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Shifts in biochemical and physiological responses by the inoculation of arbuscular mycorrhizal fungi in *Triticum aestivum* growing under drought conditions

Running title: Effects of AMF inoculation on wheat plants growing under drought

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Abstract:

BACKGROUND: A decrease in rainfall is one of the main constraints on wheat production, but the association of wheat with arbuscular mycorrhizal fungi (AMF) may be an alternative for crop production under drought conditions. Here, we used three wheat cultivars (Purple, Ilustre and Maxi Baer) inoculated with two AMF strains, one obtained from the hyperarid Atacama Desert (northern Chile; Fm) and the other obtained from southern Chile (Cc). Plants were maintained under two irrigation conditions (normal irrigation and drought), and the physiological behaviour and enzymatic and nonenzymatic antioxidant activities in the shoots were determined. In addition, the phenolic compounds were identified by HPLC-DAD-ESI-MS/MS and quantified.

RESULTS: AMF colonization produced higher levels of ΦPSII and photosynthetic pigments. High values of catalase in Purple-Cc, ascorbate peroxidase in Purple-Cc, glutathione reductase in Maxi-Cc, and superoxide dismutase in Purple-Cc, all under stress, were registered. Of the inoculated cultivars, Purple-Cc showed the highest flavonoid levels, while hydroxycinnamic acids were higher in Maxi-Fm without

drought with apigenin and luteolin being the most abundant. High levels of phenols were present in the Ilustre-Fm plants without drought. Under normal irrigation, high levels of antioxidant activities were registered in the AMF treatments, whereas under stress conditions, in general, high values were observed under the Fm inoculation.

CONCLUSION: Our results showed that the greatest antioxidant activity and phenolic content occurred in wheat plants inoculated with AMF, indicating their influence on coping with water stress, which is of importance in vast areas where global climate change is resulting in diminished rainfall.

Keywords: antioxidant activity; arbuscular mycorrhizal fungi; phenolic compounds; photosynthesis; water stress; wheat crop.

INTRODUCTION

Global warming has been continuously occurring from the last century to the present, mainly due to the continuous emissions of greenhouse gases that cause adverse effects on agroecosystems, food production, human society and the economy worldwide.[1,2] Some data provided by the Intergovernmental Panel on Climate Change (IPCC) highlight that the estimated anthropogenic global warming increased approximately 1.0±0.2 °C in 2017 from preindustrial levels and will reach 1.5 °C between 2030 and 2052.[3,4] Therefore, measures such as the United Nations Paris Agreement have emerged that focus on limiting global warming below 1.5 °C because a global warming scenario between 1.5 and 2°C can generate negative hydrological, ecological, and social responses.[5,6] One of these adverse responses is intense changes in rainfall patterns, which have increased the frequency of droughts in recent decades[7] and have had a noticeable impact on crop productivity and food security. The above is especially relevant if we consider that approximately 80% of the total crop area is associated with rainfed agricultural systems.[8]

In southern Chile, the La Araucanía region is considered the most important area where wheat (*Triticum aestivum*) is cropped, as it is the main cereal included in annual crop rotations. Wheat has been traditionally cropped under rainfed conditions but is currently affected by severe drought,[9] with a decrease of as much as 50% in rain over the last 5 years. The decrease in water availability to plants affects several biochemical and physiological processes, where the extent of the effects of water deficit depends on the intensity, duration, and genetic capacity of the plants.[10,11] It is well known that drought generates various types of damage to plants, interfering with growth, nutrient-water relationships, and photosynthesis, affecting the plant at different levels and consequently generating a significant reduction

in crop yield.[12,13] In primary metabolism, strong changes in photosynthetic mechanisms and respiration are reflected;[14] notably, the closure of stomata, the structures responsible for the highest proportion of water loss in plants,[15] and the reduction in the assimilation of CO_2 generate a decrease in the photosynthetic rate.[16] The above induces the generation of reactive oxygen species (ROS), which are common in plants exposed to different abiotic stresses.[17]

Plants also respond through antioxidant enzymes such as peroxidase (POX), ascorbate peroxidase (AP), glutathione reductase (GR), superoxide dismutase (SOD), or catalase (CAT), which promote the elimination of ROS formed by different abiotic stress conditions.[18] Furthermore, considering secondary metabolism, plants increase the synthesis of phenolic compounds such as flavonoids to contribute to stress tolerance.[19] Nevertheless, despite the intrinsic plant defence mechanisms, it is necessary to identify new strategies plants can use to cope with drought, allowing plant growth. In this context, the associations between plants and arbuscular mycorrhizal fungi (AMF) are of great importance because AMF increase a plant's resistance to water shortages.[20-23] In particular, several studies have highlighted that in a large number of plants that form AMF, their tolerance to drought improves through various mechanisms, such as i) increases in water uptake due to penetration of hyphae into very small pores inaccessible to roots and AMF mycelium contributions to water redistribution through the soil,[24] ii) the influence on modulation of aquaporin expression,[25-27] iii) improvements in the photosynthetic efficiency,[28,29], iv) increases in plant nutrient uptake,[30-32] and v) enhancements in antioxidant enzyme activities.[33] Thus, AMFs are a promising agronomic bioresource to help wheat plants cope with drought stress.

Despite the well-known role of AMF in increasing the tolerance of host plants to drought, their role in some agroecosystems currently being affected by climate change has been scarcely studied. Therefore, it is relevant to describe the effectiveness of these fungi that are used as bioinoculants in wheat, which is the main agricultural plant species cropped in the area and is currently subjected to strong changes due to increased drought. Based on the above, the aim of this study was to determine the influence of AMF on wheat genotypes in coping with water stress based on physiological responses, contrasting tolerance to drought stress as an effect of AMF colonization in a scenario of water starvation.

MATERIALS AND METHODS

Biological material and experimental design

This study was conducted in a greenhouse under controlled light (16/8 h) and temperature (25 °C/18 °C; day/night) conditions. For the study, a completely randomized 3 x 3 x 2 factorial design with three replicates was used. The main factors consisted of i) three wheat varieties (Purple, Maxi Baer and Ilustre Baer), ii) inoculation with AMF (*Funneliformis mosseae* (Fm), *Claroideoglomus claroideum* (Cc) and one control without AMF inoculation), and iii) irrigation level (field capacity at 29% volumetric water content and drought stress at 21% volumetric water content, equivalent to 50% water holding capacity).

We used seeds of three wheat varieties, which were previously selected based on a screening regarding their osmotic stress tolerance (unpublished data). The seeds were surface sterilized using a Chloramin-T solution (2% w/v) for 5 min and thoroughly washed with sterile distilled water (dH_2O). As an AMF inoculant, we used two different strains of AMF. Strain Fm was isolated from the rhizosphere soil of *Baccharis scandens* plants growing in the Atacama Desert (Camiña valley, Tarapacá Region, characterized by extreme aridity and salinity), while strain Cc was isolated from volcanic soils in southern Chile (Vilcún, La Araucanía Region). Both strains were multiplied in trap pots using a sterile mix of sand and vermiculite (1:1; v:v) as the substrate, and clover, wheat and tagetes were used as host plants for the multiplication process. After six months of growth, the plants were allowed to dry, and the substrate containing AMF spores and mycelium was used as inoculum. To produce mycorrhizal plants, we filled plastic trays (approximately 15 mL in each position) with the corresponding inoculum, and 2 seeds were sown, irrigated daily with dH_2O and maintained for three weeks after germination. The uninoculated treatments were established using the same procedure but directly in the sterile mix substrate. Then, the plantlets were transferred to 2 L pots filled with a sterile mixture of commercial sand, vermiculite and soil (1:1:2; v:v:v). The soil used was an Andisol obtained from Mahuidache locality in Freire city (30°50'32"S; 72°38'43"W, 88 m a.s.l., La Araucanía Region, Chile). The soil was previously sieved at 2 mm, mixed with sand and vermiculite, and autoclaved at 121 °C for 30 min. Both irrigation levels were maintained throughout the experiment by weighing the pots every two days. The above conditions were maintained up to 45 days after transplanting, when all the plants reached the tillering stage (Zadoks 22-23). At this time, all the data for photosynthesis traits were collected using the second youngest leaf, and the same aliquots of fresh biomass were stored in liquid nitrogen until analyses. Additionally, shoot and root biomass was measured.

Photosynthetic determinations

The determination of photosynthetic traits, such as leaf internal concentration of CO_2 (Ci: µmol mol^{-1}), photosynthesis rate (A: µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs: mmol H_2O m^{-2} s^{-1}) and water use efficiency (WUE: mmol CO_2 mol^{-1} H_2O), was carried out using Targas-1 equipment PP Systems, (Amesbury, USA). For data capture, a temperature between 22 and 24°C, an incident quantum of 185 mmol $m^{-1}s^{-1}$, and reference CO_2 of 233.7 and 317.5 µmol mol^{-1} were used. Moreover, continuous fluorescence performance with non-active light (FT: mmol m^{-2} s^{-1}) and efficiency of photosystem II (ΦPSII) were obtained using Fluorpen portable equipment (Photon Systems Instruments, Drasov, Czech Republic) using Fluorpen 1.0 software. The contents of photosynthetic pigments, carotenoids, and chlorophyll "a" and "b" were measured by extraction with 80% acetone,[34] and the analysis was carried out with an Epoch UV/Vis microplate spectrophotometer (BioTek, Winooski, U.S.A) at absorbance rates of 663, 645 and 750 nm.[35]

Total protein quantification and enzymatic antioxidant activity

All wheat leaf samples were extracted, and enzymatic determinations of CAT, APX, GR and SOD were carried out according to Aguilera et al.[18] The Epoch UV-Vis microplate spectrophotometer was used in all the determinations.

Identification and quantification of phenolic compounds

The extraction of phenolic compounds was performed according to the method described by Parada et al.,[36] and identification was performed by means of high performance liquid chromatography with diode array detector coupled to mass spectrometry HPLC-DAD-ESI-MS/MS with a Bruker HCT Ultra Ion Trap mass spectrometer and electrospray ionization (Bremen, Germany) equipped with an Agilent 1100 G1312A pump binary (Waldbronn, Germany), an Agilent 1200 G1329B ALS SL automatic injector (Waldbronn, Germany) and an Agilent 1100 G1316A DAD detector (Waldbronn, Germany). The column used was a Phenomenex Luna C_{18} (150 mm x 2.0 mm, 3 µm) (Aschaffenburg, Germany). The instrument was controlled, and data were collected using Bruker Hystar V.3.2 software (Bremen, Germany). The parameters used were a time analysis of 30 min, the temperature at 40 °C and a flow of 0.2 mL/min. The mobile phases used were 95:3:2 (v/v/v) water:acetonitrile:formic acid (mobile phase A) and 48:50:2 (v/v/v) water:acetonitrile:formic acid (mobile phase B). The detection wavelengths were between 200 and 950 nm. The parameters used for the mass spectrometer were capillary voltage of 3000 V in ESI (+) and ESI (-), nebulizer gas of 50 psi, drying gas of 10 L min^{-1}, drying temperature of 365 °C, and skimmer voltage of 20 V and 70 V of capillary output. The scan range for the analysis was

between 100 and 3000 m/z. The quantification of phenolic compounds was carried out by HPLC-DAD according to Parada et al.[36] using apigenin and chlorogenic acid for external calibration.

Total phenol determination and nonenzymatic antioxidant activity

The determination of total phenols, Trolox equivalent antioxidant capacity (TEAC), cupric reducing antioxidant capacity (CUPRAC), and antioxidant activity by the DPPH method was performed as described by Parada et al.,[36] using gallic acid as a standard for total phenol quantification. Trolox standards were used for the TEAC, CUPRAC, and DPPH methods. A UV-Vis Epoch microplate spectrophotometer (BioTek) was used for analysis, where the determinations were performed at 750 nm for total phenols, 734 nm for TEAC, 450 nm for CUPRAC, and 517 nm for the DPPH method.

Statistical analysis

According to the three-factorial experimental design, a three-way analysis of variance was performed after checking the normality and homoscedasticity of the data sets. The main sources of variation were wheat cultivar (3 levels), AMF inoculation (3 levels), irrigation (2 levels) and the multiple interactions among the above factors. The means among all the treatments (18) were compared using Tukey's multiple-range test. Moreover, the response experimental variables were subjected to a factorial analysis using the extraction of principal components (PC). In all cases, a significance level of $p<0.05$ was established as statistically significant. All analyses were carried out using IBM SPSS Statistics v. 23 (IBM Corp., New York, USA).

RESULTS

Photosynthetic variables

The FT value was higher in Purple cv. than in the other cultivars in all the treatments, especially in the non-AMF under stress treatment (5010 mmol m^{-2} s^{-1}), while under water stress, Ilustre and Maxi cvs. showed their highest values with Cc (Table 1). The ΦPSII value was similar for all treatments, with Ilustre and Maxi cvs., both with Fm and non-AMF, having the highest values, while Purple cv. showed the lowest values. The gs values highly fluctuated in most treatments, with the highest values obtained with Cc and normal irrigation for all cvs., especially for Maxi cv. (92.5 mmol H_2O m^{-2} S^{-1}). Under water stress, Ilustre and Purple cvs. showed higher results with Cc than with Fm. The A value was higher in Maxi cv. inoculated with Cc without water stress (2.53 µmol CO_2 m^{-2} s^{-1}) than in the other treatments. Under stress, Ilustre and Purple cvs. showed higher values when inoculated with Cc than when inoculated with Fm, while Maxi cv. produced a similar trend when inoculated with Fm. The Ci value was higher in Ilustre

cv. inoculated with Fm without water stress (319.8 μmol mol^{-1}) than in the other cultivar treatments, and under stress, the Ilustre and Purple cvs. showed higher values without AMF inoculation. The WUE value was higher in Maxi cv. inoculated with Fm without water stress (6.76 mmol CO_2 mol^{-1} H_2O) than in the other cultivar treatments, and under stress, the Ilustre and Purple cvs. showed higher values when inoculated with Fm than with Cc. On the other hand, pigment values were higher in Maxi cv. with Cc without water stress (0.77 μg g^{-1} of total chlorophyll and 0.29 μg g^{-1} of carotenoids) than in the other cultivar treatments, while under water stress, all cvs. showed higher pigment values when inoculated with Fm than when inoculated with Cc.

Antioxidant enzyme activities

Under normal irrigation, the Ilustre and Maxi Baer cvs. showed higher enzyme activity when inoculated with Cc rather than with Fm, while Purple was the only cv. that exhibited higher values without AMF inoculum. Moreover, in comparison to the other cultivar treatments, the Ilustre-Cc treatment presented higher enzymatic activity levels of 639.4 UA μg^{-1} (CAT), 10.33 UA μg^{-1} (APX), 0.34 UA μg^{-1} (GR) and 67.87 UA μg^{-1} (SOD) (Figure 1).

Under water stress, Ilustre and Purple cvs. had optimal enzymatic activity with the Fm and Cc inoculation, respectively, while Maxi cv. presented higher activity without AMF inoculation. Specifically, the Purple-Cc treatment was optimal under water stress, with enzyme activity levels of 772.1 UA μg^{-1} (CAT), 10.15 UA μg^{-1} (APX), 0.16 UA μg^{-1} (GR) and 51.89 UA μg^{-1} (SOD) (Figure 1).

Identification and quantification of phenolics

All the samples showed similar phenolic profile compositions, including mainly flavones as luteolin and apigenin derivatives, but the samples presented strong variations in their concentrations between treatments (Table 2). Apigenin-6-C-pentoside-8-C-hexoside (*m/z* 565, fragment ions 472.9, 443.0, 383.0, 352.9, 325.0, 296.9 uma) (peak 4) was the most important compound, reaching levels up to 1.54 mg g^{-1} in Ilustre-Cc, followed by 0.89 mg g^{-1} in Maxi-Fm and 0.84 mg g^{-1} in Purple-Fm under water stress.

The concentrations of phenolic compounds in the wheat varieties were determined by HPLC-DAD at 360 nm (Table 3) by external calibration using apigenin as a standard, with the following analytical parameters: a detection limit (DL) of 0.54 mg L^{-1}, quantification limit (QL) of 1.81 mg L^{-1}, and linear range (LR) from 1.81 to 40.0 mg L^{-1}, whereas for hydroxycinnamic acid derivatives (HCAD), 5-caffeoylquinic acid was used for external calibration with a DL of 1.01 mg L^{-1}, QL of 3.35 mg L^{-1} and

linear range from 3.35 to 125 mg L^{-1}. Under water stress, in comparison to the other treatments, the Ilustre-Cc treatment presented a higher concentration of flavones (2.18 mg g^{-1}) and HCADs (89.33 µg g^{-1}). Maxi and Purple cvs. inoculated with Fm reached total concentrations of 1.34 mg g^{-1} of flavonoids and 138.08 and 72.92 µg g^{-1} of HCAD, respectively, but with Cc inoculation, the total flavonoids decreased from 1.58 to 0.38 mg g^{-1} in Purple cv. and from 1.49 to 1.04 mg g^{-1} in Maxi cv. under normal irrigation conditions. For HCAD, the total concentrations decreased from 126.4 to 56.84 µg g^{-1} in Purple cv. and from 155.96 to 31.52 µg g^{-1} in Maxi cv. (Table 3). However, in the Ilustre-Fm treatment, the flavone concentration decreased by 32%, whereas in the Ilustre cv. the decrease was 2.6-fold under water stress compared to that under normal irrigation conditions.

Antioxidant activities determination

Under normal irrigation conditions, high levels of antioxidant activities were registered in the AMF treatments; however, Ilustre and Maxi cvs. did not show a preference for a specific AMF strain, while Purple cv. showed its highest activity when Cc was used (Figure 2). The Ilustre-Fm treatment displayed the highest phenolic concentrations (2.57 mg g^{-1}), followed by Maxi (2.45 mg g^{-1}) and Purple (2.19 mg g^{-1}) cvs. The antioxidant activity in the Purple-Cc treatment was higher than that in the other treatments, especially considering the TEAC and CUPRAC methods. However, these activities were not correlated with the phenolic concentrations. Under water stress conditions (Figure 2), high values were observed with the use of the Fm inoculation, except for TEAC for Ilustre and Purple cvs., which showed higher levels in the treatment with Cc inoculation and non-AMF, respectively.

Regarding photosynthetic traits, the FT, ΦPSII, WUE and Ci parameters showed a high association with the use of both AMF strains, especially for Purple and Ilustre cvs., while A and gs were mostly associated with the use of Fm, especially for Ilustre and Purple cvs. (Figure 3). On the other hand, the concentrations of phenolic compounds were highly correlated among the cultivars and with the nonenzymatic antioxidant activities, which were enhanced with the use of the Fm strain for all wheat varieties. Moreover, the enzymatic activity results were highly correlated among the cultivars but were mostly associated with the use of the Cc strain, especially for Purple and Ilustre cvs. A noticeable correlation was observed between the overall experimental variables and the treatments inoculated with AMF, especially with the use of the Fm strain for Ilustre and Purple cvs., except for the enzymatic antioxidant results, whereas in comparison to Fm, Cc showed a greater association with both previously mentioned cvs. In addition, the non-AMF treatments showed lower associations with the variables, and

in comparison to the other wheats cultivars, Maxi cv. showed the lowest correlation in its treatments with respect to the overall results.

DISCUSSION

Photosynthetic performance

Here, we observed that ΦPSII was usually higher in AMF-colonized plants than in non-AMF-colonized plants, with values over 0.7 μmol CO_2 μmol^{-1}. Relatedly, values near 0.4 to 0.7 have been observed in turgent and healthy plants of pea, wheat and pumpkin,[37] which means that in comparison to that in other plants, the photosynthetic apparatus in these plants was less damaged by drought due to the contribution of AMF colonization,[38] especially in the Purple and Ilustre cvs. Previous study results have shown that AMF colonization can influence bifurcation in photochemical and nonphotochemical events, specifically the ΦPSII of plants subjected and not subjected to salinity.[39] Previous results suggest protection to cope with the photoinhibition of pigments from stress, producing an increase in carotenoids, photosynthetic rate and a higher concentration of leaf chlorophyll,[40] meaning a significant improvement in physiological plant performance.[41] The total chlorophyll and carotenoid concentrations in the inoculated plants significantly increased, which results in an increased dissipation of excess light, thus avoiding photoinhibition.[42] Carotenoids, in addition to absorbing light as accessory pigments in complex light collectors, also act as photoprotective agents in the photochemical apparatus and prevent photooxidative damage to chlorophyll molecules by eliminating ROS.[43,44] The AMF inoculation of Ilustre and Maxi cvs. increased the concentration of carotenoids in the leaves, which may have played an important role in the protection of the photosynthetic apparatus and made it possible to maintain the concentration of chlorophyll in response to water shortage.

The increase in the chlorophyll content in the shoots contributed to a higher photosynthetic rate of AMF-colonized plants, which can be attributed to an increased efficiency in P acquisition, which is a well-known effect of symbiosis.[45-47] This advantageous characteristic of AMF-colonized plants is important since inorganic P is a precursor of phosphorylated intermediates (ADP, ATP and NADPH) in photosynthetic processes such as energy transfer and the metabolism of carbohydrates.[48] Therefore, in comparison to non-AMF plants, AMF plants can cope with the effect of drought on their growth through a primary nutritional role that positively impacts their ability to maintain an improved foliar area and allows a greater assimilation capacity.[13,49,50] On the other hand, *gs* has been widely associated with the photosynthesis rate[51] and the hydric status of plants.[52] For example, Santander et al.[27] found that in

comparison to non-AMF-colonized lettuce plants, AMF-colonized lettuce plants showed higher *g*s values, suggesting that AMF-enhanced photosynthetic efficiency can be partially due to higher water availability for gas exchange, which supports our results. The above implies an increase in CO_2 and water transference between the atmosphere and the mesophyll, which is fundamental to plant growth.[53] In our study, under water stress conditions, inoculation with Cc in Ilustre and Purple cvs. and inoculation with Fm in Maxi cv. resulted in improved behaviour, suggesting functional compatibility at the specific (in the case of AMF) genotypic (in the case of wheat) level.

When the entry of CO_2 into the mesophyll is interrupted by stomatal closure, there is less carboxylation and thus less consumption of the products of photochemical activities, NADPH and ATP. This mechanism promotes excessive energy pressure in the photochemical apparatus that can lead to photoinhibition and decrease the quantum efficiency of photosystem II.[54] On the other hand, stomatic closure could delay the accumulation of antioxidants, producing oxidative stress and metabolic responses associated with drought because of the inhibition of photosynthetic rates leading to a decrease in plant growth.[55] According to the above, the measurements of gas exchange in leaves allowed us to determine the net photosynthesis and transpiration and the water use efficiency at the foliar scale as the quotient of these two characteristics.[56] The above is related to the increase in water absorption, such as absorption of water by external AMF hyphae, stomatal regulation through hormonal signals, and greater osmotic adjustment in AMF-colonized plants, thus promoting turgor maintenance even at low tissue water potentials. There is also an indirect effect of AMF, including photosynthetic activity, proline accumulation and increased nutritional status, in mycorrhizal plants.[57,58] In this case, a higher WUE was registered with the use of Cc than with Fm in Ilustre and Purple cvs., which means the plants need to consume a decreased volume of water for the incorporation of atmospheric carbon.

Antioxidant enzyme activities

The contribution of AMF to increasing enzymatic activity to prevent the accumulation of ROS is well known, and the antioxidant system is one of the best ways to cope with environmental stress.[59] Regarding our results, this can be observed in Purple and Ilustre cvs. under water stress, which showed high enzymatic activity in the AMF treatments, especially in the Purple cv. inoculated with the Cc strain. However, under normal irrigation conditions, Purple cv. did not show its maximum level of activities in the AMF treatments but displayed the greatest activities without inoculation, while Ilustre and Maxi cvs. had their highest activity in the AMF treatments. The above results suggest the occurrence of a species-

specific influence of AMF depending on the hydric status of the host plant, where the activation of antioxidant enzymes by AMF does not always occur under adverse environmental conditions.[60] Moreover, AMF-enhanced antioxidant defence systems are dependent on the combination of fungal species, the host plant, and micronutrient availability.[61] In agreement with the above, it was possible to identify a close relationship between AMF and wheat but not a selective interaction between AMF and the cvs., with the latter mainly dependent on the water conditions. When a greater the volume of soil can be explored by fungal hyphae, the absorption of water and low-mobility nutrients in the soil, such as Fe, Zn and Cu,[62] which play an important role in the enzymatic activity of SOD, is enhanced.

Considering the different enzyme activities, the role of the AMF status is noticeable, where SOD has been previously reported as the main beneficial enzyme activity enhanced by AMF.[63] This enzyme is the first line of defence against ROS, converting O_2 into H_2O_2, which is further eliminated by CAT and APX as water and oxygen.[64] On the other hand, GR catalyses the reduction of GSH,[65] and its increased activity in AMF-colonized roots has also been reported.[66] Otherwise, CAT and APX are usually induced by oxidative stress but are also induced in the early stages of AMF colonization and subsequently repressed.[67]

Identification and quantification of phenolics

Even though the enhancement in antioxidant defence systems caused by AMF colonization may occur because the fungi can accumulate ROS,[68] this process may also induce a higher accumulation of other antioxidants in plants, mainly anthocyanins, carotenoids, and flavonoids and to a lesser extent of chlorophyll and phenolics, to cope with oxidative damage.[60,69] In this study, on the wheat leaves, only flavonoids were identified by HPLC-MS, and six flavones (five luteolin and one apigenin derivative) and one flavanol (kaempferol) were identified. Our results are similar to those reported by Olenichenko et al.[70] where mainly C-glycosides of two flavones, apigenin and luteolin, and their O-derivatives were identified. Furthermore, these compounds have also been found in the leaves of two cultivars of spring wheat.[70] More specifically, the most abundant compound reported was apigenin-C-hexoside-pentoside, which is commonly found in herbs, fruits, and vegetables in the form of a yellow pigment. This compound is considered to be a phytoestrogen due to its pharmacological and biological characteristics.[71] Here, the shifts in concentrations generated by each AMF strain depending on the wheat cultivar were remarkable.

Antioxidant activity determinations

In general, the AMF-colonized plants showed increased activities, which highlights the influence of AMF on the synthesis of secondary metabolites such as flavonoids and the increase in antioxidant activity in plants. Under water stress conditions, the antioxidant activities increased with Fm inoculation, which can increase plant tolerance to environmental stresses[72] and could also have been related to an increased synthesis of total phenols.[73] However, it should be noted that none of the flavonoids identified here could be clearly correlated with an increase in antioxidant activities. Moreover, under normal irrigation, it was not possible to observe an influence of a specific AMF strain, showing that AMF under non-water stress conditions have a less determining effect on plant growth and antioxidant response.

At the photosynthetic level, FT and Ci were highly associated with the use of Fm in Ilustre cv. under normal irrigation and Purple cv. under drought. On the other hand, the concentrations of pigments such as chlorophyll and carotenoids were grouped preferentially with individuals in the Ilustre-Fm treatment. Finally, *gs* and A were highly associated with the Purple-Fm and Maxi-Fm treatments under water stress. Notably, Fm was the strain that showed the highest responses for all wheat cvs., which could be partially explained by its origin in environments subjected to strong osmotic stress in the Atacama Desert, the most hyperarid ecosystem in the world. The above results also support the beneficial role of AMF (and other beneficial microorganisms) on photosynthetic performance.[74,75] On the other hand, the enzymatic assays (CAT, APX, GR and SOD) were mainly associated with Purple cv., especially in the treatments under water stress inoculated with Cc and in the control that was not inoculated with AMF, where Purple-Cc reached values higher than the other treatments. Regarding the antioxidant activity, TEAC, CUPRAC, DPPH and total phenols were highly associated with the treatments Purple-Fm under drought and Purple under normal irrigation, which is not entirely consistent with the concentrations of some flavonoids (peaks 1-6) and HCADs, since these included the Maxi-Fm treatments under water stress and Maxi-Cc and Ilustre both under normal irrigation. Moreover, the correlation values were generally low (between 0.26 and 0.5), where the highest values were for total phenols and DPPH with respect to apigenin 6-C-pentoside-8-C-hexoside (0.355 and 0.373, respectively), while CUPRAC and TEAC showed better correlations with luteolin 6-C hexose O-hexose (0.25 and 0.425, respectively). Based on the above, it was not possible to confirm an increased antioxidant effect from flavonoids in wheat plants, which suggests the participation of other compounds present in this plant species that also increase antioxidant activity. Some previous reports have indicated that melatonin (N-acetyl-5-methoxytryptamine) could have an important antioxidant capacity, therefore

helping cope with drought stress,[76] which was demonstrated by its direct interaction with ROS and the modulation of enzyme antioxidant activities and antioxidant capacity in response to excessive ROS.[77] As described by Cui et al.[77] a melatonin treatment remarkably increased the drought tolerance of wheat seedlings, as determined by increased antioxidant capacity, decreased endogenous ROS levels and increased enzyme activity, such as GSH, among other plant benefits. Previous research has reported that when applied exogenously, melatonin alleviates oxidative damage by scavenging ROS and stimulating antioxidant systems under various environmental stresses, such as ozone damage, cold stress, drought stress, UV damage, photooxidation and copper toxicity; thus, melatonin could be another generator of antioxidant activity closely related to the production of phenols. The above allowed us to suggest that cvs. Ilustre and Maxi respond with higher nonenzymatic antioxidant activity, and Purple cv. presents an intrinsically higher enzymatic response.

CONCLUSIONS

Inoculation with different strains of arbuscular mycorrhizal fungi (AMF), irrespective of the origin, generates a significant contribution to plant performance, as evidenced by an improvement in photosynthetic behaviour and antioxidant response. However, the above improvement can be categorized at different levels depending on the specific combination of wheat cultivar and AMF strain, which suggests the presence of functional compatibility at the subspecies level. Noticeably, the beneficial responses mostly increased under drought conditions with the fungus *Funneliformis mosseae*, which was isolated from the Atacama Desert, suggesting that an intrinsic capacity of this fungus to cope with osmotic stress is transferred to host plants, as was observed especially for Ilustre and Maxi cvs. Our results not only provide more evidence about the beneficial role of AMF in providing tolerance to water shortages but also represent support for the potential to increase the beneficial responses of arbuscular mycorrhizal symbiosis, optimizing the use of both the host and the fungus even at the subspecies level.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

1. Wu S, Wang L, Cai Q, Yan X, Potential impact assessment for China's dry and wet areas under global warming targets. *Phys Chem Earth* **116**:102838 (2020).
2. Zhang F, Zou YN, Wu QS, Kuča K, Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. *Environ. Exp. Bot* **171**:103926 (2020).
3. Li Z, Fang G, Chen Y, Duan W, Mukanov Y, Agricultural water demands in Central Asia under 1.5 °C and 2.0 °C global warming. *Agric. Water Manag* **231**: 106020 (2020).
4. Wijesiri B, Liu A, Goonetilleke A, Impact of global warming on urban stormwater quality: From the perspective of an alternative water resource. *J. Clean. Prod* **262**:121330 (2020).
5. Ma X, Zhao C, Tao H, Zhu J, Kundzewicz ZW, Projections of actual evapotranspiration under the 1.5 °C and 2.0 °C global warming scenarios in sandy areas in northern China. *Sci. Total Environ* **645**:1496–1508 (2018).
6. Templ B and Calanca P, Critical increase in the occurrence of heat stress during reproductive growth in Russian wheat beyond 1.5 C global warming. *Weather. Clim. Extremes* **30**:100281 (2020).
7. Bai X, Shen W, Wu X, Wang P, Applicability of long-term satellite-based precipitation products for drought indices considering global warming. *J. Environ. Manage* **255**:109846 (2020).
8. Mphande W, Kettlewell PS, Grove IG, Farrell AD, The potential of antitranspirants in drought management of arable crops: A review. *Agric. Water Manag* **236**:106143 (2020).
9. Zambrano-Bigiarini M, Baez-Villanueva OM, Characterizing meteorological droughts in data scare regions using remote sensing estimates of precipitation, in *Extreme Hydroclimatic Events and Multivariate Hazards in a Changing Environment - A remote sensing approach*, ed. by Maggioni V, Massari C, Elsevier, Amsterdam, pp. 221–246 (2019).
10. Bernardo L, Carletti P, Badeck FW, Rizza F, Morcia C, Ghizzoni R, Rouphael Y, Colla G, Terzi V, Lucini L, Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. *Plant Physiol Biochem* **137**:203–212 (2019).
11. Ihuoma SO, Madramootoo, CA, Recent advances in crop water stress detection. Comput Electron Agr **141**:267–275 (2017).

12. Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J, Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Front. Plant Sci* **8**:1147 (2017).
13. Santander C, Aroca R, Ruiz-Lozano JM, Olave J, Borie F, Cornejo P, Arbuscular mycorrhiza effects on plant performance under osmotic stress. *Mycorrhiza* **27**:639–657 (2017).
14. Guan K, Pan M, Li H, Wof A, Wu J, Medvigy D, Caylor K, Sheffield J, Wood E, Malhi Y, Liang M, Kimball J, Saleska S, Berry J, Joiner J, Lyapustin A, Photosynthetic seasonality of global tropical forests constrained by hydroclimate. *Nat. Geosci* **8**:284–289 (2015).
15. Taiz L and Zeiger E, Water Balance of Plants, in *Plant Physiology*, ed. by Taiz L, Zeiger E. Sinauer Associates, Sunderland, p.57 (2010).
16. Saibo NJM, Lourenço T, Oliveira MM, Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. *Ann. Bot* **103**:609–623 (2008).
17. Cruz de Carvalho MH, Drought stress and reactive oxygen species. *Plant Signal. Behav* **3**:156–165 (2008).
18. Aguilera A, Tereucán G, Ercoli S, Cornejo P, Gomez MR, Uhlmann L, Guigas C, Esatbeyoglu T, Ruiz A, Influence of Organic and Chemical Fertilisation on Antioxidant Compounds Profiles and Activities in Fruits of *Fragaria ananassa* var. Camarosa. *J. Soil Sci. Plant Nutr* **20**:715–724 (2020).
19. Santander C, Ruiz A, García S, Aroca R, Cumming J, Cornejo P, Efficiency of two arbuscular mycorrhizal fungal inocula to improve saline stress tolerance in lettuce plants by changes of antioxidant defense mechanisms. *J. Sci. Food Agric* **100**:1577–1587 (2020).
20. Cornejo P, Pérez-Tienda J, Meier S, Valderas A, Borie F, Azcón-Aguilar C, Ferrol N, Copper compartmentalization in spores as a survival strategy of arbuscular mycorrhizal fungi in Cu-polluted environments. *Soil Biol Biochem* **57**:925–928 (2013).
21. Cornejo P, Seguel A, Aguilera P, Meier S, Larsen J, Borie F, Arbuscular Mycorrhizal Fungi Improve Tolerance of Agricultural Plants to Cope Abiotic Stress Conditions, in *Plant-Microbe Interactions in Agro-Ecological Perspectives*, ed. by Singh DP, Singh HB, Prabha R. Springer, Singapore, pp. 55–80 (2017).
22. de Novais CB, Sbrana C, da Conceição Jesus E, Rouws LFM, Giovannetti M, Avio L, Siqueira JO, Saggin Júnior OJ, da Silva EMR, de Faria SM, Mycorrhizal networks facilitate the colonization of legume roots by a symbiotic nitrogen-fixing bacterium. *Mycorrhiza* **30**:389–396 (2020).

23. Emmanuel OC and Babalola OO, Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. *Microbiol. Res* **239**:126569 (2020).
24. Rydlová J and Püschel D, Arbuscular mycorrhiza, but not hydrogel, alleviates drought stress of ornamental plants in peat-based substrate. *Appl. Soil Ecol* **146**:103394 (2020).
25. Bárzana G, Aroca R, Bienert GP, Chaumont F, Ruiz-Lozano JM, New Insights into the Regulation of Aquaporins by the Arbuscular Mycorrhizal Symbiosis in Maize Plants Under Drought Stress and Possible Implications for Plant Performance. *Mol Plant Microbe Interact* **27**:349–363 (2014).
26. Mbodj D, Effa-Effa B, Kane A, Manneh B, Gantet P, Laplaze L, Diedhiou AG, Grondin A, Arbuscular mycorrhizal symbiosis in rice: establishment, environmental control and impact on plant growth and resistance to abiotic stresses. *Rhizosphere* **8**:12–26 (2018).
27. Santander C, Aroca R, Cartes R, Vidal G, Cornejo P, Aquaporins and cation transporters are differentially regulated by two arbuscular mycorrhizal fungi strains in lettuce cultivars growing under salinity conditions. *Plant Physiol Biochem* **158**:396–409 (2021).
28. Rozpądek P, Rąpała-Kozik M, Wężowicz K, Grandin A, Karlsson S, Ważny R, Anielska T, Turnau K, Arbuscular mycorrhiza improves yield and nutritional properties of onion (*Allium cepa*). *Plant Physiol. Biochem* **107**:264–272 (2016).
29. Zhang T, Hu Y, Zhang K, Tian C, Guo J, Arbuscular mycorrhizal fungi improve plant growth of *Ricinus communis* by altering photosynthetic properties and increasing pigments under drought and salt stress. *Ind Crops Prod* **117**:13–19 (2018).
30. Begum N, Ahanger MA, Zhang L, AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in *Nicotiana tabacum* by up-regulating antioxidant metabolism and osmolyte accumulation. *Environ. Exp. Bot* **176**:104088 (2020).
31. Ferrol N, Azcón-Aguilar C, Pérez-Tienda J, Review: Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: An overview on the mechanisms involved. *Plant Sci* **280**:441–447 (2019).
32. Tarraf W, Ruta C, Tagarelli A, De Cillis F, De Mastro G, Influence of arbuscular mycorrhizae on plant growth, essential oil production and phosphorus uptake of *Salvia officinalis* L. *Ind Crops Prod* **102**:144–153 (2017).

33. Benhiba L, Fouad MO, Essahibi A, Ghoulam C, Qaddoury A, Arbuscular mycorrhizal symbiosis enhanced growth and antioxidant metabolism in date palm subjected to long-term drought. *Trees* **29:**1725–1733 (2015).
34. Lichtenthaler HK, Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes, in *Methods in Enzymology*, ed. by Douce R and Packer L. Academic Press, New York, pp. 350–382 (1987)
35. Strain HH, Svec WA, Extraction, Separation, Estimation, and Isolation of the Chlorophylls, in *The Chlorophyll*, ed. by Vernon LP and Seely GR. Academic Press, New York, pp. 21–66 (1966).
36. Parada J, Valenzuela T, Gómez F, Tereucán G, García S, Cornejo P, Winterhalter P, Ruiz A, Effect of fertilization and arbuscular mycorrhizal fungal inoculation on antioxidant profiles and activities in *Fragaria ananassa* fruit. *J. Sci. Food Agric* **99**:1397–1404 (2019).
37. Yudina L, Sukhova E, Gromova E, Nerush V, Vodeneev V, Sukhov V, A light-induced decrease in the photochemical reflectance index (PRI) can be used to estimate the energy-dependent component of non-photochemical quenching under heat stress and soil drought in pea, wheat, and pumpkin. *Photosynth. Res* **146:**175–187 (2020).
38. Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F, Antioxidative defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol* **119**:1091–1099 (1999).
39. Sheng M, Tang M, Chen H, Yang B, Zhang F, Huang Y, Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* **18**:287–296 (2008).
40. Abo-Ghalia H, Khalafallah A, Reponses of wheat plants associated with arbuscular mycorrhizal fungi to short-term water stress followed by recovery at three growth stages. *J Appl Sci* **4**:570–580 (2009).
41. Steele MR, Gitelson AA, Rundquist DC, A comparaison of two techniques for nondestructive measurement of chlorophyll content in grapevine leaves. *J. Agron* **100**:779–782 (2008).
42. Muñoz P, Briones M, Munné-Bosch S, Photoinhibition and photoprotection during flower opening in lilies. *Plant Science* **272**:220-229 (2018).
43. Rossini Oliva S, Mingorance MD, Valdés B, Leidi EO, (2010). Uptake, localisation and physiological changes in response to copper excess in *Erica andevalensis*. *Plan Soil* **328**:411–420 (2010).

44. Houri T, Khairallah Y, Zahab AA, Osta B, Romanos D, Haddad G, Heavy Metals Accumulation Effects on The Photosynthetic Performance of Geophytes in Mediterranean Reserve. *Journal of King Saud University – Science* **32**:874–880 (2020).
45. Borie F, Aguilera P, Castillo C, Valentine A, Seguel A, Barea JM, Cornejo P, Revisiting the Nature of Phosphorus Pools in Chilean Volcanic Soils as a Basis for Arbuscular Mycorrhizal Management in Plant P Acquisition. *J Soil Sci Plant Nutr* **19**:390–401 (2019).
46. Tran CTK, Watts-Williams SJ, Smernik RJ, Cavagnaro TR, Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching. *Sci. Total Environ* **722**: 137847 (2020).
47. de Souza Campos PM, Borie F, Cornejo P, Meier S, López-Ráez JA, López-Garcia A, Seguel A, Wheat root trait plasticity, nutrient acquisition and growth responses are dependent on specific arbuscular mycorrhizal fungus and plant genotype interactions. *J. Plant Physiol* **256**:153297 (2020).
48. Singh SK, Badgujar GB, Reddy VR, Fleisher DH, Timlin DJ, Effect of phosphorus nutrition on growth and physiology of cotton under ambient and elevated carbon dioxide. *J Agron Crop Sci* **199**:436–448 (2013).
49. Oyewole BO, Olawuyi OJ, Odebode AC, Abiala MA, Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. *Biotechnol. Rep* **14**:8–15 (2017).
50. Arpanahi AA, Feizian M, Mehdipourian G, Khojasteh DN, Arbuscular mycorrhizal fungi inoculation improve essential oil and physiological parameters and nutritional values of *Thymus daenensis* Celak and *Thymus vulgaris* L. under normal and drought stress conditions. *Eur. J. Soil Biol* **100**:103217 (2020).
51. Faralli M, Matthews J, Lawson T, Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement. *Curr. Plant Biol*. **49**:1–7 (2019).
52. Quiroga G, Erice G, Ding L, Chaumont F, Aroca R, Ruiz-Lozano JM, The arbuscular mycorrhizal symbiosis regulates aquaporins activity and improves root cell water permeability in maize plants subjected to water stress. *Plant Cell Environ* **42**:2274–2290 (2019).
53. Xiong D, Douthe C, Flexas J, Differential coordination of stomatal conductance, mesophyll conductance and leaf hydraulic conductance in response to changing light across species. *Plant Cell Environ* **41**:436–450 (2018).

54. Sales CRG, Ribeiro RV, Silveira JAG, Machado EC, Martins MO, Lagôa AM, Superoxide dismutase and ascorbate peroxidase improve the recovery of photosynthesis in sugarcane plants subjected to water deficit and low substrate temperature. *Plant Physiol Biochem* **73**:326–336 (2013).
55. Flexas J, Niinemets U, Gallé A, Barbour MM, Centritto M, Diaz-Espejo A, Douthe C, Galmés J, Ribas-Carbo M, Rodriguez PL, Rosselló F, Soolanayakanahally R, Tomas M, Wright IJ, Farquhar GD, Medrano H, Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynth. Res* **117**:45–59 (2013).
56. Yu L, Gao X, Zhao X, Global synthesis of the impact of droughts on crops' water-use efficiency (WUE): Towards both high WUE and productivity. Agric. Syst. **177**:102723 (2020).
57. Zhu X, Song F, Liu S, Liu T, Zhou X, Arbuscular mycorrhizae improve photosynthesis and water status of *Zea mays* L. under drought stress. *Plant Soil Environ* **58**:186–191 (2012).
58. Santander C, Sanhueza M, Olave J, Borie F, Valentine A, Cornejo P, Arbuscular mycorrhizal colonization promotes the tolerance to salt stress in lettuce plants through an efficient modification of ionic balance. *J. Soil Sci. Plant Nutr* **19**:321–331 (2019).
59. Gao W, Zhang Y, Feng Z, Bai Q, He J, Wang Y, Effects of Melatonin on Antioxidant Capacity in Naked Oat Seedlings under Drought Stress. *Molecules* **23**:1580 (2018).
60. Abbaspour H, Saeidi-Sar S, Afshari H, Abdel-Wahhab MA, Tolerance of Mycorrhiza infected Pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions. *J. Plant Physiol* **169**:704–709 (2012).
61. Ruíz-Lozano JM, Porcel R, Azcón C, Aroca R, Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *J. Exp. Bot* **63**:695–709 (2012).
62. Navarro García A, del Pilar Bañón Árias S, Morte A, Sánchez-Blanco MJ, Effects of nursery preconditioning through mycorrhizal inoculation and drought in *Arbutus unedo* L. plants. *Mycorrhiza* **21**:53–64 (2011).
63. Huang YM, Srivastava AK, Zou YN, Ni QD, Han Y, Wu QS, Mycorrhizal-induced calmodulin mediated changes in antioxidant enzymes and growth response of drought-stressed trifoliate orange. *Front. Microbiol* **5** (2014).
64. Sharma P, Jha AB, Dubey RS, Pessarakli M, Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Bot*. **2012**:1–26 (2012).

65. Mallick N, Mohn FH, Reactive oxygen species: response of algal cells. *J. Plant Physiol* **157**:183–193 (2000).
66. Wu QS, Xia RX, Zou YN, Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. *J. Plant Physiol* **163**:1101–1110 (2006).
67. Gianinazzi-Pearson V, Plant cell response to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. *Plant Cell* **8**:1871–1883 (1996).
68. Fester T and Hause G, Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. *Mycorrhiza* **15**:373–379 (2005).
69. Baslam M and Goicoechea N, Water deficit improves the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. *Mycorrhiza* **22**:347–359 (2012).
70. Olenichenko NA, Ossipov VI, Zagoskina NV, Effect of cold hardening on the phenolic complex of winter wheat leaves. *Russ. J. Plant Physiol* **53**:495–500 (2006).
71. Liu XL, Zhang HF, Qiao GJ, Cao W, Zheng JB, Determination of Apigenin by LC with Electrochemical Detection. *Chromatographia* **68**:147–150 (2008).
72. Gholinezhad E, Darvishzadeh R, Siavash Moghaddam S, Popović-Djordjević J, Effect of mycorrhizal inoculation in reducing water stress in sesame (Sesamum indicum L.): The assessment of agrobiochemical traits and enzymatic antioxidant activity. *Agric. Water Manag* **238:**106234 (2020).
73. Naikoo MI, Dar MI, Raghib F, Jaleel H, Ahmad B, Raina A, Khan FA, Naushin F, Role and Regulation of Plants Phenolics in Abiotic Stress Tolerance, in *Plant Signaling Molecules*, ed. by Khan MIR, Reddy PS, Ferrante A, Khan NA. Woodhead Publishing, Sawston, pp. 157–168 (2019).
74. Mathur S, Sharma MP, Jajoo, A. Improved photosynthetic efficacy of maize (*Zea mays*) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. *J Photochem Photobiol B* **180**:149-154 (2018).
75. Moustakas M, Bayçu G, Sperdouli I, Eroglu H, Eleftheriou EP, Arbuscular Mycorrhizal Symbiosis Enhances Photosynthesis in the Medicinal Herb *Salvia fruticosa* by Improving Photosystem II Photochemistry. *Plants* **9:**962 (2020).

76. Meng JF, Xu TF, Wang ZZ, Fang YL, Xi ZM, Zhang ZW, The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. *J. Pineal Res* **57**:200–212 (2014).

77. Cui G, Zhao X, Liu S, Sun F, Zhang C, Xi Y, Beneficial effects of melatonin in overcoming drought stress in wheat seedlings. *Plant Physiol Biochem* **118**:138–149 (2017).

Figure captions

Figure 1: Specific activity (UA μg^{-1}) of enzymes catalase (A, E), ascorbate peroxidase (B, F), glutathione reductase (C, G) and superoxide dismutase (D, H) growing without (upper subfigures) or with drought conditions (lower subfigures). The arbuscular mycorrhizal fungi species inoculated were *Claroideoglomus claroideum*, *Funneliformis mosseae*, and treatments non-inoculated (No AMF). The wheat cultivars evaluated corresponded to Ilustre Baer, Maxi Baer and Purple. Different lowercase letters indicate statistic difference according to Tukey's multiple range test ($p < 0.05$). Data are the mean ± SE, n = 3.

Figure 2: Antioxidant activities (mmol Trolox equivalents g^{-1}) of TEAC (A, E), CUPRAC (B, F), DPPH (C, G) and total phenols (D, H) growing without (upper subfigures) or with drought conditions (lower subfigures). The arbuscular mycorrhizal fungi species inoculated were *Claroideoglomus claroideum*, *Funneliformis mosseae*, and treatments non-inoculated (No AMF). The wheat cultivars evaluated corresponded to Ilustre Baer, Maxi Baer and Purple. Different lowercase letters indicate statistic difference according to Tukey's multiple range test ($p < 0.05$). Data are the mean ± SE, n = 3.

Figure 3: (A) Principal component (PC) scores for the experimental variables determined in plants of three cultivars of *Triticum aestivum* inoculated or not with different strains of arbuscular fungi (AMF) and growing under drought or non-limiting water conditions. The percentage values in parentheses indicate the variation explained by each PC. The plot shows the distribution of the experimental individuals according to the PCs and grouped according to (B) the wheat cultivar, (C) the type of AMF inoculum, and (D) the water condition. CC = *Claroideoglomus claroideum*, FM = *Funneliformis mosseae*, NI = Non-inoculated. Experimental variables corresponds to: AP: Ascorbate peroxidase, Cat: Catalase, SOD: Superoxide dismutase, GR: Glutathione reductase; Flav: Flavonoids; AHC1: Hydroxycinnamic acid, CUPRAC, DPPH, Folin, TEAC (Non-enzymatic assays), Continuous fluorescence performance in non-actinic light (FT), quantum yield of photosystem II (ΦPSII), internal CO_2 concentration in the leaf (Ci), rate of photosynthesis (A), stomatal conductance (gs), water use efficiency (WUE), Total chlorophylls (ChlT), chlorophyll concentration a (Chla), chlorophyll concentration b (Chlb) and carotenes (Carot).

Table 1. Photosynthetic traits measured in plants of *Triticum aestivum* (wheat) of three commercial cultivars inoculated or not with two arbuscular mycorrhizal fungi (AMF) and growing with or without drought stress. F-values and significances for the main effect of influencing variables are included. Different letters in a column represent statistic significant differences according with Tukey's test (P<0.05).

Wheat cultivar	AMF	Drought	FT	ΦPSII	Gs	A	Ci	WUE	Chl-a	Chl-b	Chl-total	Carotenoids
Ilustre	*C. claroideum*	NO	3287 ± 156 ^{g}	0.71 ± 0.02 ^{b}	65.7 ± 7.1 ^{ab}	1.78 ± 0.21 ^{abc}	250.6 ± 3.1 ^{bc}	2.10 ± 0.13 ^{b}	0.36 ± 0.04 ^{cdef}	0.11 ± 0.01 ^{cdef}	0.48 ± 0.05 ^{cdef}	0.21 ± 0.02 ^{def}
		YES	3451 ± 72 ^{efg}	0.75 ± 0.01 ^{ab}	55.8 ± 8.5 ^{bcd}	1.80 ± 0.25 ^{abc}	240.4 ± 2.5 ^{bcd}	2.85 ± 0.14 ^{b}	0.44 ± 0.03 ^{bcd}	0.14 ± 0.02 ^{bc}	0.58 ± 0.05 ^{bc}	0.25 ± 0.01 ^{abcde}
	F. mosseae	NO	3603 ± 97 ^{defg}	0.76 ± 0.01 ^{ab}	58.1 ± 12.2 ^{abc}	2.20 ± 0.27 ^{ab}	319.8 ± 8.2 ^{a}	3.12 ± 0.34 ^{b}	0.30 ± 0,02 ^{ef}	0.08 ± 0.01 ^{f}	0.37 ± 0.03 ^{ef}	0.25 ± 0.01 ^{abcde}
		YES	3358 ± 113 ^{g}	0.76 ± 0.00 ^{ab}	27.5 ± 8.7 ^{cde}	1.60 ± 0.16 ^{abc}	203.8 ± 28.6 ^{cd}	5.67 ± 0.91 ^{ab}	0.47 ± 0.02 ^{abc}	0.15 ± 0.01 ^{bc}	0.61 ± 0.03 ^{bc}	0.26 ± 0.01 ^{abc}
	No AMF	NO	3469 ± 86 ^{efg}	0.77 ± 0.00 ^{a}	31.0 ± 1.8 ^{bcde}	1.47 ± 0.22 ^{bc}	260.7 ± 8.6 ^{abc}	3.05 ± 0.40 ^{b}	0.37 ± 0.02 ^{cdef}	0.11 ± 0.01 ^{cdef}	0.48 ± 0.02 ^{cdef}	0.22 ± 0.01 ^{cde}
		YES	3426 ± 72 ^{fg}	0.77 ± 0.01 ^{ab}	28.0 ± 3.8 ^{cde}	1.49 ± 0.15 ^{bc}	287.5 ± 13.1 ^{ab}	3.16 ± 0.18 ^{b}	0.41 ± 0.01 ^{cd}	0.12 ± 0.00 ^{cde}	0.53 ± 0.01 ^{cd}	0.24 ± 0.00 ^{bcde}
Maxi	*C. claroideum*	NO	3805 ± 43 ^{cdefg}	0.77 ± 0.00 ^{ab}	92.5 ± 6.5 ^{a}	2.53 ± 0.14 ^{a}	263.1 ± 7.8 ^{abc}	2.82 ± 0.23 ^{b}	0.56 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.77 ± 0.01 ^{a}	0.29 ± 0.00 ^{a}
		YES	3994 ± 177 ^{bcde}	0.71 ± 0.04 ^{ab}	35.1 ± 7.1 ^{bcde}	1.27 ± 0.23 ^{bc}	269.9 ± 12.0 ^{abc}	3.92 ± 0.65 ^{b}	0.39 ± 0.03 ^{cde}	0.11 ± 0.01 ^{cdef}	0.50 ± 0.03 ^{cdef}	0.23 ± 0.01 ^{cde}
	F. mosseae	NO	3644 ± 55 ^{cdefg}	0.76 ± 0.00 ^{ab}	17.5 ± 9.9 ^{de}	1.19 ± 0.30 ^{bc}	172.5 ± 24.4 ^{ab}	6.76 ± 2.91 ^{a}	0.52 ± 0.01 ^{ab}	0.18 ± 0.01 ^{ab}	0.70 ± 0.02 ^{ab}	0.29 ± 0.00 ^{ab}
		YES	3758 ± 90 ^{cdefg}	0.77 ± 0.01 ^{ab}	44.4 ± 7.8 ^{bcde}	1.85 ± 0.15 ^{abc}	245.0 ± 9.8 ^{bc}	2.83 ± 0.31 ^{b}	0.45 ± 0.02 ^{abcd}	0.15 ± 0.01 ^{bc}	0.59 ± 0.03 ^{bc}	0.26 ± 0.01 ^{abcd}
	No AMF	NO	3522 ± 75 ^{defg}	0.77 ± 0.00 ^{ab}	30.3 ± 4.7 ^{cde}	1.62 ± 0.20 ^{abc}	270.2 ± 11.3 ^{abc}	3.35 ± 0.40 ^{b}	0.40 ± 0.01 ^{cde}	0.12 ± 0.00 ^{cde}	0.52 ± 0.01 ^{cd}	0.24 ± 0.01 ^{bcde}
		YES	3818 ± 150 ^{cdefg}	0.74 ± 0.01 ^{ab}	14.8 ± 2.3 ^{e}	1.27 ± 0.10 ^{bc}	174.2 ± 16.0 ^{d}	4.85 ± 0.54 ^{ab}	0.39 ± 0.01 ^{cde}	0.14 ± 0.00 ^{cd}	0.52 ± 0.01 ^{cde}	0.24 ± 0.00 ^{bcde}
Purple	*C. claroideum*	NO	4459 ± 113 ^{b}	0.74 ± 0.01 ^{ab}	46.9 ± 9.8 ^{bcde}	1.73 ± 0.26 ^{abc}	238.7 ± 14.4 ^{bcd}	2.82 ± 0.46 ^{b}	0.28 ± 0.02 ^{f}	0.09 ± 0.01 ^{ef}	0.36 ± 0.03 ^{f}	0.17 ± 0.01 ^{f}
		YES	3958 ± 34 ^{bcdefg}	0.74 ± 0.01 ^{ab}	46.5 ± 6.1 ^{bcde}	1.81 ± 0.20 ^{abc}	256.9 ± 4.2 ^{abc}	2.47 ± 0.16 ^{b}	0.27 ± 0.01 ^{f}	0.09 ± 0.00 ^{ef}	0.36 ± 0.01 ^{f}	0.16 ± 0.01 ^{f}
	F. mosseae	NO	4187 ± 62 ^{bc}	0.74 ± 0.01 ^{ab}	41.3 ± 6.7 ^{bcde}	1.80 ± 0.30 ^{abc}	240.9 ± 4.9 ^{bcd}	2.68 ± 0.17 ^{b}	0.37 ± 0.03 ^{cde}	0.12 ± 0.01 ^{cde}	0.49 ± 0.03 ^{cdef}	0.21 ± 0.01 ^{cdef}
		YES	4375 ± 125 ^{b}	0.72 ± 0.01 ^{ab}	29.6 ± 6.0 ^{cde}	1.58 ± 0.19 ^{abc}	285.2 ± 20.4 ^{ab}	5.81 ± 1.00 ^{ab}	0.36 ± 0.02 ^{cdef}	0.12 ± 0.01 ^{cde}	0.49 ± 0.03 ^{cdef}	0.22 ± 0.02 ^{cde}
	No AMF	NO	4065 ± 151 ^{bcd}	0.74 ± 0.01 ^{ab}	24.4 ± 2.7 ^{cde}	1.22 ± 0.10 ^{bc}	250.6 ± 10.9 ^{bc}	3.43 ± 0.53 ^{b}	0.35 ± 0.03 ^{cdef}	0.12 ± 0.01 ^{cdef}	0.47 ± 0.04 ^{cdef}	0.21 ± 0.02 ^{ef}
		YES	5010 ± 148 ^{a}	0.71 ± 0.01 ^{ab}	28.2 ± 3.6 ^{cde}	0.95 ± 0.10 ^{c}	275.0 ± 3.4 ^{ab}	2.18 ± 0.14 ^{b}	0.29 ± 0.02 ^{df}	0.10 ± 0.00 ^{def}	0.39 ± 0.02 ^{def}	0.17 ± 0.01 ^{f}
ANOVA (F-value)		**DF**										
Wheat cultivar (WC)		2	106.75 ***	5.68 **	2.05 NS	1.52 NS	0.72 NS	2.99 NS	53 ***	49 ***	53 ***	70 ***
AM fungus (AMF)		2	0.64 NS	2.56 NS	30.21 ***	9.00 ***	1.21 NS	8.14 ***	6.06 **	5.51 **	6.02 **	18.07 ***
Drought (D)		1	5.68 *	1.04 NS	12.05 ***	4.86 *	6.37 *	0.61 NS	0.25 NS	0.20 NS	0.25 NS	1.80 NS
WC X AMF		4	3.76 **	2.25 NS	1.40 NS	1.31 NS	4.57 **	0.13 NS	6.86 ***	7.95 ***	7.33 ***	2.51 *
WC X D		2	2.55 NS	3.77 *	1.78 NS	0.33 NS	12.87 ***	2.75 NS	26.4 ***	33.7 ***	29.2 ***	11.2 ***
AMF X D		2	7.33 ***	0.73 NS	2.89 NS	0.99 NS	3.82 *	0.77 NS	3.25 *	5.88 **	3.93 *	0.69 NS
WC X AMF X D		4	8.31 ***	2.74 *	7.99 ***	5.85 ***	9.69 ***	7.71 ***	4.91 **	11.9 ***	6.69 ***	4.42 **

Abbreviation conventions: FT = continuous fluorescence performance with non-active light (mmol m^{-2} s^{-1}); ΦPSII = quantum yield of photosystem II (mmol CO_2 μmol^{-1} absorbed photons); gs = stomatal conductance (mmol H_2O m^{-2} s^{-1}); A = photosynthesis rate (µmol CO_2 m^{-2} s^{-1}); leaf internal concentration of CO_2 (µmol mol^{-1}); WUE = water use efficiency (mmol CO_2 mol^{-1} H_2O). Significance conventions: NS = non-significant; *P<0.05; **P<0.01; ***P<0.001.

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Table 2. Phenolic compounds identified by HPLC-ESI-MS/MS in wheat plant.

peak	t_R (min)	$[M-H]^-$ (m/z)	Fragments (m/z)	Compound
1	11.0	609.1	446.9; 429; 410.9; 392.9; 386.8; 368.9; 356.9; 326.9	Luteolin 6-C-hexoside-O-hexoside
2	12.0	579.1	561; 543; 519.1; 489; 471.1; 459; 441; 429; 410.9	Luteolin C-hexoside C-pentoside
3	12.5	609.1	591.1; 429; 350.9; 308.9	Luteolin O-hexoside C-hexoside
4	13.2	563.0	472.9; 443; 383; 352.9; 325; 296.9	Apigenin 6-C-pentoside 8-C-hexoside
5	13.6	476.2	459.2; 415.1; 371; 220.9; 176.9	Not identified
6	15.0	623.1	443; 323; 322.9; 307.9	Luteolin C-deoxihexosyl O-hexoside
7	15.7	461.0	340.9; 298.9; 297.8	Kaempferol-3-glucuronide
8	16.0	608.3	591.2; 503.2; 463; 353.1; 318	Not identified
9	17.0	755.1	635.1; 593; 579; 429; 411; 356.9	Luteolin O-(O-caffeoyl-hexoside) C-hexoside

Note. t_R: retention time; [M-H] - (m / z): compounds mass in negative ionization mode

Table 3. Concentrations of phenolic compounds (flavons in mg g^{-1} dry weight and HCAD in µg g^{-1} dry weight) measured in plants of *Triticum aestivum* (wheat) of three commercial cultivars inoculated or not with two arbuscular mycorrhizal fungi (AMF) and growing with or without drought stress. Identifications of phenolic compounds according to Table 2. F-values and significances for the main effect of influencing variables are included. Different letters in a column represent statistic significant differences according with Tukey's test (P<0.05).

Wheat cultivar	AM fungus	Drought	Flav.1*	Flav.2	Flav.3	Flav.4	Flav.5	Flav.6	Flav.7	Flav.8	Flav.9	Flav. 9a	HCAD
Ilustre	*C. claroideum*	NO	0.06±0.01^{bcdef}	0.08±0.02^{cd}	0.06±0.02^{bcd}	0.07±0.01^{def}	0.59±0.19^{cdef}	Nd	0.04±0.01^{bc}	nd	0.05±0.02^{ef}	0.07±0.03^{def}	nd
		YES	0.10±0.01^{a}	0.15±0.01^{ab}	0.17±0.01^{a}	0.13±0.01^{ab}	1.54±0.08^{a}	nd	0.08±0.01^{a}	nd	0.11±0.01^{abc}	0.14±0.01^{a}	89.3±24.9^{abc}
	F. mosseae	NO	0.04±0.00^{cdef}	0.09±0.00^{c}	0.07±0.02^{bcd}	0.08±0.01^{cde}	0.99±0.05^{bc}	nd	0.04±0.01^{bc}	nd	0.07±0.00^{bcdef}	0.08±0.00^{bcdef}	23.9±6.1^{d}
		YES	0.03±0.00^{ef}	0.06±0.01^{cde}	0.06±0.00^{bcd}	0.05±0.01^{defg}	0.66±0.08^{cdef}	nd	0.04±0.00^{cd}	nd	0.06±0.00^{def}	0.06±0.01^{ef}	23.5±11.8^{d}
	No AMF	NO	0.08±0.00^{abc}	0.18±0.02^{a}	0.10±0.02^{b}	0.13±0.00^{a}	1.30±0.02^{ab}	nd	0.09±0.00^{a}	nd	0.12±0.00^{a}	0.12±0.00^{ab}	61.4±15.4^{bcd}
		YES	0.04±0.00^{def}	0.07±0.01^{cd}	0.04±0.01^{bcd}	0.06±0.00^{def}	0.43±0.04^{def}	0.12±0.06^{a}	0.05±0.00^{b}	nd	0.07±0.00^{bcdef}	0.07±0.00^{def}	nd
Maxi	*C. claroideum*	NO	0.09±0.01^{ab}	0.08±0.02^{cd}	0.04±0.01^{bcd}	0.07±0.01^{def}	1.19±0.20^{ab}	nd	0.04±0.01^{bc}	0.04±0.01^{ab}	0.08±0.01^{abcde}	0.10±0.01^{bcde}	156.0±23.9^{a}
		YES	0.02±0.00^{f}	0.02±0.00^{e}	0.02±0.00^{d}	0.02±0.00^{g}	0.26±0.01^{f}	nd	0.01±0.00^{c}	0.02±0.00^{bc}	0.03±0.00^{f}	0.04±0.00^{g}	31.5±3.5^{cd}
	F. mosseae	NO	0.09±0.02^{ab}	0.07±0.01^{cd}	0.07±0.02^{bcd}	0.08±0.02^{cd}	0.83±0.24^{bcd}	nd	0.07±0.01^{a}	0.04±0.01^{ab}	0.10±0.02^{abcde}	0.11±0.02^{abcd}	137.9±40.2^{a}
		YES	0.08±0.00^{abc}	0.07±0.00^{cd}	0.08±0.00^{bcd}	0.07±0.00^{de}	0.84±0.02^{bcd}	nd	0.06±0.00^{a}	0.06±0.00^{a}	0.11±0.00^{abc}	0.11±0.00^{abc}	138.1±4.0^{ab}
	No AMF	NO	0.03±0.00^{f}	0.03±0.00^{de}	0.04±0.00^{bcd}	0.04±0.00^{efg}	0.39±0.08^{def}	nd	0.04±0.00^{b}	nd	0.05±0.00^{def}	0.05±0.00^{fg}	32.1±16.0^{cd}
		YES	0.04±0.01^{ef}	0.03±0.01^{de}	0.03±0.01^{cd}	0.03±0.01^{fg}	0.35±0.11^{ef}	0.08±0.05^{b}	0.04±0.00^{b}	0.01±0.01^{c}	0.06±0.01^{cdef}	0.07±0.01^{cdef}	33.6±16.9^{cd}
Purple	*C. claroideum*	NO	0.07±0.01^{abcde}	0.10±0.01^{bc}	0.09±0.01^{bc}	0.12±0.02^{abc}	1.03±0.10^{abc}	nd	0.06±0.01^{a}	0.04±0.01^{ab}	0.11±0.01^{ab}	nd	126.4±7.0^{ab}
		YES	0.05±0.00^{bcdef}	0.09±0.01^{c}	0.02±0.01^{d}	0.08±0.01^{cd}	0.65±0.07^{cdef}	nd	0.05±0.00^{b}	0.04±0.01^{ab}	0.10±0.00^{abc}	nd	56.8±14.4^{bcd}
	F. mosseae	NO	0.06±0.01^{bcdef}	0.07±0.01^{cd}	0.07±0.01^{bcd}	0.09±0.01^{bcd}	0.85±0.05^{bcd}	nd	0.05±0.00^{b}	0.05±0.01^{ab}	0.10±0.01^{abcd}	nd	87.3± 0.9^{abc}
		YES	0.08±0.01^{abcd}	0.10±0.01^{bc}	0.06±0.01^{bcd}	0.09±0.00^{abc}	0.89±0.02^{bcd}	nd	0.06±0.00^{a}	0.02±0.01^{ab}	0.12±0.01^{a}	nd	89.2±6.4^{abc}
	No AMF	NO	0.04±0.01^{def}	0.08±0.00^{cd}	0.02±0.01^{d}	0.07±0.00^{def}	0.45±0.05^{def}	nd	0.06±0.00^{a}	0.02±0.01^{bc}	0.08±0.01^{abcd}	nd	32.9±16.4^{cd}
		YES	0.06±0.01^{bcdef}	0.07±0.02^{cd}	0.05±0.01^{bcd}	0.07±0.02^{def}	0.44±0.07^{def}	nd	0.06±0.01^{a}	0.02±0.01^{bc}	0.09±0.02^{abcd}	nd	72.9±21.7^{abc}
ANOVA (F-value)		**DF**											
Wheat cultivar (WC)		2	0.08 NS	42.67 ***	14.71 ***	35.47 ***	11.47 ***	1.67 NS	5.92 **	32.92 ***	15.11 ***	175.93 ***	18.71 ***
AM fungus (AMF)		2	10.32 ***	1.10 NS	5.82 *	4.98 NS	16.76 ***	5.90 **	3.31 **	12.48 ***	3.54 **	1.53 NS	12.58 ***
Drought (D)		1	2.65 NS	7.80 NS	0.31 NS	11.56 ***	12.66 ***	5.90 *	0.73 NS	0.30 NS	0.15 NS	0.42 NS	3.05 NS
WC X AMF		4	13.93 ***	11.44 ***	7.06 ***	13.99 ***	3.83 NS	1.67 NS	13.61 ***	5.45 ***	12.65 ***	11.37 ***	5.94 ***
WC X D		2	4.58 *	3.96 *	2.79 NS	1.06 NS	2.26 NS	1.67 NS	2.08 NS	0.75 NS	1.22 NS	0.73 NS	3.51 *
AMF X D		2	1.08 NS	6.79 *	1.10 NS	1.78 NS	1.77 NS	5.90 *	2.82 NS	0.47 NS	0.98 NS	1.31 NS	1.92 NS

WC X AMF X D	4	14.30 ***	20.21 ***	13.14 ***	19.16 ***	27.19 ***	1.67 NS	10.98 ***	3.20 *	11.96 ***	16.84 ***	11.84 ***

*The identifications are according to Table 2. HCAD = unidentified hydroxycinnamic acid derivative. Significance conventions: NS = non-significant; *P<0.05; **P<0.01; ***P<0.001.

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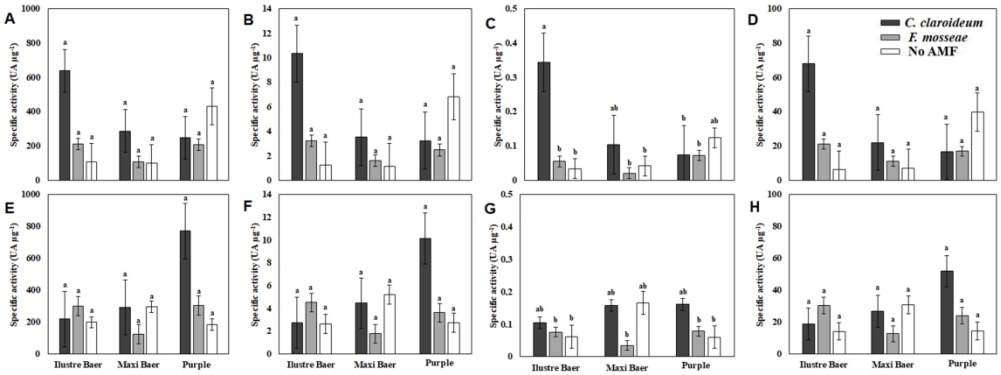

Figure 1: Specific activity (UA µg-1) of enzymes catalase (A, E), ascorbate peroxidase (B, F), glutathione reductase (C, G) and superoxide dismutase (D, H) growing without (upper subfigures) or with drought conditions (lower subfigures). The arbuscular mycorrhizal fungi species inoculated were Claroideoglomus claroideum, Funneliformis mosseae, and treatments non-inoculated (No AMF). The wheat cultivars evaluated corresponded to Ilustre Baer, Maxi Baer and Purple. Different lowercase letters indicate statistic difference according to Tukey's multiple range test ($p < 0.05$). Data are the mean ± SE, n = 3.

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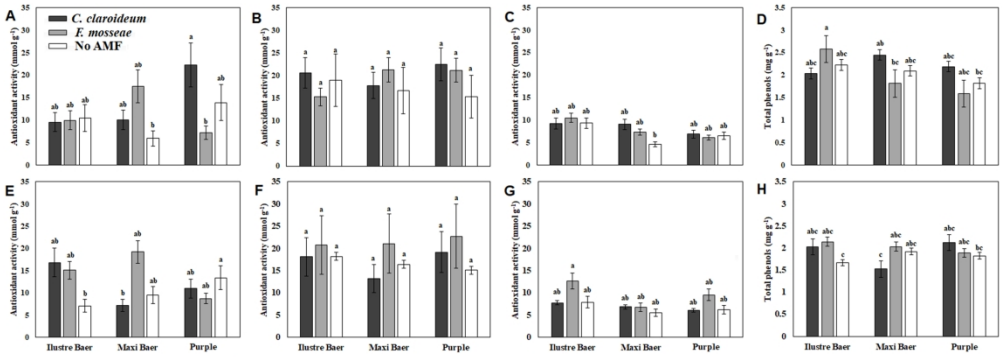

Figure 2: Antioxidant activities (mmol Trolox equivalents g-1) of TEAC (A, E), CUPRAC (B, F), DPPH (C, G) and total phenols (D, H) growing without (upper subfigures) or with drought conditions (lower subfigures). The arbuscular mycorrhizal fungi species inoculated were Claroideoglomus claroideum, Funneliformis mosseae, and treatments non-inoculated (No AMF). The wheat cultivars evaluated corresponded to Ilustre Baer, Maxi Baer and Purple. Different lowercase letters indicate statistic difference according to Tukey's multiple range test ($p < 0.05$). Data are the mean ± SE, n = 3.

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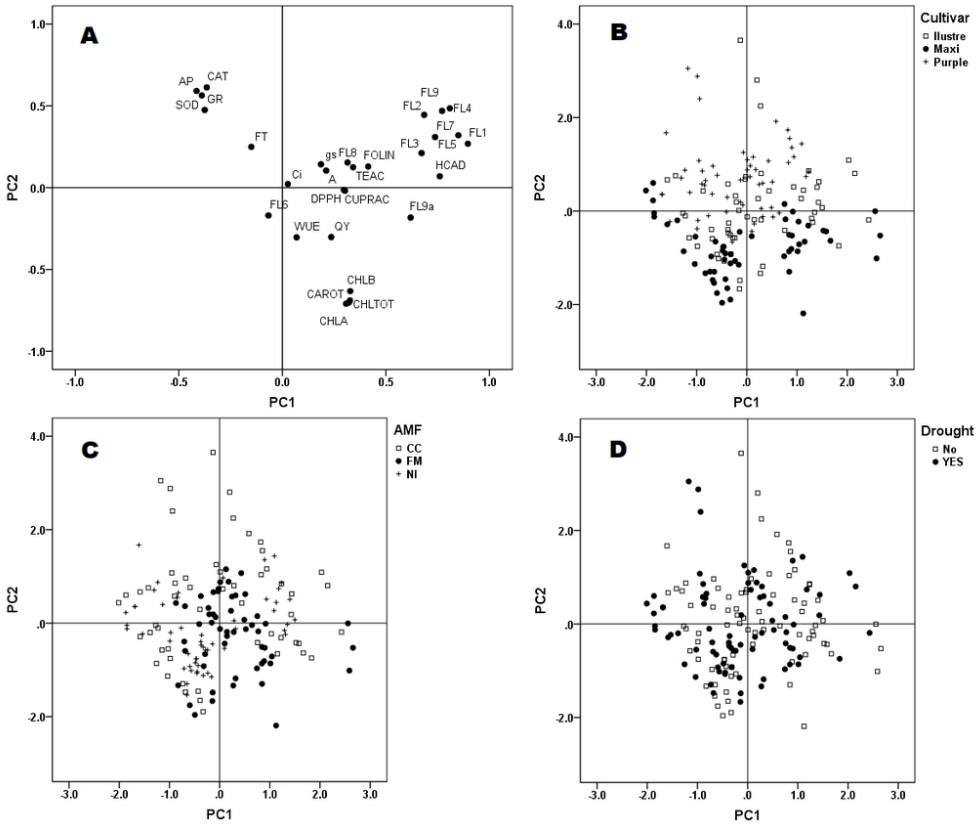

Figure 3: (A) Principal component (PC) scores for the experimental variables determined in plants of three cultivars of Triticum aestivum inoculated or not with different strains of arbuscular fungi (AMF) and growing under drought or non-limiting water conditions. The percentage values in parentheses indicate the variation explained by each PC. The plot shows the distribution of the experimental individuals according to the PCs and grouped according to (B) the wheat cultivar, (C) the type of AMF inoculum, and (D) the water condition. CC = Claroideoglomus claroideum, FM = Funneliformis mosseae, NI = Non-inoculated. Experimental variables corresponds to: AP: Ascorbate peroxidase, Cat: Catalase, SOD: Superoxide dismutase, GR: Glutathione reductase; Flav: Flavonoids; AHC1: Hydroxycinnamic acid, CUPRAC, DPPH, Folin, TEAC (Non-enzymatic assays), Continuous fluorescence performance in non-actinic light (FT), quantum yield of photosystem II (ΦPSII), internal CO2 concentration in the leaf (Ci), rate of photosynthesis (A), stomatal conductance (gs), water use efficiency (WUE), Total chlorophylls (ChlT), chlorophyll concentration a (Chla), chlorophyll concentration b (Chlb) and carotenes (Carot).

86x71mm (300 x 300 DPI)