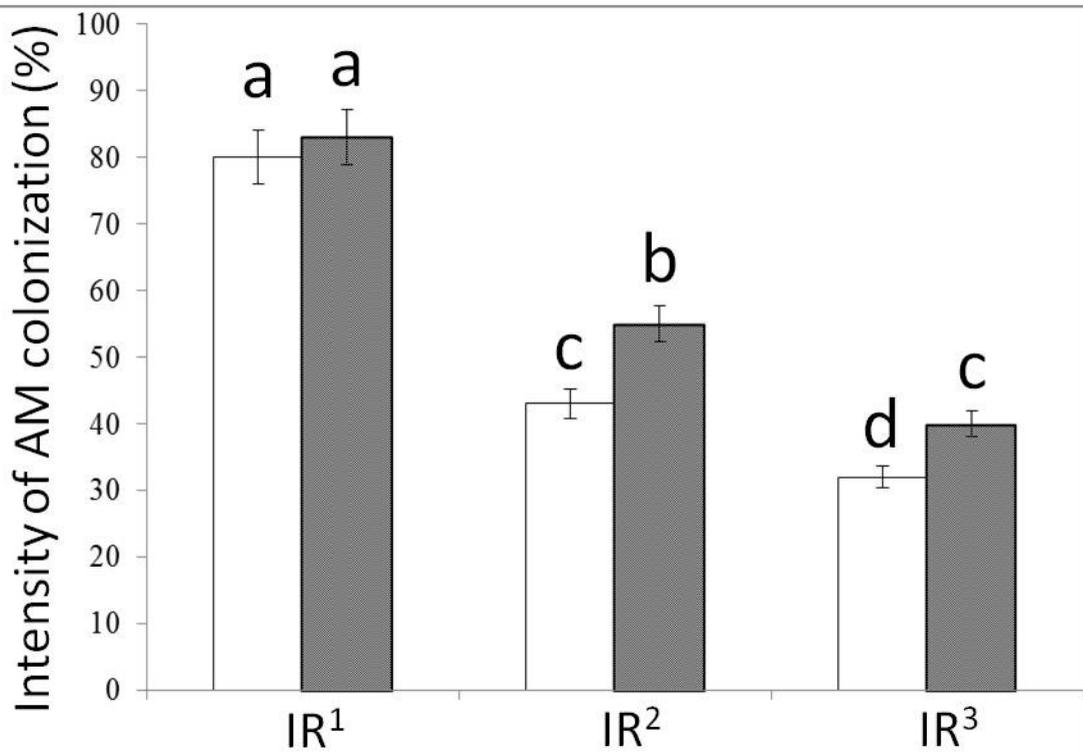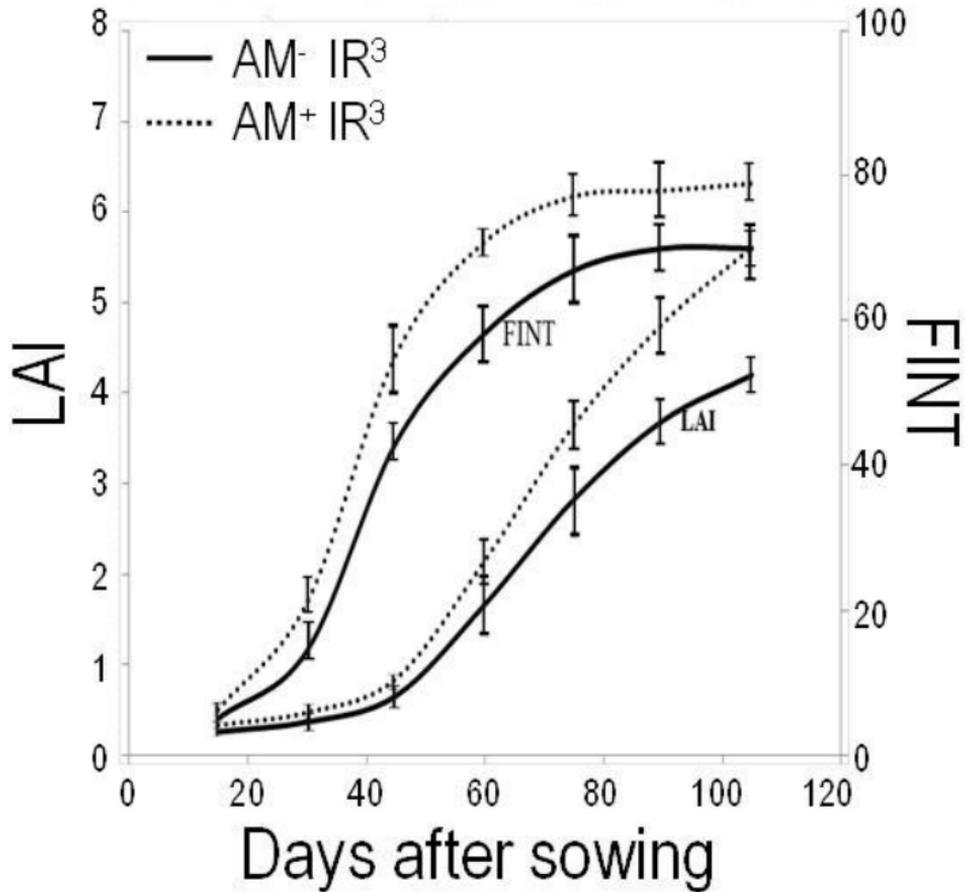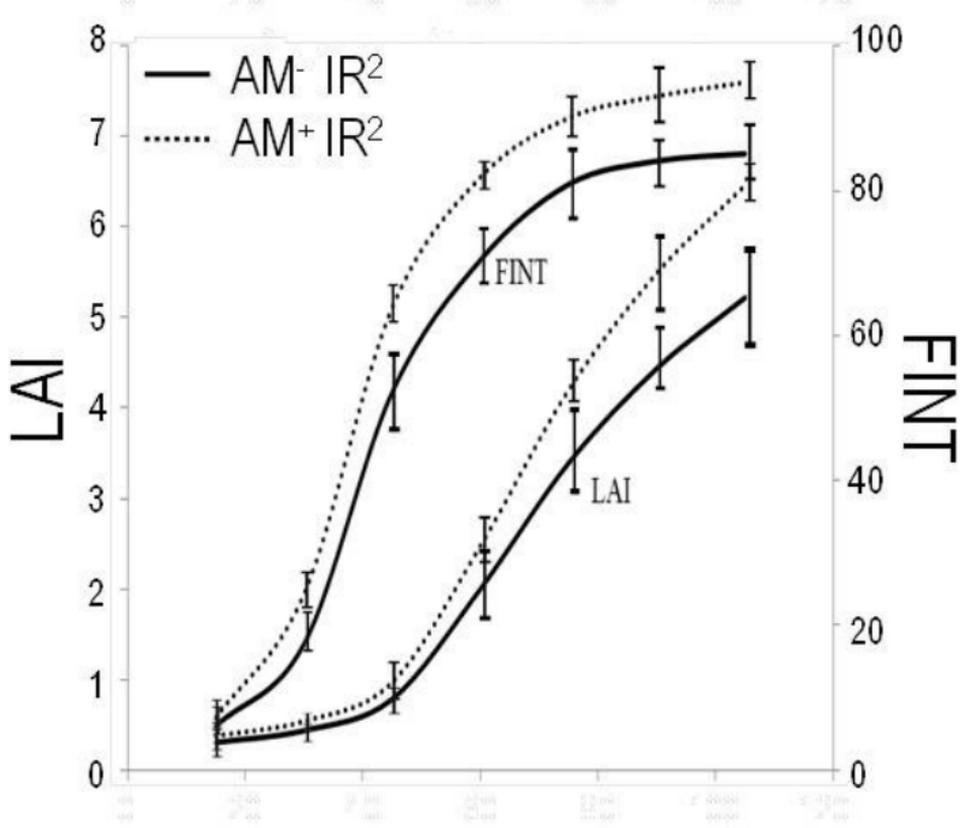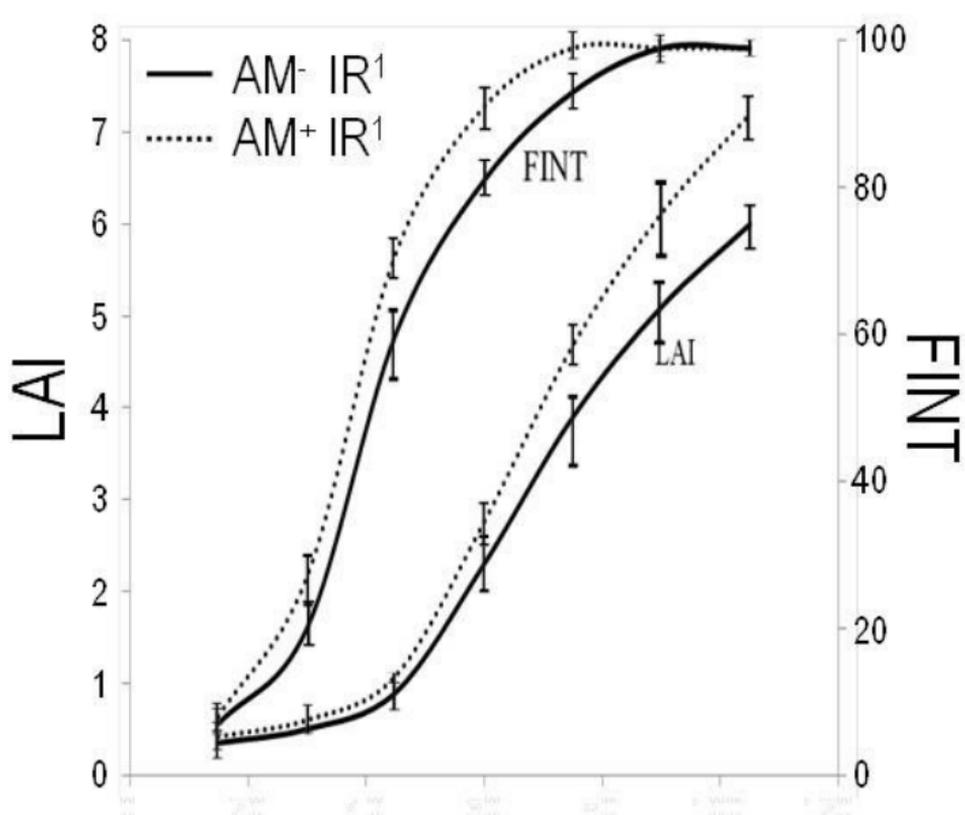
Table 1. Mean temperature, rainfall and total volume of irrigation water in both chicory growing seasons.

	Mean temperatures (°C)		Rainfall (mm)	
	2017	2018	2017	2018
March	16	17	30	27
April	17	19	20	19
May	20	20	8	7
June	22	23	4	2
July	24	25	3	2
Irrigation levels			Water volume (mm)	
IR^1 (40% MAD)			270	290
IR^2 (65% MAD)			210	225
IR^3 (90% MAD)			110	120

Irrigation rate, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of maximum allowable depletion (MAD), respectively;

1 **Table 2.** The interaction effects of irrigation levels × AM inoculation × ecotypes on some growth traits of chicory crop at harvesting
2 (data averaged over 2 years, 2017 and 2018).
3
4

Treatment		RD (cm)		NLP (no. plant⁻¹)		RDM (kg ha⁻¹)		SDM (kg ha⁻¹)		RL (cm)	
		AM⁻	AM⁺	AM⁻	AM⁺	AM⁻	AM⁺	AM⁻	AM⁺	AM⁻	AM⁺
IR¹	E¹	2.3 aB	2.6 aA	35 aA	37 aA	904.3 aB	988.7 aA	3798.2 aB	4080.3 aA	25.4 aA	26.1 aA
	E²	2.4 aA	2.6 aA	34 aA	36 aA	909.8 aA	1000.6 aA	3880.6 aB	4100.2 aA	26.2 aA	27.5aA
IR²	E¹	2.0 bB	2.4 aA	26 bB	30 bA	708.4 bcB	829.1 bA	3110.6 bB	3513.2 bA	20.1 cB	23.5 cA
	E²	2.2 aB	2.5 aA	28 bB	33 abA	760.9 bB	850.2 bA	3325.6 bB	3600.0 bA	23.6 bB	25.7 bA
IR³	E¹	1.5 cB	2.0 bA	17 cB	23 cA	568.2 cB	710.5 cA	2525.3 dB	3000.3 dA	16.1 dB	18.3 eA
	E²	1.8 bB	2.2 bA	21 cB	29 bA	650.9 cB	795.1 bA	2810.8 cB	3312.5 cA	20.1 cB	21.8 dA
ANOVA											
IR		**		*		*		*		*	
AM		*		*		*		*		*	
E		*		ns		ns		ns		*	
IR × AM		**		*		*		*		*	
IR × E		*		*		*		*		*	
AM × E		*		*		*		*		*	
IR × AM × E		*		*		*		*		*	

5
6 Values belonging to the same characteristic without common letters in row for AM inoculation (upper case letter) and in columns for irrigation
7 rate of each ecotype (lower case letter) are statistically different according to LSD (0.05). RD is root diameter, NLP is no. of leaves plant⁻¹, RDM
8 is root dry matter, SDM is shoot dry matter, RL is root length. Irrigation rate, IR¹, IR² and IR³ are the 40, 65, and 90% of maximum allowable
9 depletion, respectively; AM⁺ and AM⁻ are chicory plant inoculated with arbuscular mycorrhizal, without inoculation, respectively; E¹ and E² are
10 Sefid Isfahan and Siyah Shiraz ecotypes, respectively. ns, not significant; * and **, significant at the 0.05 and 0.01 level of probability,
11 respectively.
12
13

Table 3. The interaction effects of irrigation levels × AM inoculation × ecotypes on nutrient content in leaves of chicory crop at
harvesting (data averaged over 2 years, 2017 and 2018).

Treatment		N (g plant^{-1})		P (g plant^{-1})		Mg (g plant^{-1})		K (g plant^{-1})	
		AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$
IR^1	E^1	2.4 aB	2.7 aA	0.151 aB	0.172bA	0.139 aB	0.181 aA	1.6 aB	2.1 abA
	E^2	2.4 aB	2.8 aA	0.169 aB	0.195 aA	0.141 aB	0.188 aA	1.7 aB	2.3 aA
IR^2	E^1	2.2 bB	2.4 bA	0.111 cB	0.140 cA	0.129 aB	0.161 abA	1.4 abB	1.9 abA
	E^2	2.3 abB	2.5 bA	0.138 bB	0.165 bA	0.132 aB	0.175 aA	1.5 aB	2.1 abA
IR^3	E^1	1.9 cB	2.2 cA	0.082 dB	0.125 cA	0.100 bB	0.136 bA	1.1 bB	1.7 bA
	E^2	2.1 cB	2.3 bcA	0.119 cB	0.149 bcA	0.119 abB	0.159 abA	1.3 abB	1.8 bA
ANOVA									
IR		*		*		*		*	
AM		*		*		*		*	
E		ns		*		ns		ns	
IR × AM		*		*		*		*	
IR × E		*		*		*		*	
AM × E		*		*		*		*	
IR × AM × E		*		*		*		*	

Values belonging to the same characteristic without common letters in row for AM inoculation (upper case letter) and in columns for
irrigation rate of each ecotype (lower case letter) are statistically different according to LSD (0.05). Irrigation rate, IR^1, IR^2 and IR^3 are
the 40, 65, and 90% of maximum allowable depletion, respectively; AM$^+$ and AM$^-$ are chicory plant inoculated with arbuscular
mycorrhizal, without inoculation, respectively; E^1 and E^2 are Sefid Isfahan and Siyah Shiraz ecotypes, respectively. ns, not significant;
* and **, significant at the 0.05 and 0.01 level of probability, respectively.

Table 4. The interaction effects of irrigation levels × AM inoculation × ecotypes on photosynthesis parameters and pigments in leaves of chicory crop at harvesting (data averaged over 2 years, 2017 and 2018).

Treatment		CER (μmol CO_2 m^{-2} s^{-1})		Gs (mol H_2O m^{-2} s^{-1})		E (mol H_2O m^{-2} s^{-1})		Chlorophyll (mg g^{-1} plant^{-1})		Carotenoids (mg g^{-1} plant)	
		AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$
IR^1	E^1	14.6 abB	16.5 abA	0.145 abB	0.168 abA	1.25 aB	1.89 aA	1578 abB	1798 aA	280 abB	359 aA
	E^2	15.5 aA	17.0 aA	0.150 aB	0.174 aA	1.26 aB	2.00 aA	1658 aB	1840 aA	303 aB	380 aA
IR^2	E^1	13.6 bB	15.5 bA	0.124 bB	0.155 abA	1.07 abB	1.70 abA	1414 cB	1600 bcA	239 abB	320 abA
	E^2	14.5 abB	16.1 bA	0.139 abB	0.163 abA	1.11 abB	1.81 aA	1541 bB	1656 bA	255 abB	349 aA
IR^3	E^1	10.9 dB	12.7 dA	0.090 cB	0.130 bA	0.81 bB	1.42 bA	1204 dB	1402 dA	189 bB	270 bA
	E^2	12.5 cB	14.5 cA	0.120 bB	0.151 bA	0.95 abB	1.72 abA	1435 cB	1546 cA	227 bB	315 abA
ANOVA											
IR		**		**		**		*		*	
AM		*		*		*		*		*	
E		ns		ns		ns		ns		ns	
IR× AM		*		*		*		*		*	
IR × E		*		*		*		*		*	
AM × E		*		*		*		*		*	
IR × AM × E		**		*		*		*		*	

Values belonging to the same characteristic without common letters in row for AM inoculation (upper case letter) and in columns for irrigation rate of each ecotype (lower case letter) are statistically different according to LSD (0.05). CER is carbon exchange rate; Gs is Stomatal conductance; E is transpiration rate. Irrigation rate, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of maximum allowable depletion, respectively; AM$^+$ and AM$^-$ are chicory plant inoculated with arbuscular mycorrhizal, without inoculation, respectively; E^1 and E^2 are Sefid Isfahan and Siyah Shiraz ecotypes, respectively. ns, not significant; * and **, significant at the 0.05 and 0.01 level of probability, respectively.

1 **Table 5.** The interaction effects of irrigation levels × AM inoculation × ecotypes on relative water content, hydrogen peroxide content
2 and malondialdeyhde in leaves of chicory crop at harvesting (data averaged over 2 years, 2017 and 2018).
3

Treatment		RWC (%)		H_2O_2 (nmol g^{-1} FW)		MDA (nmol g^{-1} FW)	
		AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$
IR^1	E^1	90.3 aA	94.2 aA	19.0 bA	15.1 bB	139.7 bcA	105.6 bB
	E^2	91.0 aA	95.9 aA	17.5 bA	13.9 bB	127.2 cA	100.8 bB
IR^2	E^1	72.1 cB	83.6 bcA	23.5 abA	15.9 bB	159.9 bA	116.6 abB
	E^2	81.9 bB	86.8 bA	20.1 bA	15.0 bB	142.3 bcA	110.0 bB
IR^3	E^1	60.8 dB	70.2 dA	25.4 aA	19.8 aB	181.6 aA	138.7 aB
	E^2	70.9 cB	82.6 cA	22.0 abA	16.0 bB	152.7 bcA	116.2 abB
ANOVA							
IR		**		*		*	
AM		*		*		*	
E		ns		ns		ns	
IR × AM		*		*		*	
IR × E		*		*		*	
AM × E		*		*		*	
IR × AM × E		**		*		*	

4
5 Values belonging to the same characteristic without common letters in row for AM inoculation (upper case letter) and in columns for
6 irrigation rate of each ecotype (lower case letter) are statistically different according to LSD (0.05). RWC is relative water content;
7 H2O2 is shoot hydrogen peroxide content; MDA is malondialdehyde. Irrigation rate, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of
8 maximum allowable depletion, respectively; AM$^+$ and AM$^-$ are chicory plant inoculated with arbuscular mycorrhizal, without
9 inoculation, respectively; E^1 and E^2 are Sefid Isfahan and Siyah Shiraz ecotypes, respectively. ns, not significant; * and **, significant
10 at the 0.05 and 0.01 level of probability, respectively.

Table 6. The interaction effects of irrigation levels × AM inoculation × ecotypes on antioxidant enzymes activities, accumulation of antioxidant compounds and proline content in leaves of chicory crop at harvesting (data averaged over 2 years, 2017 and 2018).

Treatment		SOD (U g^{-1} FW)		POD (U g^{-1} FW)		Glutathione (nmol g^{-1} DW)		Ascorbate (nmol g^{-1} DW)		Proline (mg g^{-1} DW)	
		AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$
IR^1	E^1	10.1 cB	12.3 cA	3.5	4.1	17.9 bB	42.9 bA	10.0 bB	26.0 bA	10.5 cB	16.8 bA
	E^2	10.5 cB	12.8 cA	3.9	4.2	20.1 bB	50.0 bA	12.2 bB	28.0 bA	11.0 cB	18.7 bA
IR^2	E^1	11.5 cB	13.9 bcA	3.9	4.5	25.3 abB	51.6 bA	16.1 bB	32.9 abA	15.4 bB	19.7 bA
	E^2	12.1 bcB	14.1 bcA	4.3	4.6	32.4 abB	59.1 abA	20.6 abB	36.7 abA	18.7 abB	22.7 abA
IR^3	E^1	13.1 bB	15.0 bA	4.5	4.5	30.7 abB	61.1 abA	20.8 abB	36.2 abA	17.3 abB	21.5 abA
	E^2	15.1 aB	17.0 aA	4.8	4.8	40.4 aB	71.4 aA	28.6 aB	41.0 aA	20.1 aB	24.5 aA
ANOVA											
IR		*		ns		*		**		*	
AM		*		ns		*		*		*	
E		ns		ns		ns		ns		*	
IR × AM		*		ns		*		*		*	
IR × E		*		ns		*		*		*	
AM × E		*		ns		*		*		*	
IR × AM × E		*		ns		*		*		*	

Values belonging to the same characteristic without common letters in row for AM inoculation (upper case letter) and in columns for irrigation rate of each ecotype (lower case letter) are statistically different according to LSD (0.05). SOD is superoxide dismutase; POD is peroxidase. Irrigation rate, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of maximum allowable depletion, respectively; AM$^+$ and AM$^-$ are chicory plant inoculated with arbuscular mycorrhizal, without inoculation, respectively; E^1 and E^2 are Sefid Isfahan and Siyah Shiraz ecotypes, respectively. ns, not significant; * and **, significant at the 0.05 and 0.01 level of probability, respectively.

Table 7. The interaction effects of irrigation levels × AM inoculation × ecotypes on percent of inulin, mean inulin degree of polymerization ABA and ethylene content in leaves of chicory crop at harvesting (data averaged over 2 years, 2017 and 2018).

Treatment		IP (%)		DP		ABA (ng g FW^{-1})		Ethylene (µmol g^{-1} FW h^{-1})	
		AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$	AM$^-$	AM$^+$
IR^1	E^1	67.6 bB	71.3 bA	26.3 aB	28.6 aA	74.2 bA	55.4 bB	0.5 eA	0.4 dA
	E^2	67.8 bB	72.1 bA	26.5 aB	28.7 aA	74.1 bA	54.9 bB	0.5 eA	0.5 dA
IR^2	E^1	74.5 aB	78.2 aA	26.0 aB	28.5 aA	81.2 bA	60.1 bB	0.8 dA	0.7 cB
	E^2	75.1 aB	79.1 aA	25.9 aB	28.8 aA	82.7 bA	61.3 bB	0.9 cA	0.8 cB
IR^3	E^1	60.0 dB	68.5 cA	21.2 cB	26.8 bA	121.8 aA	90.2 aB	1.1 bA	1.0 bB
	E^2	64.9 cB	68.7 cA	25.0 bB	26.9 bA	135.9 aA	100.4 aB	1.6 aA	1.3 aB
ANOVA									
IR		*		*		*		*	
AM		*		*		*		*	
E		ns		ns		ns		ns	
IR × AM		*		*		*		*	
IR × E		*		*		*		*	
AM × E		*		*		*		*	
IR × AM × E		*		*		*		*	

Values belonging to the same characteristic without common letters in row for AM inoculation (upper case letter) and in columns for irrigation rate of each ecotype (lower case letter) are statistically different according to LSD (0.05). IP is the percent of inulin; DP is mean inulin degree of polymerization. Irrigation rate, IR^1, IR^2 and IR^3 are the 40, 65, and 90% of maximum allowable depletion, respectively; AM$^+$ and AM$^-$ are chicory plant inoculated with arbuscular mycorrhizal, without inoculation, respectively; E^1 and E^2 are Sefid Isfahan and Siyah Shiraz ecotypes, respectively. ns, not significant; * and **, significant at the 0.05 and 0.01 level of probability, respectively.