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Research Article

The role of mycorrhizal fungi on physiology and biochemistry of *Salvia hispanica* L. grown under different soil pH.

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Abstract

The current study aimed to examine whether arbuscular mycorrhizal fungi (AMF- MC10, *Glomus mosseae*) can ameliorate the physiology and biochemistry of chia plants (*Salvia hispanica* L.), cultivated on different soil pH. The experiment included six treatments i.e. control- non arbuscular mycorrhizal fungi-NAMF (pH 7.1), control- arbuscular mycorrhizal fungi-AMF (pH7.1), acid-NAMF (pH 5.1), acid-AMF (pH 5.1), alkaline-NAMF (pH 8.2), and alkaline-AMF (pH 8.2). Our results showed that strongly acidic (pH <5.5) or alkaline (pH >8) soil stress adversely affected physiological and biochemical parameters, namely, the nutrient uptake, photosynthesis, chlorophyll concentration and membrane lipid peroxidation. Although extreme soil pH inhibited nutrient accumulation in the plants, the use of AMF inoculation at both soil pH significantly increased P, N, K, Na, Fe, Zn, Mn, and Cu concentrations in the shoots and roots. The mechanism by which AMF inoculation increases absorption and translocation of the nutrients in tissues, could be the decreased membrane lipid peroxidation (MDA) and the increased water use efficiency observed mainly in alkaline soil. Vitality index (Rfd) was significantly increased in AMF inoculated chia under alkaline and acidic environments compared to the NAMF inoculated plants by 26 and 59%, respectively. Photochemical quenching revealed a sharp increase of 26% in AMF inoculated plants compared to NAMF inoculated plants under pH 8.2. The protective role of AMF colonization focused also on the improvement of photosynthetic efficiency in inoculated plants by the activation of light reactions of photosynthesis and the enhanced stomatal conductivity resulting in higher light energy conversion to CO_2 fixation.

1. Introduction

Plants as sequestered organisms are affected by changing environments and frequent biotic and abiotic stresses, which induce redistribution of energy and carbon fluxes to adapt to these abnormal conditions. Soil acidity is a major environmental and economic concern having a serious effect on crop production. In very acidic soils, all the major plant nutrients (nitrogen, phosphorus, potassium, sulphur, calcium, manganese) and also the trace element molybdenum may be unavailable, or only available in insufficient quantities [1]. Another serious threat in developed and developing countries is the alkaline soils caused by the intensive use of agricultural practices. Alkaline soils affect plant growth, development and ultimately affect the yield, but also disrupt the ecological balance of the area. These soils are characterized by reduced nutrient availability. Particularly, iron deficiency (iron chlorosis) is a very

common problem and is the direct result of high pH soils reducing the availability of iron to plants [2].

The beneficial fungi, arbuscular mycorrhizal fungi (AMF) develop a mutual association with plant roots that receive the products of photosynthesis made by plants and then assist them by improving the absorption of nutrients, protecting them against stresses, improving the accumulation of biomass and photosynthesis as well [3, 4]. The term "arbuscular" originates from the formation of arbuscules, the tree-shaped subcellular structures within plant cells, as a result of the symbiotic development between the two organisms. These structures are thought to be the main site of nutrient exchange between the fungal and plant symbiotic partners [5]. Approximately 80% of terrestrial plants including cereals, vegetables and fruit trees, have symbiotic relations with fungi, where shrubs and hyphae are formed and fungal hyphae percolate into the cortical cells of plant roots [6, 7]. AM hyphae also produce a water-insoluble protein (glomalin) in soil (GRSP, glomalin-related soil protein), generally associated with insoluble humus or mineral fractions and contributing to stabilizing soil aggregates [8]. GRSP is considered to contribute to pools of soil organic carbon and provide positive impacts on plant growth. That way, AM fungi supply mineral nutrients to plants, especially phosphorus, which is precipitated by ions such as Ca, Mg, Zn [9], they also play an important role in membrane stability, stimulating plants to produce their own defense compounds, enhance the photosynthetic pigments and maintain the osmotic and ionic balance of the cell [2]. However, several biotic and abiotic factors have been reported to have a great influence on the functional performance of AMF populations. Soil pH seems to be among the most influential abiotic variables in shaping the AMF community structure [10, 11]. Generally, AMF are considered an important tool in modern environmentally friendly agriculture in the 21st century for the improvement of crop yield and quality and for the decrease of mineral fertilizers and pesticides/herbicides [12]. If mycorrhizal colonization can promote the development of roots in alkaline and acidic soils, then the impact of the stress upon the host plants may be reduced.

Salvia hispanica L., an annually cultivated plant, is categorized under the mint family, and it has its origin from Central American civilizations [11,13]. Chia seed has up to 34% total dietary fiber, 31% total lipids, 16% protein and 5.8% moisture. The predominant fatty acids of chia leaves are palmitic acid (18.3%), a-linolenic (17.1%), pentadecanoic (11%), linoleic (7.5%), oleic (7.5%) and stearic (6.3) [11]. Being a re-emerged crop, chia has the potential to diversify the local agricultural economy as a profitable addition or even alternative to traditional crops. Since it can be grown in arid environments, where water availability is the main limitation to crop production, it has been highly recommended as an alternative crop for the field crop industry [14].

Therefore, the aim of the study was to evaluate the ability of AMF inoculation to improve plant performance focusing on nutritional status and photosynthetic activity in chia plants under different soil pH. Other parameters related to extreme soil conditions like pigment content, ascorbic acid, lipid peroxidation and chlorophyll fluorescence have been also studied in inoculated and non-inoculated plants.

2. Materials and methods

2.1. Plant material and growth conditions

This study was conducted in a non-heated greenhouse at the Institute of Food Technology, Lycovrissi, Attiki Greece, during the spring and summer of 2012 and 2013 (latitude 23°46′35″ E, longitude 38°4′9″ N; altitude: 202 m). Greenhouse conditions were as follows: temperature 12 °C (min), 32.5 °C (max) and 24.3 °C (average); average relative humidity 65%; average photosynthetic photon flux density at leaf level (PPFD) 350±40 μmol m^{-2} s^{-1}. Seven days old *Salvia hispanica* L. uniform seedlings, with 8cm average shoot height, were transferred in pots containing 5L (three plants per pot) of soil, differing in pH values and physicochemical characteristics (Table 1). Soil analysis was carried out in duplicate subsamples. Particle size distribution was carried out using the Bouyoucos method [15]; pH and electrical conductivity were measured in the paste extract using pH/EC meter equipped with a glass electrode; organic matter was determined by dichromate oxidation (ISO 14235:1998); carbonates by using Bernard calcimeter; total N by the Kjeldahl method (ISO 11261:1995); available phosphorus using sodium hydrogen carbonate extraction (ISO 11263:1994); exchangeable K^+, Ca^{2+}, and Mg^{2+} using $BaCl_2$ extraction (ISO 11260:1994). K, Ca, and Mg were measured in a Varian AA-220 Atomic Absorption, P was measured in a HITACHI U3010 Spectrophotometer and Na in a

Table 1. Physico-chemical properties of the three different soil types used as growth medium

Soil characteristics		Control-7.1	Alkaline-8.2	Acid-5.1
Sand	(%)	36	68	52
Silt	(%)	38.4	14	22.4
Clay	(%)	25.6	18	25.6
Soil texture		L	SL	SCL
pH		7.1	8.2	5.1
EC	mS cm^{-1}	0.5	0.7	0.3
Organic matter	%	2.7	1.4	3.2
$CaCO_3$	%	17.6	38	-
Total N	%	0.2	0.32	0.2
P-Olsen	(m kg^{-1})	8	4	54.8
Exchangeable K	(meq $100g^{-1}$)	0.59	0.8	0.6
Exchangeable Mg	(meq $100g^{-1}$)	1.17	1.4	0.9
Exchangeable Na	(meq $100g^{-1}$)	0.34	0.3	0.3
Available Cu	(mg kg^{-1})	17.2	0.7	3.9
Available Fe	(mg kg^{-1})	19.4	3.7	98.9
Available Zn	(mg kg^{-1})	0.5	0.39	0.6
Available Mn	(mg kg^{-1})	5.4	3.4	9.6
Available B	(mg kg^{-1})	0.2	0.2	1.7

Korning Flame photometer. Determination of NH_4^+, NO_3^-, Cl^-, PO_4^{3-}, and SO_4^{2-} was performed in 1:10 water extracts using a Dionex-100 Ionic Chromatography. Methanol extractable phenol compounds were quantified by means of the Folin–Ciocalteu colorimetric method [16]. Available Mn, Fe, Cu, and Zn were determined using DTPA extraction according to ISO 14870: 2001. Soil B was extracted with boiling water using the azomethine-H method [17]. Available Cu, Mn, Fe, Zn were measured in a Varian SPECTRAA- 220 Atomic Absorption.

The experimental design included six treatments i.e. control- non arbuscular mycorrhizal fungi-NAMF (pH 7.1), control- arbuscular mycorrhizal fungi-AMF (pH 7.1), acidic-NAMF (pH 5.1), acidic-AMF (pH 5.1), alkaline-NAMF (pH 8.2), and alkaline-AMF (pH 8.2). Non mycorrhizal soils were used as references for each soil pH. The inoculum (namely MC10) used belongs to the collection of the University of Thessaly and consist of *Glomus mosseae* spores, hyphae and colonized maize roots (kindly provided by I. Ipsilantis). Inoculation was performed by placing ~10g of the inoculum in the transplant hole. Plants were irrigated once a week with tap water (500ml for each pot), without the use of fertilizers.

The experiments were set up in completely randomized block design with 6 different treatments and 4 replications each year. Twenty-four plants were grown in each replication and plant tissues were harvested 90 days after transplantation. By the end of the two cultivation periods (almost three months each), physiological and biochemical characteristics of chia leaves, were recorded.

2.2. Elemental composition

Tissues (shoot and root) were gently cleaned with a mild detergent solution, shaken to remove excess water, and immediately rinsed thoroughly in tap water and distilled water. The samples were dried in an oven at 60 ◦C until constant weight and ground with a Wiley mill to pass through a 1-mm mesh screen before mineral concentration analysis. Shoot and root samples were analyzed for total N, P, K, Na, Fe, Mn, Zn, and Cu [18]. Phosphorus was determined by the vanadomolybdophosphoric yellow color method [19]. Total N was determined by the Kjeldhal method. Prior to measuring the other nutrients, tissue samples were dry ashed at 550 ◦C for 5 h. Mineral contents of Na, K, Fe, Zn, Cu, and Mn were measured by atomic absorption spectroscopy and colorimetry using the azomethine H spectrophotometric method for B [17].

2.3. Chlorophyll content

Chlorophylls (a+b) of the youngest fully expanded leaf were quantitatively measured in 100 % acetone extract by spectrophotometry using the re-determined extinction coefficients [20].

2.4. Ascorbic acid content

The ascorbic acid content of chia leaves was estimated by macerating the sample mechanically with a stabilizing agent (5% metaphosphoric acid) as described by Ouzounidou and Asfi [21]. Data were

expressed as mg $100g^{-1}$ FW. The leaf moisture content was determined according to the AOAC method [22].

2.5. H_2O_2 assay

H_2O_2 quantification is based on the formation of a titanium peroxide complex [23]. Chia leaf samples were homogenized in cold acetone (1:6 w/v), and after filtration and centrifugation the supernatant was discarded and the pellet was dissolved in 3mL of 2N H_2SO_4. The absorbance of the solution was read at 410 nm and H_2O_2 concentration was calculated using a standard curve with concentration ranging from 0.1 to 1mM. H_2O_2 content was expressed as nmol g^{-1} FW.

2.6. Determination of lipid peroxidation

At the end of the experiment, the level of lipid peroxidation in chia leaves was measured as malondialdehyde (MDA) content determined by reaction with 2-thiobarbituric acid (TBA) reactive substances according to Ouzounidou et al. [23]. The tissue was homogenized in 0.3% TBA in 10% trichloracetic acid (TCA) at 4°C. The concentration of MDA was calculated from the difference of the absorbance at 532 nm and 600 nm using the extinction coefficient of 155 $mmol^{-1}$ cm^{-1} and expressed as nmol (MDA) g^{-1} of fresh weight.

2.7. Gas exchange- chlorophyll fluorescence measurements

Leaf gas exchange measurements were coupled with measurements of chlorophyll fluorescence using an open gas exchange portable system (LI-6400; LI-COR, Inc., Lincoln, NE) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-COR, Inc.) [24]. Measurements on the youngest mature leaves were conducted between 9:00 and 12:00 h. Leaf temperature inside the cuvette was maintained between 25 and 27°C and the cuvette relative humidity was about 60%. The CO_2 concentration at the reference infrared gas analyzer (IRGA) was maintained at 400 μmol mol^{-1} by means of a 12g CO_2 cylinder and the 6400-01 CO_2 injector, with the airflow rate through the chamber maintained at 400 μmol s^{-1}. Leaf gas exchange measurements were calculated by the LI-6400 operating software, according to the method of von Caemmerer and Farquhar [25].

In vivo chlorophyll fluorescence was measured on the upper surface of the fully expanded younger leaves after they were left for 30 min to dark adaptation, at room temperature. Different values were selected in order to determine any structural and functional changes of the photosynthetic apparatus under different soil pH: the redox state of the plastoquinone pool (*qp*, photochemical quenching), which is the fraction of open PSII reaction centres as = (Fm'-Fs)/(Fm'-Fo') and *Rfd* (vitality index) = (Fm-Fs) /Fs.

2.8. Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple comparison tests were performed to compare means at a significance level P=0.05. Data shown are means and SE of two different experiments with four replicates per treatment per experiment.

3. Results

3.1. Elemental composition

AMF inoculation of chia plants grown under the three pH, generally resulted in an increase in nutrient concentrations compared to NAMF chia plants (Table 2). More precisely, the significance increase of P accumulation in shoots and roots was observed in the three pH treatments in inoculated plants with the higher values +34% and +23% in alkaline environments in shoots and roots respectively, compared to the non-inoculated plants. The N concentration revealed a a significant drop under pH 5.1 by about 22% in AMF roots compared to the NAMF roots, while in neutral and alkaline environments, inoculated tissues showed significantly higher N concentrations. The K concentration showed slight fluctuations under the three pH treatments both to AMF and NAMF plants. The lowest values were recorded under acidic environment regardless AMF inoculation. On the contrary, Na concentration in AMF shoots and roots was significantly increased by 2-2.5 folds in neutral pH and by about 63-69% in alkaline pH, while in acid pH no change both in AMF and NAMF was observed. Under inoculation the micronutrients Fe, Zn, Mn, and Cu tended to be increased (Table 2). The greater Fe increases approximately by 43% and by 47% of the NAMF were detected in shoots of pH 5.1 and roots of pH 8.2 in AMF, respectively. Plants grown under alkaline pH and inoculation showed an over doubling and a significant increase (66%) in Zn contents. An increase but on a lower scale under neutral and acidic environment was observed in inoculated plants. Similar results were found for Mn concentration, while the AMF roots under neutral and alkaline treatment accumulated 4,5- and 3,2-folds higher Cu concentrations compared to NAMF roots. Under acid

Table 2. Elements concentration in shoot and root of chia plants grown on different soil pH with (AMF) or without (NAMF) inoculation.

Treatments	Name	N (% DW)	P (% DW)	K (% DW)	Na (% DW)	Fe (μg g^{-1} DW)	Zn (μg g^{-1} DW)	Mn (μg g^{-1} DW)	Cu (μg g^{-1} DW)
Shoot									
Control soil-pH 7.1	NAMF	3.35b	6.83b	5.52a	0.10c	176.00c	73.35d	116.00d	24.50b
	AMF	4.22a	7.80a	5.87a	0.25a	205.50b	124.06b	145.24c	25.65b
Alkaline soil-pH 8.2	NAMF	3.45b	5.35c	5.08b	0.11c	169.31c	70.00d	92.00e	20.55c
	AMF	4.87a	7.18a	5.04b	0.18b	234.04a	168.32a	136.60d	25.58b
Acidic soil-pH 5.1	NAMF	3.12c	4.24d	4.36c	0.09c	104.57e	78.50d	279.26a	35.55a
	AMF	3.05c	5.24c	4.85b	0.11c	149.50d	95.37c	250.50b	27.18b
Root Control soil-pH 7.1	NAMF	0.64d	2.04b	1.85a	0.33c	3812.37bc	32.20c	140.00c	25.00d
	AMF	0.87c	2.33a	1.92a	0.72a	5280.00a	36.85c	184.00a	112.00a
Alkaline soil-pH 8.2	NAMF	1.00b	1.75c	1.45c	0.45b	3885.42b	51.15b	127.65d	31.93c
	AMF	1.27a	2.15ab	1.60b	0.76a	5707.50a	84.63a	183.33a	102.71a
Acidic soil-pH 5.1	NAMF	0.68d	0.75e	1.32c	0.37c	3335.80d	37.26c	173.81b	35.80bc
	AMF	0.53e	0.94d	0.93d	0.38c	3570.00cd	54.00b	197.94a	39.60b

Mean values of n=3, followed by different letters in the same column are significant different at P<0.05, for shoot and root.

pH and inoculation an important reduction in Cu content by about 24% of non-inoculation, was measured.

3.2. CO_2 assimilation

AMF inoculation caused alleviation of net photosynthesis (A) and stomatal conductance (gs) of chia leaves under neutral and alkaline treatment compared to NAMF inoculated leaves. CO_2 assimilation and stomatal conductance were enhanced by 32 and 40% at pH 8.2, respectively (Fig. 1).

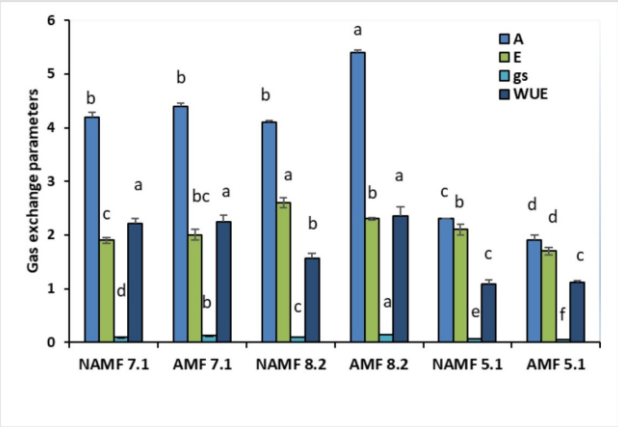

Figure 1. The net assimilation (A, μmol CO_2 $m^{-2}s^{-1}$), transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$), stomatal conductance (gs, mol H_2O $m^{-2}s^{-1}$) and water use efficiency (WUE, μmol CO_2 $mmol^{-1}$ H_2O) of the youngest fully expanded chia leaf during exposure to control soil pH-7.1, alkaline soil pH-8.2, acidic soil pH-5.1 with (AMF) or without (NAMF) inoculation.

In contrast, under pH 5.1 and AMF inoculation, A and gs dropped by 17% of NAMF. Transpiration rate (E) was significantly reduced under alkaline and acid pH and AMF by 12% and 20% compared to NAMF, respectively. Under pH 7.1, the AMF and NAMF leaves had similar transpiration rates. The significant net photosynthesis increases in AMF leaves under pH 8.2 induced significant increase in WUE, an indicator of the metabolic use efficiency of water. Under the rest of the treatments eg at pH 7.1 pH 5.1, AMF and NAMF, no changes were observed. The significant increases of stomatal conductance of AMF inoculated leaves under pH 7.1 and pH 8.2 induced significant decrease of the ratio A/gs (intrinsic water use efficiency), by 21 and 6%, respectively (data not shown).

3.3. Chlorophyll fluorescence

Chlorophyll fluorescence parameters were estimated after three months of chia growth under different pH exposure to examine possible mechanisms related to the alleviation of AMF inoculation (Fig. 2). Vitality index (Rfd) was significantly increased in AMF inoculated plants under alkaline and acidic environments compared to the NAMF inoculated plants by 26 and 59%, respectively. Lower value of Rfd was observed in AMF inoculated plants under pH 7.1. In parallel, the fraction of open PSII reaction centers, which is the redox state of the plastoquinone pool (*qp*), differed between inoculated and non-inoculated chia leaves under the three pH treatments. Photochemical quenching revealed a sharp increase of

Table 3. Effect of different soil pH on ascorbic acid, moisture, total chlorophyll (Chl a+b), lipid peroxidation (MDA) and H_2O_2 concentrations of chia leaves grown with (AMF) or without (NAMF) inoculation.

Treatments	Name	Ascorbic acid (% DW)	Moisture (% DW)	Chl a+b (mg g^{-1} FW)	MDA (nmol g^{-1} FW)	H_2O_2 (nmol g^{-1} FW)
Control soil-pH 7.1	NAMF	33.4 ± 0.9a	85.4 ± 1.3a	7.31 ± 0.02a	21.21 ± 0.9d	3.31 ± 0.11e
	AMF	30.2 ± 1.1b	86.2 ± 0.8a	6.60 ± 0.04bc	19.40 ± 0.4d	3.20 ± 0.09e
Alkaline soil-pH 8.2	NAMF	26.2 ± 1.8c	84.8 ± 0.9a	6.24 ± 0.03c	53.24 ± 1.0b	8.84 ± 0.10c
	AMF	29.6 ± 0.7b	86.1 ± 1.1a	7.00 ± 0.02ab	32.30 ± 0.7c	5.00 ± 0.06d
Acidic soil-pH 5.1	NAMF	26.0 ± 1.7c	83.8 ± 0.5a	4.91 ± 0.01d	68.21 ± 0.9a	12.10 ± 0.12a
	AMF	20.9 ± 1.3d	83.6 ± 0.8a	3.31 ± 0.03e	55.31 ± 0.6b	10.31 ± 0.08b

Data are the means ± SE of three replications, values followed by different letters in the same column are significant different at P<0.05.

26% in AMF inoculated plants compared to NAMF inoculated plants under pH 8.2 (Fig. 2). Under neutral and acidic environments, a significant decline was observed in AMF inoculated plants, by 18 and 44%, respectively.

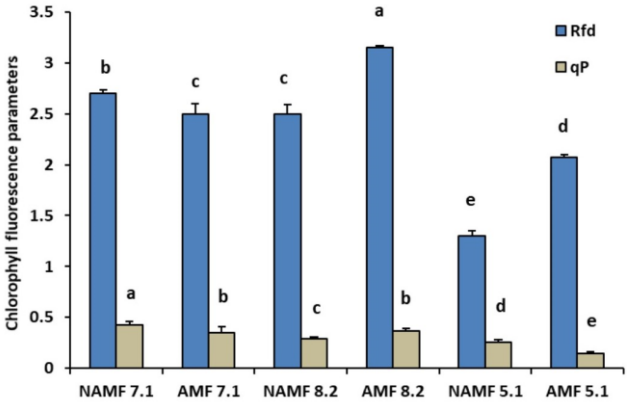

Figure 2. The vitality index (Rfd) and the photochemical quenching (qP) of the youngest fully expanded chia leaves during exposure to control soil pH-7.1, alkaline soil pH-8.2, acidic soil pH-5.1 with (AMF) or without (NAMF) inoculation.

3.4. Biochemistry

No moisture changes were induced in plants grown under either inoculation or non-inoculation and different pH treatments (Table 3). On the contrary, ascorbic acid and total chlorophyll showed differential responses to pH and inoculation. Under alkaline treatment and inoculation, significant augmentation by 13% of the non-inoculated plants, was observed. Ascorbic acid concentrations were significantly reduced under neutral and acid pH and AMF-inoculation, by 10 and 20% of NAMF, respectively. Total chlorophyll dropped by 10 and 32% in AMF inoculated leaves compared to NAMF inoculated under pH 7.1 and 5.1; while it increased by 13% under AMF inoculation and pH 8.2 (Table 3). The leaves in acidic pH were clearly distinguished by a yellow-green color and stunted growth. MDA in the inoculated chia leaves decreased by 8.5% compared to non-inoculated under pH 7.1, but this change was not significant (Table 3). On the contrary, the AMF-inoculation in pH 8.2 and 5.1, significantly reduced the MDA concentrations, by 40 and 19% respectively, compared to the non-inoculated leaves. Lipid peroxidation in non-inoculated leaves displayed more than 2 and 3 times increase in alkaline and acidic pH, respectively, compared to the neutral values (Table 3). H_2O_2 content in leaves under neutral pH did not show any changes under AMF-inoculation (Table 3). However, a sharp increase in H_2O_2 content of NAMF leaves, under pH 8.2 and 5.1 was observed, by 2.6 and 3.5 folds, compared to pH 7.1. The inoculation induced a significant reduction in H_2O_2 content by 43 and 15% in alkaline and acidic pH, respectively compared to the values of NAMF leaves at the same pH.

4. Discussion

Arbuscular mycorrhizal fungi (AMF) enhance plant resilience under abiotic stresses such as salinity, extreme soil and climate events like heat, elevated CO2, and biotic stress involving bacterial and fungal pathogens [11, 26, 27]. AMF symbiosis is established upon a nutrient economy in which host plants dedicate a portion of their photosynthates to feed AMF colonizing their roots in exchange for mineral nutrients, especially phosphorus and nitrogen. These are provided through the extraradical hyphae, which serve as an extension of the root system to form an alternative route for nutrient uptake called the mycorrhizal pathway [28].

As it was previously shown by Ouzounidou et al. [11], AMF colonization was observed either by species

indigenous to the soil used in each soil treatment or by MC10 inoculum tested in the study. Chia plants grown in neutral and alkaline soil exhibited the highest percentage of root colonization values, while an inhibition of AMF colonization in plants grown under low pH values was found. In our present experiments, the two extreme soil pH (8.2 and 5.1) without AMF inoculation, negatively affected both the physiological and nutritional status of chia plants, while under MC10-inoculation plants showed a consistent trend to enhanced the physiology and biochemistry, especially in an alkaline environment. Although AMF is restricted morphologically to the roots, physiological and metabolic alterations in the root caused by AMF also influence the physiology of the entire plant. AM fungi promoted plant growth as it refers to different species from various stresses [11, 12, 29-33].

Our results clearly showed that acid and alkaline soil stress adversely affects physiological and biochemical parameters, namely, nutrient uptake, photosynthesis, chlorophyll concentration and membrane lipid peroxidation. Soils that are strongly acidic (pH <5.5) or alkaline (pH >8) present a spectrum of challenges for the plant, including nutrient availability, ion toxicities, and nutrient imbalances influencing the ion relations and nutrition within the plant itself [34]. However, it is evident that inoculation of AMF can significantly enhance the concentration of various macro- and micro-nutrients, which leads to increased photosynthetic production and hence increased biomass accumulation [3, 4].

Indeed, although extreme soil pH inhibited nutrient accumulation in the plants, the use of AMF inoculation at both soil pH resulted in improved chia plant nutrition, which is in line with recent findings [29, 35-37]. Phosphorus, N, K, Na, Fe, Zn, Mn, and Cu concentrations in the shoots and roots of chia plants were highly increased by the inoculation under both acid and alkaline soil. It seems that AMF is particularly important in mobilizing not only phosphorus, but nitrogen, potassium, zinc, iron, manganese, copper and other essential nutrients, as it was also reported by Begum et al. [12]. Mycorrhizal symbiosis positively increased the concentrations of N, P, and Fe in *Pelargonium graveolens* L. under drought stress [12, 38]. Gomez-Bellot et al. [39] reported improved levels of P, Ca, and K in *Euonymus japonica* under salinity stress due to instant fungus attachment. Furthermore, plants possessing AMF show enhanced uptake of P, Ca^{2+}, N, Mg^{2+}, and K^{+} in the AMF-treated *Cucumis sativus* plants compared with those in the uninoculated plants under salt stress conditions [40].

According to Basiru et al. [28], arbuscular mycorrhizal fungi work together with other soil microorganisms, which help the fungus extract more nutrients for the plant acting as a bridge between the plant root's internal environment and the surrounding soil, extending beyond the root's influence zone. The decreased Mn, Fe, Zn, Cu concentrations in chia leaves under extreme soil conditions resulted in lower photosynthetic activity. AMF inoculation of the plants induced higher nutrient absorption and accumulation especially under alkaline soil pH, with a concomitant enhanced photosynthesis, transpiration, stomatal conductance and vitality index. These nutrients have a key role as metal components of various enzymes or as functional, structural or regulatory cofactors associated with photosynthesis, metabolism and protein synthesis [23, 41, 42]. The mechanism by which AMF inoculation increases absorption and translocation of the nutrients in chia tissues, could be the decreased membrane lipid peroxidation and the increased water use efficiency.

We used gas exchange measurements to quantify CO_2 and water fluxes across the leaf surface under extreme soil pH and AMF inoculation. The high gs observed under an alkaline environment in AMF inoculated chia leaves were accompanied by the highest rates of the net photosynthesis and the higher efficiency of water use. In contrast, the low gs values observed under acid pH on both AMF and NAMF leaves may indicate that the stomatal conductance was reduced by the stomatal closure to prevent excessive water loss. This closure negatively influenced the photosynthetic rate and the water use efficiency whereas, the decreased photosynthetic rates can be also explained by the lower levels of chl (a + b) occurred mainly under pH 5.1 (Fig. 1). Janah et al. [35] and Zhu et al. [43] also reported that the colonization with AMF has a positive effect in stomatal behavior and enhanced gas exchange ability under salinity and drought.

Photochemical quenching represents the activation of enzymes involved in carbon metabolism and in the opening of stomata, as well as it is an indication of the proportion of PSII reaction centers that are open [23, 44]. Electrons flow from the active PS II center to

NADP+, reducing it to NADPH then enter the Calvin cycle and assimilate CO_2 to synthesize organic compounds. In the present study, qP changes are in line with those of stomatal conductance under the three pH treatments and AMF inoculation (Figs 1, 2). The inoculated chia plants exhibited significantly higher stomatal conductance than the non-inoculated, under alkaline pH (Fig.1), with a concomitant higher net photosynthesis and water use efficiency. Based on our results of chlorophyll fluorescence and photosynthesis, plants in acidic and non-inoculated showed fewer energy absorbed by PSII reaction center is used for electron transfer and activation of PSI reaction center, then photosynthetic fixation of CO_2 was limited resulting in a reduction of plant metabolism. In addition, the ratio of the fluorescence decreases Fd, to the steady state fluorescence Fs (i.e., Rfd = Fd/Fs), known as vitality index, measuring the whole photosynthetic efficiency from the primary photochemical event to the activity of Calvin cycle enzymes [21, 45], displayed significant inhibition under the extreme soil environments (pH 8.2 and 5.1). These observations clearly indicate that direct and indirect effects are caused mainly by the acid pH on the photosynthetic function of chia plants. The higher values of Rfd induced by inoculation with MC 10 at the two soil pH reflect the higher photosynthetic capacity and CO_2 fixation rates of the plants under AMF symbiosis. These symbiotic effects of mycorrhizal fungi under stress conditions improve the chloroplast cycle and protect pigments from damage caused by photosystem reaction centers and stress by increasing energy absorption efficiency and this could be a key mechanism of the ameliorative role of AMF. Rasouli et al. [46], showed the improvement of water status and stomatal opening in AMF-inoculated savory plants and the increase of the efficiency of PSII (Fv/Fm) under conditions of drought and AMF colonization. Application of AMF on stevia and sage has also a beneficial effect on the efficiency of the photosystem II under stressed conditions as it was observed by Moustakas et al. [30] and, Janah et al. [35].

Extreme soil conditions seem to act by intercepting electrons from the photosynthetic electron transport chain, resulting in the production of toxic reactive oxygen species (ROS). To combat the harmful effects of ROS, cells have evolved an effective system of enzymatic and non-enzymatic antioxidant defenses [47, 48]. Ascorbic acid belongs to the non-enzymatic antioxidant defense system and it can significantly lower ROS and prevent cell damage [49]. Indeed, the higher levels of ascorbic acid and chlorophyll(a+b) observed in AMF-inoculated plants under alkaline soil in combination with the lowered malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations, display the protective role of these substances to cell oxidation under pH 8.1. On the other side, the content of MDA and H_2O_2 showed a significant increase in chia leaves, in the absence of AMF inoculation, showing severe oxidative stress response especially under extremely acidic conditions (Table 3). MDA is one of the products of membrane lipid peroxidation, which indirectly reflects ROS damage to cells and organisms and it is used as one of the detection indexes of cell senescence when facing and enduring damage [23]. It seems likely, that chia plants subjected to soil stress revealed accumulation of MDA and H_2O_2, which in turn alter basic cellular properties such as membrane fluidity and ion transport, while enzyme inactivation, DNA damage, pigment decolorization can be also induced [50-52]. Meanwhile, the presence of mycorrhizae is able to induce an alleviation mechanism against oxidative damage during pH stress conditions, since MDA and H_2O_2 showed their lower values regardless of the pH treatment (Table 3). Hu et al. [53] also reported that mycorrhizal plants had lower levels of malondialdehyde than non-mycorrhizal plants, which is in line with our data. In agreement are also, the results of Chandrasekaran [54] which clearly showed that AMF inoculation decreased the level of H_2O_2, which, in turn, reduced lipid peroxidation by MDA content and membrane stability by the reduced level of EL. Moreover, AMF plants induced high levels of carotenoids, which enhanced their soluble sugars and ABA levels better and faster than non-AM plants. Thus, the application of mycorrhizae can be used as a strategy to mitigate cell membrane damage and cell death by decreasing H_2O_2 content under extreme soil pH stress.

5. Conclusions

Overall, our findings suggested that the ameliorative effects of AMF symbiosis are more expressed on exposure to alkaline soil pH than to acidic and neutral. It seems that the mycorrhizal pathway mitigated the eco-physiological adaptation of chia plants under

alkaline soil conditions through the significant increase of P, N, Na and the other micronutrient accumulation in plant tissues. The protective role of AMF colonization focused also on the improvement of photosynthetic efficiency in inoculated plants by the activation of light reactions of photosynthesis and the enhanced stomatal conductivity resulting in higher light energy conversion to CO_2 fixation and water use efficiency, as well. Furthermore, the key role of AMF inoculation against membrane oxidation and ROS limitation under extreme soil conditions is very important. Since crop production is constantly exposed to various biotic and abiotic stresses, new and effective strategies are needed to improve agricultural productivity and overcome the stresses faced. In this study, we have provided new insights into the protective role of mycorrhizal symbiosis with plants under different soil pH, but more research needs to be done about the equilibrium between costs and benefits raised by the mutualistic symbiosis.

Authors' contributions

Performed the research work and data analysis, M.A. and G.O.; manuscript drafting, G.O. and V.K.; figures drawn, G.O. and M.A. critically revised the work, G.O. and V.K.

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Availability of data and materials

Data supporting this study are included within the article.

Conflicts of interest

The authors have no conflicts of interest or competing interests to declare that are relevant to the content of this article.

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