Preprints.org

Article

Not peer-reviewed version

The Influence of Salinity and Arbuscular Mycorrhizal Fungi (AMF) on the Growth and Yield Performances of Flax (Linum usitatissimum L.)

Ioanna Kakabouki *, Panteleimon Stavropoulos, Ioannis Roussis, Antonios Mavroeidis, Dimitrios Bilalis *

Posted Date: 6 September 2023

doi: 10.20944/preprints202309.0345.v1

Keywords: AMF root colonization; commercial arbuscular mycorrhizal inoculant; leaf relative water content; natural difference vegetation index (NDVI); soil plants analysis development (SPAD); seed yield; stem fiber yield

Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

The Influence of Salinity and Arbuscular Mycorrhizal Fungi (AMF) on the Growth and Yield Performances of Flax (*Linum usitatissimum* L.)

Ioanna Kakabouki *, Panteleimon Stavropoulos, Ioannis Roussis, Antonios Mavroeidis and Dimitrios Bilalis *

Laboratory of Agronomy, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece; i.kakabouki@aua.gr (I.K.); stavropoulosp@aua.gr (P.S.); roussis@aua.gr (I.R.); p1152016@aua.gr (A.M.); stud118148@aua.gr (A.T.)

* Correspondence: i.kakabouki@aua.gr; bilalis@aua.gr

Abstract: Throughout the world, salinity is a major environmental issue that limits agricultural productivity, particularly in arid and semi-arid regions. In addition, climate change is the most important reason for the salinization of agricultural soils in the world, so it is now essential to find solutions to increase salinity tolerance in plants. This study investigated the potential of arbuscular mycorrhizal fungi (AMF) inoculation to enhance the growth and yield performances of flax under different salinity levels by conducting a pot experiment. The experiment was laid out in a two-factor completely randomized design including AMF inoculation (AMF+: with and AMF-: without inoculation) and irrigation water salinity (0, 50, 100, and 150 mM NaCl). According to the results, it is evident that salt stress caused negative physiological effects, including limited growth, reduced photosynthesis, and decreased nitrogen (N) and phosphorus (P) content in shoots and roots of flax plants. Moreover, mycorrhizal association improved the salt tolerance of the plants by increasing chlorophyll content, enhancing N and P shoot and root contents, and consequently yield parameters, such as seed, and stem fiber yield, particularly at moderate salt concentrations (50 and 100 mM NaCl). As a result of using AMF, flax plants grown under salt stress exhibited tolerance, suggesting that AMF could be applied in saline environments to maintain ecological stability.

Keywords: AMF root colonization; commercial arbuscular mycorrhizal inoculant; leaf relative water content; natural difference vegetation index (NDVI); soil plants analysis development (SPAD); seed yield; stem fiber yield

1. Introduction

Flax (*Linum usitatissimum* L.) is one of about 230 species in the Linaceae family, and it has considerable potential due to its versatile uses and adaptability [1,2]. In spite of its origins in the Mediterranean region and southwestern Asia, its ability to adapt to differing climatic conditions enabled its cultivation throughout the Middle East, India, Canada, and several European countries [2,3]. As a multipurpose crop, flax is cultivated either for its fiber or for its seeds and the oil produced from them [2]. Fibers from flax have been considerably used for a variety of industrial applications, ranging from dressing fabrics and bedsheets to twine and ropes [4]. A variety of high-quality textiles (also known as linen fabrics), such as damasks and lace, are made from best grades of flax fibers [4,5]. Additionally, these fibers are used in the paper industry and, interestingly, in the production of banknotes [6]. It is notable that flax seeds contain a variety of bioactive compounds, including alpha-linolenic acid (ALA), proteins, and lignans, which make them a valuable source of nutrition for both humans and animals [7,8]. Flaxseeds can be consumed as whole grains or as ground powders [9] and are high in oil (40%) [5,10]. The oil is edible and highly nutritious, as well as having a pleasant taste and aroma [11,12]. It comprises a high percentage of linolenic acid (from 48.5% to 68.5%), a low percentage of saturated fatty acids, and a high percentage of omega-3 and omega-6 fatty acids [5,12].

© 2023 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

Throughout the world, salinity is a major environmental issue that limits agricultural productivity, particularly in arid and semi-arid regions [13]. There are two main reasons for the detrimental effects of sodium chloride (NaCl) on plants: the reduced availability of water caused by sodium accumulation in the soil reducing soil water potential and the toxic effects of sodium and chlorine ions on plant. In response to reduced nutrient and water uptake, osmotic stress, ion toxicity, and nutritional imbalances result in significantly reduced plant growth and crop production [14–16]. High salinity conditions adversely affect plant growth and photosynthesis as a result of elevated ethylene levels in roots, ionic imbalance, and hyperosmotic conditions [16,17]. Salt accumulation in the soil decreases the osmotic potential, thereby interfering with the absorption of water by roots and affecting cell growth and metabolism [16]. Moreover, high salinity levels decrease the growth of plants by causing oxidative damage to them as a result of the formation of reactive oxygen species (ROS) [14].

Aside from employing some intrinsic mechanisms of adaptation, plants exposed to soil salinity can enhance their tolerance by developing mutualistic relationships with the microorganisms living in the rhizosphere [18,19]. The arbuscular mycorrhizal fungi (AMF) are among the soil microorganisms that have symbiotic relationships with the majority of terrestrial plants [20]. Specifically, AMF have been proven to enhance salt tolerance in host plants by improving nutrient uptake, increasing chlorophyll synthesis, as well as enhancing enzyme activity of protection systems [21,22]. It is estimated that 70–90% of terrestrial plant species are associated with these beneficial microorganisms in soil [23]. These organisms play an important role in ecosystems by providing water and cycling nutrients [23,24], both of which have a significant impact on carbon, nitrogen, and phosphorus biogeochemical cycles [25]. It is well known that AMF are able to take up nutrients from the soil and then transfer them to the host plants as compensation for photosynthetically fixed carbon [26,27]. Furthermore, 5 to 10% of the photosynthetically active compounds of host plants are assigned to the AMF partner [28], and when soil phosphorus levels are low, roots and AMF transfer more carbon and phosphorus to each other [23,29]. Under drought conditions, AM fungi can absorb and supply more water and nitrogen to their host plants via their hyphae [27]. The presence of AMF increases the tolerance of their host plants to biotic and abiotic stresses, specifically drought [30]. Plant-mycorrhizal fungi symbiotic interaction has been demonstrated to increase plant yield and help plants resist biotic and abiotic stresses in agriculture [31], making the symbiotic relationship among plants and mycorrhizal fungi agriculturally and ecologically important [32,33].

Climate change is the most important reason for the salinization of agricultural soils in the world, so it is now essential to find solutions to increase salinity tolerance in plants. In addition, despite the significant industrial and nutritional importance of flax, limited research has been conducted on the effect of salinity induced by different salts on plant growth and performance in this plant species. Therefore, the aim of the present work was to determine the potential of AMF inoculation to enhance the growth, seed and stem fiber yield of flax under different salinity levels by conducting a pot experiment.

2. Materials and Methods

A greenhouse pot experiment was conducted in 2023 (from January to May) at the Agricultural University of Athens (latitude 37°59′N, longitude 23°42′, 30 m from the sea surface), Greece, using a fiber flax cultivar (*Linum usitatissimum* L. cv. Eden) in order to determine the effect of inoculation with an introduced AM fungal inoculum under the influence of irrigation water salinity. The average lowest and highest recorded temperatures during the experiment was 13.1°C and 35.9°C, respectively (average 15.6°C), whereas the relative humidity (RH) ranged from 42% to 84% (average 72%). In addition, the plants were subjected to a natural daylight cycle ranging between 10-14 h during the current study.

The experiment was conducted in a two-factor completely randomized design including AMF inoculation and irrigation water salinity. Mycorrhizal inoculation included two levels: control treatment without inoculation (AMF–) and inoculation with AM inoculum (AMF+). The inoculum used was the commercial microgranulated-based root inoculant product Micoseeds Plus (Microspore

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

Hellas – Sacom Hellas, Athens, Greece), which is based on microorganisms that predominantly contains arbuscular mycorrhizal fungi (AMF) spores of *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus aggregatum* and *Rhizophagus intraradices*. In addition to the aforementioned AMF spores, there are fungi and bacterial species belonging to the genera *Trichoderma*, *Streptomyces*, *Bacillus* and *Pseudomonas*. The inoculation dosage was 2 g of inoculum per plant according to the recommendations of the manufacturer of the inoculant product. For the inoculation group of pots, the inoculum was put 3 cm below the surface of the substrate before sowing. Four levels of water salinity were applied (according to the screening results of the preliminary experiment): 0, 50, 100, and 150 mM NaCl. In general, a total of eight treatments were formed, with thirty pots planted in each treatment.

The seeds were sown directly 2 cm deep in plastic pots with a volume of 7 L containing 3 kg of homogenous mixture (2:1:1) of clay loam soil (non-sterilized), peat moss (sterilized) and perlite (sterilized). The soil was collected from the experimental field next to the greenhouse. Some physicochemical properties of the substate used are presented in Table 1. Five seeds were sown in each pot on 17th January 2023, and seedlings were thinned to two per pot after emergence, which was on 26th January 2023. Water salinity treatments were started at 20 days after sowing (DAS). For the salinity groups, in order to avoid osmotic shock, each group was treated with different concentrations of NaCl solution (10 mM, 20 mM, or 30 mM) every 2 days until reaching the final concentration, with 5 replicates. As for the control (0 mM NaCl) group, it was treated with sterilized water. According to the pre-experiment, treatments of 50 and 100 mM NaCl water salinity level were set as moderate concentration in the present research study.

Table 1. Physicochemical characteristics of the soil substrate.

Substrate properties	Soil, peat, and perlite (2:1:1)
Soil type	Clay loam (28.4% clay, 33.7% silt, 37.9% sand)
pH (1:2 H_2O)	7.42
Organic matter (%)	0.41
$CaCO_3$	15.48
P (Olsen) (mg kg^{-1})	9.87
NO_3^-	114.8
Na^+	107.2

The traits that were taken into account in order to assess the flax plants were the plant growth parameters and biomass allocation, AMF root colonization, leaf relative water content, total chlorophyll content, SPAD and NDVI readings, total proline content, nitrogen (N) and phosphorus (P) absorption and distribution, seed yield, biological yield, harvest index and stem fiber yield. The plant growth parameters and biomass allocation were measured using twenty plant samples from each treatment at 90 DAS. Specifically, the following plant growth parameters were evaluated: plant height, leaf area per plant, and fresh and dry weight of the plant aboveground parts (shoot) and roots. For the determination of dry weight, the samples were oven-dried for 72 h at 65°C. The leaf area per plant were measured by placing the collected samples on an Epson Perfection V330 Photo Scanner (Seiko Epson Inc., Nagano-ken, Japan) using Delta–T software (Delta–T Scan ver. 2.04; Delta–T Devices Ltd., Burwell, Cambridge, UK). The biomass allocation of each part was calculated according to the following formula: root to shoot ratio = underground biomass/aboveground biomass. Moreover, the arbuscular mycorrhizal fungi (AMF) colonization rate in roots for each plant sample was also determined microscopically by gridline-intersection method at 30-40× magnification [34], a technique that involves cleaning roots in 10%(w/v) KOH for 10 minutes at 90°C and staining them with lactophenol containing 0.05% (w/v) trypan blue [35].

Leaf relative water content was determined at 90 DAS utilizing the method of Yamasaki and Dillenburg [36]. Specifically, for each treatment, a total of 100 mg of fresh leaf samples were placed in Petri dishes containing distilled water for four hours at room temperature (23°C). After the samples

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

were taken out and dried off, the turgor weight (TW) was also recorded, after which the samples were dried in an oven at 70°C overnight. The dry weight (DW) was then measured. The relative water content was calculated using the following equation:

$$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100$$

where, FW is the fresh weight of the leaf tissue.

For the estimation of total chlorophyll content, fresh leaf samples collected at 90 DAS from the youngest fully expanded leaves were carefully divided into tiny pieces (about 0.1 g), ground to a powder in 10 mL of 80% acetone also centrifuged for five minutes at 10,000 rpm. The process was reiterated after collecting the supernatant till the remainder had no color. The absorbance was read at 645 and 663 nm using a near infra-red spectrometer (Rapid Analyzer XDS; Foss Tecator AB, Höganas, Sweden) with a spectral range from 400 to 2498 nm. The blank solution contained 80% acetone. The total chlorophyll content was determined according to the following formula [37]:

$$\text{Total chlorophyll content} = 20.2\ (A_{645}) + 8.02\ (A_{663})\ \frac{V}{1000 \times W}$$

where, A is the absorbance at specific wave length; V is the final volume of chlorophyll content in 80% acetone, and; W is the fresh weight of the leaf tissue extract.

Soil plants analysis development (SPAD) values of the third fully expanded leaves were determined at 90 DAS (anthesis stage) using SPAD 502 plus (Konica Minolta Optics Inc., Tokyo, Japan). Based on the manufacturer's instructions, measurements were taken from the center of each leaf, avoiding the main vein. The mean value of six flax plants was considered as one replication. The normalized difference vegetation index (NDVI) of the total plant biomass in the pot was also measured at 90 DAS, by means of a Trimble GreenSeeker portable handheld spectroradiometer (Trimble Agriculture Division, Westminster, CO, USA). At each pot, the sensor head was placed 100 cm above the surface of the pot, covering the entire area of the plant. About 100 NDVI measurements were taken at each pot. In order to soil-adjust the measurements of NDVI, measurements made in pots containing only soil substrate were subtracted from measurements made in pots containing plants. The proline content was determined spectrophotometrically according to the method of Bates et al. [38]. Specifically, 500 mg fresh leaf material at 90 DAS was taken and homogenized in 10 mL of sulfosalicylic acid. To the extract, 2 mL each of glacial acetic acid and ninhydrin acid were added, and the samples were heated at 100°C. The mixture was extracted with 4 mL toluene and the free toluene was spectrophotometrically quantified at 520 nm using L-proline as a standard.

To determine the total nitrogen (N) content of aerial and root biomass, dried samples collected for plant growth parameter measurements at 90 DAS were ground to a fine powder and applied to the Kjeldahl procedure using a Kjeltec 8400 autoanalyzer (Foss Tecator AB, Höganas, Sweden). The phosphorus (P) content was determined by selecting ten plant samples per treatment at 90 DAS, dissolving them in a $HNO_3H_2O_2$ solution, and heating them under pressure in a CEM MDS 2000 microwave (CEM Ltd., Buckingham, UK). In order to analyze the extract, an iCAP 6500 DUO ICP-emission spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used.

Finally, the plants were harvested at full seed maturity (seed moisture 12%) on 22nd May 2023 (125 DAS). The seed and biological yields were determined by ten randomly selected plants from each treatment. Harvest index (HI) was calculated as the ratio between seed yield and above-ground biomass (biological) yield (whole weight of plants derived for seed yield). In addition, fiber yield per plant was determined by the method described by Khan et al. [39]. In particular, fibers were extracted by water retting process. For each treatment, twelve plants were taken and tied to make a bundle. Bundles were dipped into fresh water tank for 15 days complete degradation of cell wall material due to microbial action. After complete retting, the fibers were separated from woody material by means of an operation known as scutching.

Data were subjected to two-way analysis of variance (ANOVA) using SigmaPlot v.12.0 (Systat Software Inc., San Jose, CA, USA). The differences between means were separated using the Tukey's honestly significant difference (HSD) test. Correlation analyses were used to describe the

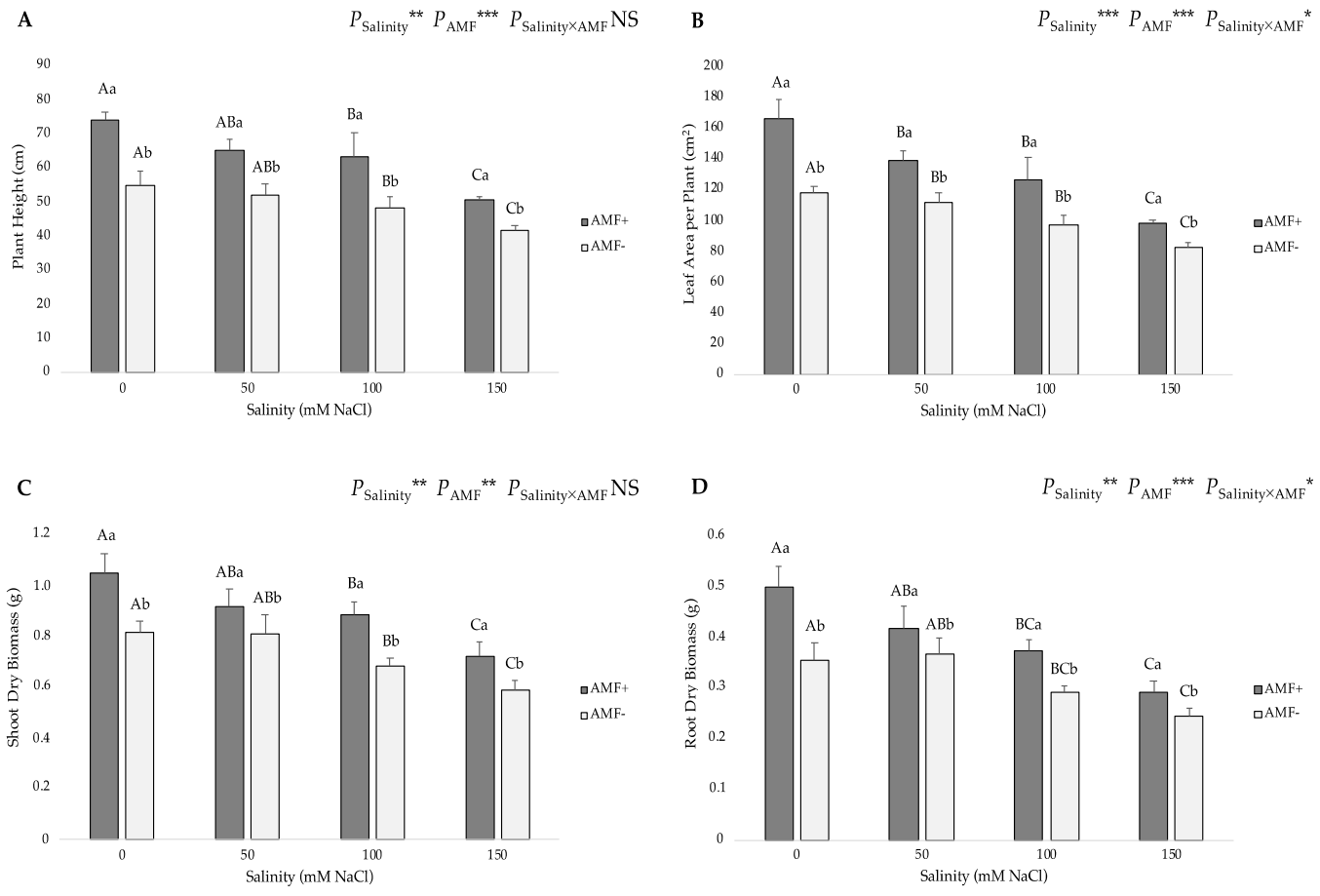
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

relationships among all the studied measurements using Pearson's correlation. All comparisons were made at the 5% level of significance ($p < 0.05$).

3. Results

3.1. Growth Parameters and AMF Root Colonization

Based on the two-way ANOVA, the effects of salinity and AMF inoculation were significant on plant height, leaf area per plant, shoot dry biomass, root dry biomass and root to shoot ratio, but there was no significant interactive effect on plant height, shoot dry biomass and root to shoot ratio (Figure 1). Inoculation with AMF increased all the above-mentioned growth parameters under all salinity levels versus the non-inoculated plants. Although salinity reduced the values of these studied parameters in mycorrhizal plants, they were higher than those of non-inoculated plants. The highest plant height was related to the AMF-inoculated (AMF+) plants at 0 mM NaCl salinity (74.2 cm) and the lowest one (41.7 cm) was recorded at the 150 mM NaCl level in non-inoculated (AMF–) plants (Figure 1A). Leaf area per plant was higher in the AMF+ plants at the 0 mM salt level (166.25 cm^2) and showed the lowest (82.58 cm^2) in AMF– plants under 150 mM NaCl (Figure 1B). The highest shoot and root dry biomass values were found in the AMF+ plants at the 0 mM salt level (1.04 and 0.49 g, respectively), whereas the lowest values of these traits were obtained at 150 mM NaCl in the AMF– plants (0.59 and 0.24 g for shoot and dry biomass, respectively) (Figure 1C and 1D, respectively). As for root to shoot ratio, the highest value was observed in the AMF-inoculated plants at the 0 mM salt level (0.470), whereas the lowest one (0.403) recorded in non-inoculated plants at the 150 mM salt level; however, the differences among AMF treatments for the same salinity level were not statistically significant (Figure 1E). Consequently, the findings of the growth parameters of the present investigation support the hypothesis that AMF inoculation stimulates plant growth and development under salt stress.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

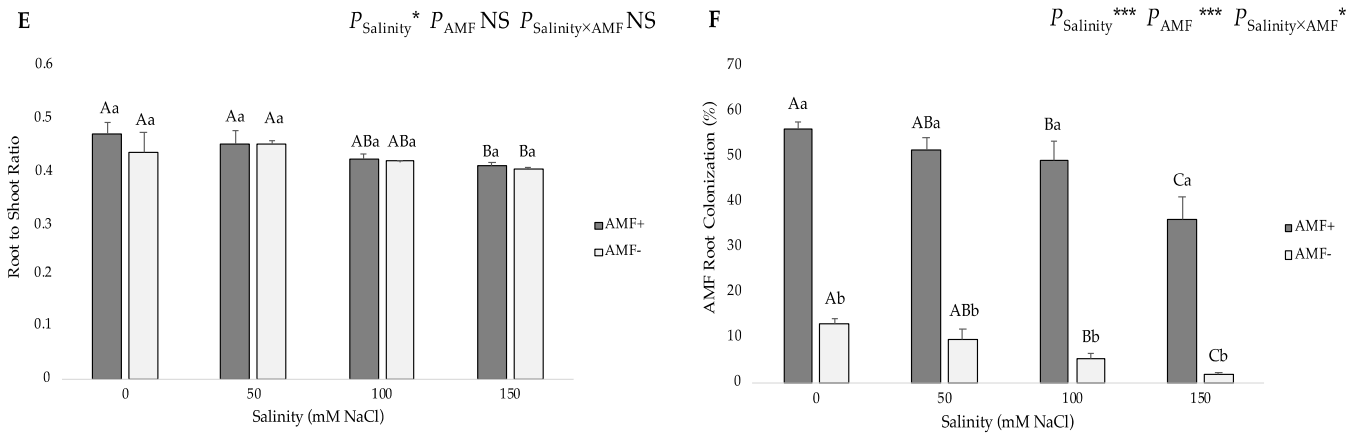


Figure 1. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on (**A**) plant height, (**B**) leaf area per plant, (**C**) shoot dry biomass, (**D**) root dry biomass, (**E**) root to shoot ration, and (**F**) AMF root colonization of flax at different salinity (0, 50, 100, and 150 mM NaCl) levels. AMF+: with AM inoculum; (AMF–): without inoculation. NS: No significant effect; *, ** and ***: Significant at 5%, 1% and 0.1% probability levels, respectively. Values are means ± standard error. The different capital letters indicate significant differences among different salinity levels, and the different lowercase letters for the same salinity level indicate significant differences among different AMF inoculation treatments according to the Tukey's HSD test ($p \leq 0.05$).

Salt stress caused significant declines in the AMF root colonization rate in flax plants under NaCl stress from 50 to 150 mM (Figure 1F). The values of AMF root colonization percentage in AMF-inoculated plants decreased from 56.17% (in the 0 mM NaCl treatment) to 35.93% (in the 150 mM NaCl treatment). In addition, the analysis of AMF colonization revealed that the non-inoculated plants presented the lowest mycorrhizal infection. Specifically, the AMF colonization on AMF– plants was kept below 14%, with the highest value recorded at the 0 mM salt level (13.19%), while the lowest one (1.82%) was recorded at the highest (150 mM) salt level.

3.2. Physiological and Biochemical Parameters

The role of the treatments on the leaf relative water content (RWC), total chlorophyll content and soil plants analysis development (SPAD) index was similar to those found for the shoot dry biomass. Increasing salinity concentrations in the irrigation water from 0 to 150 mM NaCl caused a reduction on the aforementioned traits (Figure 2). AMF inoculation had also a significant on these parameters. In particular, the highest RWC was related to the AMF+ plants at 0 mM NaCl salinity (86.04%), while the lowest value (61.16%) was obtained from AMF– plants at the 150 mM NaCl level (Figure 2A). The highest total chlorophyll content was recorded in the AMF+ plants at the 0 mM salt level (1.45 mg g^{-1} FW), and the lowest one (0.81 mg g^{-1} FW) was obtained from AMF– plants at 150 mM NaCl level (Figure 2B). Concerning the SPAD index, a non-destructive measurement of the chlorophyll content, the highest value was observed in the AMF+ plants at the 0 mM salt level (20.48 SPAD Units), whereas the lowest one (11.03 SPAD Units) found in the non-AMF-inoculated plants at the 150 mM salt level (Figure 2C).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

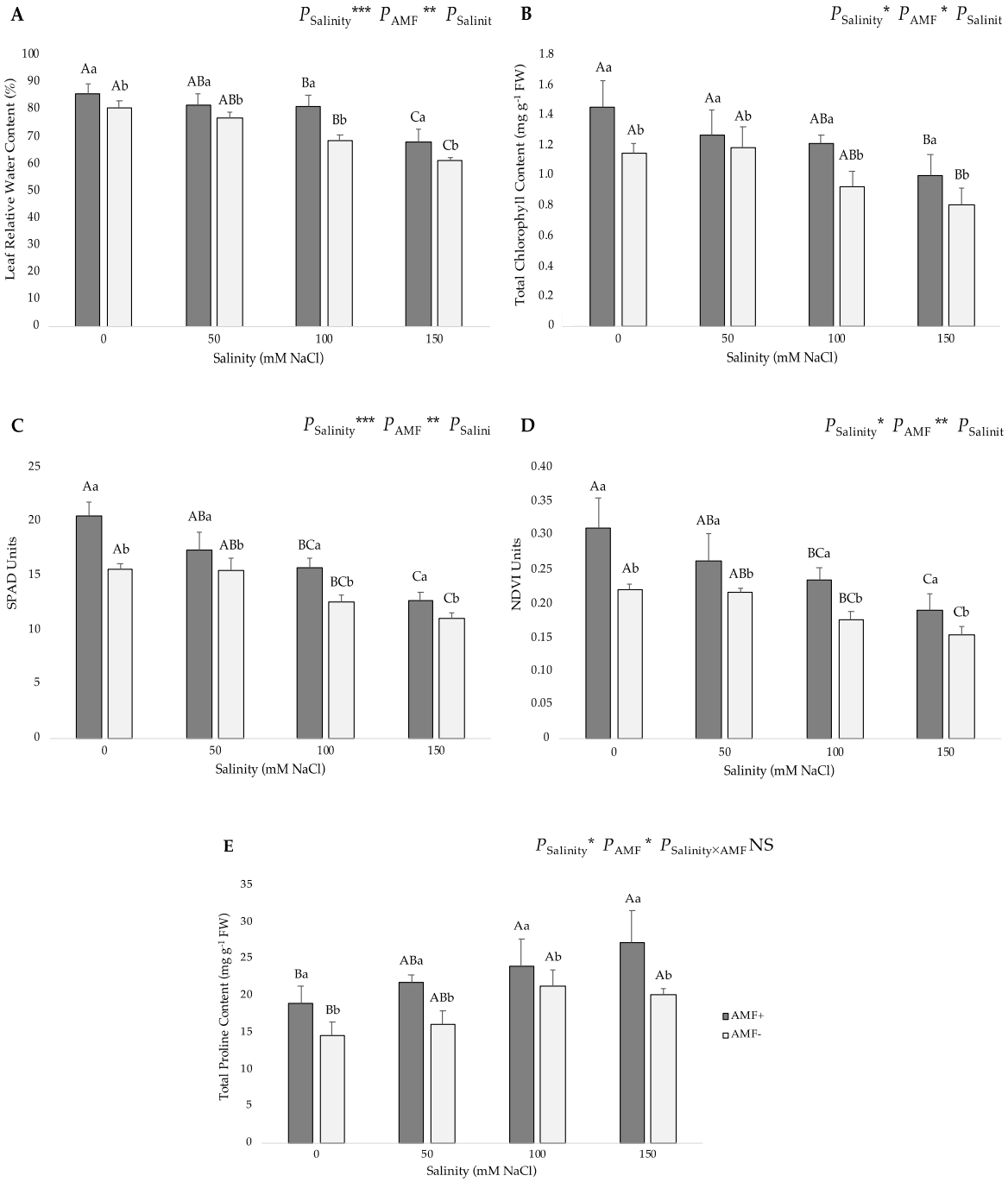


Figure 2. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on (**A**) leaf relative water content, (**B**) total chlorophyll content, (**C**) soil plants analysis development (SPAD), (**D**) natural difference vegetation index (NDVI), and (**E**) total proline content in leaves of flax at different salinity (0, 50, 100, and 150 mM NaCl) levels. AMF+: with AM inoculum; (AMF–): without inoculation. NS: No significant effect; *, ** and ***: Significant at 5%, 1% and 0.1% probability levels, respectively. Values are means ± standard error. The different capital letters indicate significant differences among different salinity levels, and the different lowercase letters for the same salinity level indicate significant differences among different AMF inoculation treatments according to the Tukey's HSD test ($p \leq 0.05$).

The natural difference vegetation index (NDVI) is a remote sensing method that uses the reflectance of light in red and near-infrared light and can be utilized to determine chlorophyll content by measuring the transmission of these light wavelengths through leaves. As expected, NDVI index

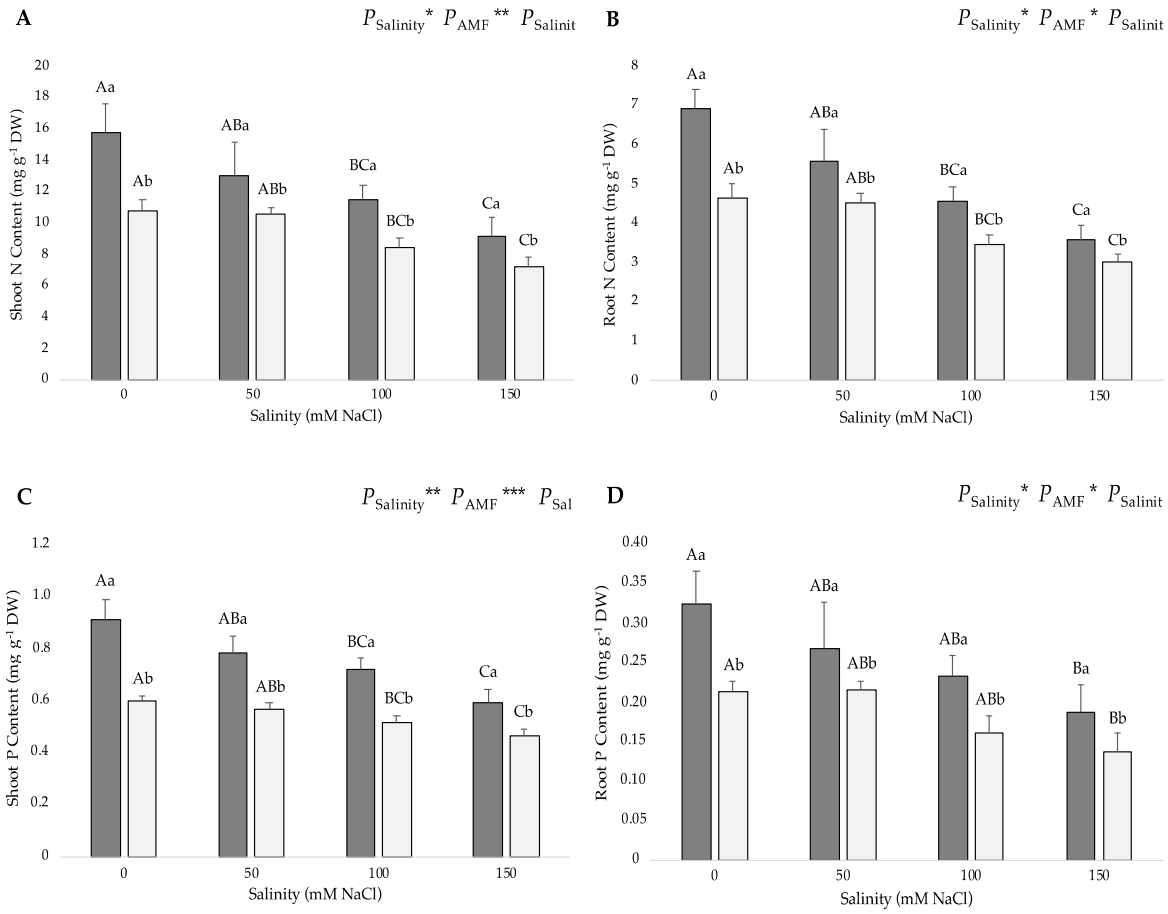
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

presented a similar trend as the total chlorophyll content. The highest NDVI value was observed in the AMF+ plants at the 0 mM NaCl level (0.31 NDVI Units), whereas the lowest value (0.15 NDVI Units) was obtained from AMF– plants at 150 mM NaCl level (Figure 2D).

The pattern of total proline content in leaves showed that salinity caused a significant increase in this trait (Figure 2E). AMF inoculation significantly reduced total proline content in flax plants (14.52, 16.09, 21.32 and 20.23 mg g^{-1} FW at 0, 50, 100 and 150 mM NaCl, respectively). The lowest and highest total proline content (14.52 and 27.29 mg g^{-1} FW) occurred in non-inoculated plants at 0 mM NaCl and AMF-inoculated plants at 150 mM NaCl, respectively.

3.3. Nitrogen and Phosphorus Distribution

The two-way ANOVA suggested that salinity and AMF inoculation had significant influence on nitrogen (N) and phosphorus (P) contents in shoots and roots, and the interaction of two factors significantly affected shoot P content. Gradually increasing salinity levels from 0 to 150 mM NaCl lead to gradually decrease in N and P contents of both AMF-inoculated and non-inoculated flax plants (Figure 3). As compared with roots, shoots accumulated obviously more N and P as well. In addition, N and P content of shoots and roots of AMF-inoculated plants were significantly higher than those of non-inoculated plants at all studied NaCl levels. Under salt stress, AMF inoculation significantly increased N content by 46.19%, 23.81%, 35.98% and 26.32% at 0, 50, 100 and 150 mM NaCl in shoots (Figure 3A), whereas it increased by 48.82%, 23.94%, 31.03% and 19.33% at 0, 50, 100 and 150 mM NaCl in roots, respectively (Figure 3B). Moreover, compared with non-inoculated plants, AMF-inoculated plants had significantly higher shoot P content (52.94%, 37.43%, 39.22% and 28.35% at 0, 50, 100 and 150 mM NaCl, respectively) (Figure 3C), and higher root P content (52.60%, 24.77%, 45.38% and 35.76% at 0, 50, 100 and 150 mM NaCl, respectively) (Figure 3D).

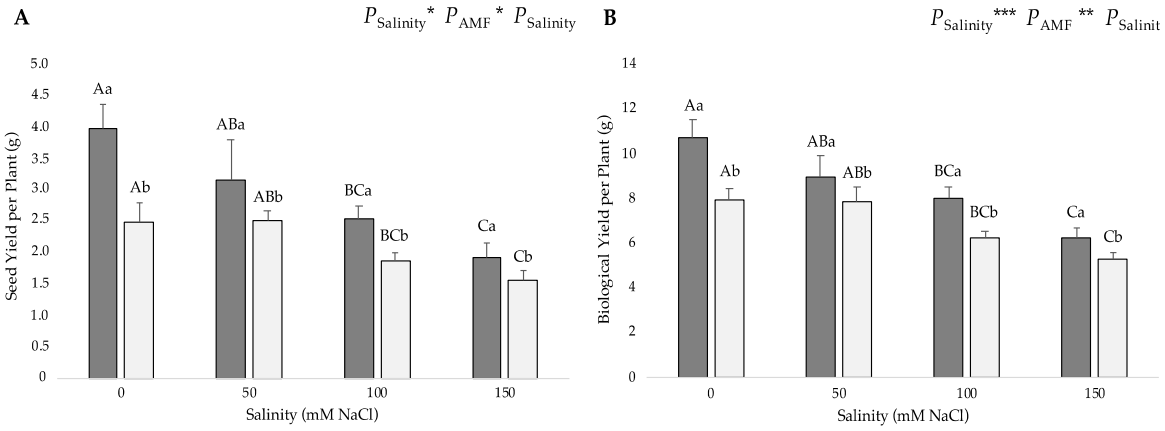


Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

Figure 3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on (**A**) shoot nitrogen (N) content, (**B**) root N content, (**C**) shoot phosphorus (P) content, and (**D**) root P content of flax at different salinity (0, 50, 100, and 150 mM NaCl) levels. AMF+: with AM inoculum; (AMF–): without inoculation. NS: No significant effect; *, ** and ***: Significant at 5%, 1% and 0.1% probability levels, respectively. Values are means ± standard error. The different capital letters indicate significant differences among different salinity levels, and the different lowercase letters for the same salinity level indicate significant differences among different AMF inoculation treatments according to the Tukey's HSD test ($p \leq 0.05$).

3.4. Yield Parameters

Seed and biological yields per plant were significantly affected by salinity regimes and AMF colonization. Increasing salt levels in the irrigation water from 0 to 150 mM NaCl caused significant declines in these yield parameters (Figure 4). AMF inoculation had also a significantly positive impact on these traits. Specifically, the highest seed yield per plant was related to the AMF+ plants at 0 mM NaCl salinity (3.98 g), while the lowest value (1.57 g) was recorded in the AMF– plants at the 150 mM NaCl level (Figure 4A). The highest biological yield per plant was recorded in the AMF+ plants at the 0 mM NaCl level (10.79 g), whereas the lowest one (5.35 g) was found in the AMF– plants at 150 mM NaCl level (Figure 4B). In regard to harvest index (HI), there were no significant differences among the studied salinity regimes and AMF inoculation treatments (Figure 4C); however, under salt stress, the values of AMF-inoculated plants were slightly higher (0.358, 0.344, 0.317 and 0.304 at 0, 50, 100 and 150 mM NaCl, respectively) than those of non-inoculated plants (0.313, 0.321, 0.299 and 0.292 at 0, 50, 100 and 150 mM NaCl, respectively). As for stem fiber yield per plant, this trait presented similar trends as descripted on seed and biological yields per plant values. Salt stress had a negative effect on stem fiber yield per plant reflected by obvious reduction at 100 and 150 mM of AMF-inoculated and non-inoculated flax plants, and this reduction increased with the NaCl levels increased gradually. In addition, AMF inoculation positively affected this trait under salt stress. The highest stem fiber yield per plant was observed in the AMF+ plants at the 0 mM NaCl level (1.51 g), while the lowest value (0.85 g) was obtained from AMF– plants at 150 mM NaCl level (Figure 4D).



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

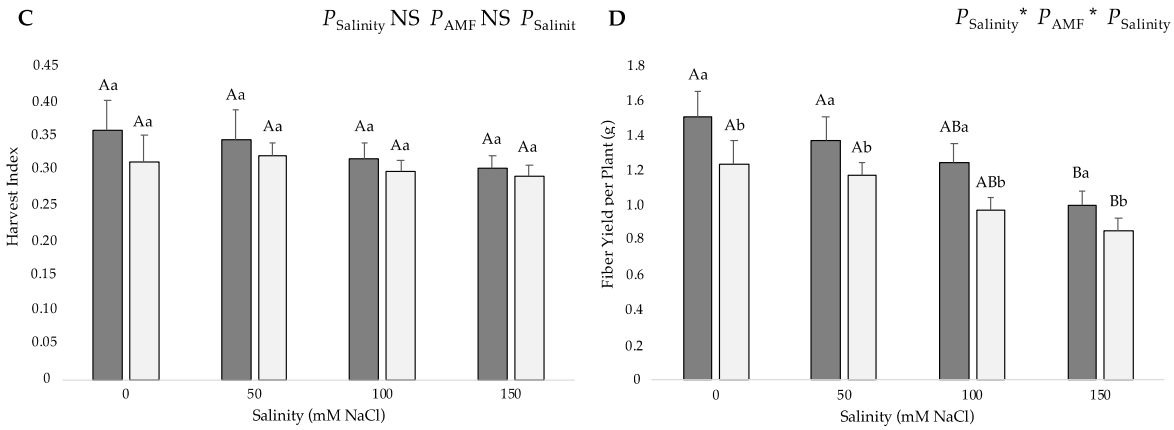


Figure 4. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on (**A**) seed yield per plant, (**B**) biological yield per plant, (**C**) harvest index, and (**D**) stem fiber yield per plant of flax at different salinity (0, 50, 100, and 150 mM NaCl) levels. AMF+: with AM inoculum; (AMF–): without inoculation. NS: No significant effect; *, ** and ***: Significant at 5%, 1% and 0.1% probability levels, respectively. Values are means ± standard error. The different capital letters indicate significant differences among different salinity levels, and the different lowercase letters for the same salinity level indicate significant differences among different AMF inoculation treatments according to the Tukey's HSD test ($p \leq 0.05$).

4. Discussion

With the advent of natural calamities and the increasing impact of global warming, there is a growing need for food security, plant productivity, and food safety in order to sustain the global population and crop production. A significant environmental stress affecting plant growth is salt stress [40,41]. It is common for plants growing in saline areas to be affected by ion toxicity [41,42]. Salt ions can cause great damage to enzyme structure, disrupt photosynthesis, and result in nutrient deficiencies as a result of their toxic effects [18,21]. Salt stress conditions are a result of global warming, and salt stress management is necessary for better crop production and food security. Consequently, sustainable practices for managing crop production have been emphasized worldwide in recent years. AMF is one of the most widely used symbiotic associations for the promotion of plant growth and the resistance of plants to salt [41,43,44]. To explore the potential function of AMF in flax plants under abiotic stress, the present research examined growth, physiological, biochemical, and yield parameters, as well as nutrient distribution within the plants exposed to salt stress and AMF inoculation.

Salinity decreased plant height with a larger effect at higher salinity levels, whereas AMF-inoculated plants produced higher heights than non-inoculated plants (Figure 1A). These results are consistent with those presented by Mathur et al. [45] on moth bean (*Vigna aconitifolia* L.), Jamil et al. [46] on radish plant (*Raphanus sativus* L.), Kapoor and Srivastava [47] on black gram (*Vigna mungo* L.), Begum et al. [48] on maize (*Zea mays* L.), Bernardo et al. [49] on wheat (*Triticum* sp. L), Hashem et al. [50] on chickpeas (*Cicer arietinum* L.), and Jerbi et al. [51] on hulless barley (*Hordeum vulgare* ssp. *nudum* L.), where they indicated that increasing the concentrations of sodium chloride (NaCl) resulted in the plants decreasing in length. The observed decrease in the plant height of flax plants after the treatment with NaCl solution could be the result of the negative impact of this salt on the rate of photosynthesis, changes in enzyme activity that directly affect protein synthesis, and a decrease in carbohydrates and growth hormones, both of which can inhibit the plant growth [52,53].

Plants can benefit from the symbiosis between AMFs and host plants in a number of ways, including the promotion of growth and development as well as the enhancement of their resistance to many abiotic stresses. The application of AMF can reduce the inhibition of plant functions caused by salinity stresses, as well as increase plant height and growth generally [53–55]. As observed in a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

previous study [56], salinity-induced detrimental effects on plant height, leaf area, and number of leaves were mitigated by inoculation with AMF.

The leaf area per plant was significantly influenced by both salinity and AMF-inoculation. Regarding the salinity effect, it was observed that with increasing salt levels, the leaf area per plant decreased. In terms of AMF-inoculation, this had a positive effect on the leaf area, with the highest values being found in AMF-inoculated plants. The results of Bernardo et al. [49] on wheat and Hashem et al. [50] on chickpeas support our findings that NaCl treatment reduced leaf area when compared to the control (0 mM NaCl) plants. As a consequence of exposure to higher concentrations of sodium chloride, the present study observed a significant decrease in leaf area that may be explained by the negative effect salt has on photosynthesis, resulting in a reduction in leaf growth, chlorophyll content, and plant growth [52].

Salt-induced osmotic stress results in a reduction in leaf area, as well as a decrease in stomatal conductance and mesophyll conductance, thus limiting CO_2 assimilation and availability [53,57]. A symbiotic relationship with AMF appears to alleviate the negative effects of salinity on the photosynthetic capacity of plants. It has been demonstrated that mycorrhizal symbiosis prevents salt stress from negatively affecting photosynthesis by improving the water status of mycorrhizal plants, which increases leaf area and stomatal conductance, and thereby results in better CO_2 assimilation [54,55]. Additionally, the increase in leaf area following AMF inoculation can also be attributed to the increased activity of cytokinin, a growth promoter that appears to increase leaf growth through its effect on cell division and expansion [57].

Furthermore, leaf area per plant was linearly related to the biological and seed yield per plant ($r = 0.930$, $p < 0.001$; $r = 0.819$, $p < 0.001$, respectively) (Figure 5), suggesting that leaf area is an important yield component that should be considered during selection of promising accessions. There is evidence that plants with a large number of leaves and a large leaf area are more likely to have a higher ability to perform photosynthetic duties, which contributes to the development and growth of the plant [33,44,45].

Trait	Coefficient of correlation (*r*)																		
	Plant Height	Leaf Area per Plant	Shoot Dry Biomass	Root Dry Biomass	Root to Shoot Ratio	AMF Coloni-zation	Leaf Relative Water Content (RWC)	Total Chloro-phyll Content	SPAD	NDVI	Total Proline Content	Shoot N Content	Root N Content	Shoot P Content	Root P Content	Seed Yield per Plant	Biologi-cal Yield per Plant	Harvest Index (HI)	Fiber Yield per Plant
Plant Height	1.000	0.948***	0.891***	0.803***	0.261^{ns}	0.759***	0.627**	0.679***	0.814***	0.673***	–0.136^{ns}	0.689***	0.625**	0.838***	0.807***	0.634***	0.814***	0.169^{ns}	0.674**
Leaf per Plant	0.948***	1.000	0.919***	0.923***	0.534**	0.721***	0.727***	0.765***	0.929***	0.823***	–0.181^{ns}	0.837***	0.814***	0.923***	0.743***	0.819***	0.930***	0.409*	0.648***
Shoot Dry Biomass	0.891***	0.919***	1.000	0.950***	0.435*	0.598**	0.731***	0.936***	0.960***	0.881***	–0.052^{ns}	0.891***	0.831***	0.889***	0.846***	0.833***	0.962***	0.434*	0.683***
Root Dry Biomass	0.803***	0.923***	0.950***	1.000	0.690***	0.249^{ns}	0.768***	0.927***	0.994***	0.952***	–0.069^{ns}	0.961***	0.944***	0.925***	0.902***	0.951***	0.994***	0.638***	0.821***
Root to Shoot Ratio	0.261^{ns}	0.534**	0.435*	0.690***	1.000	0.630**	0.574**	0.520**	0.647***	0.751***	–0.120^{ns}	0.701***	0.790***	0.593**	0.692***	0.804***	0.649***	0.836***	0.799***
AMF Colonization	0.759***	0.721***	0.598**	0.249^{ns}	0.630**	1.000	0.652**	0.499*	0.581**	0.564**	0.269^{ns}	0.568**	0.514*	0.771***	0.518**	0.529**	0.581**	0.293^{ns}	0.520**
RWC	0.627**	0.727***	0.731***	0.768***	0.574**	0.652**	1.000	0.728***	0.776***	0.688***	–0.125^{ns}	0.693***	0.689***	0.667***	0.690***	0.698***	0.776***	0.427*	0.811***
Total Chlorophyll Content	0.679***	0.765***	0.936***	0.927***	0.520**	0.499*	0.728***	1.000	0.926***	0.917***	0.055^{ns}	0.920***	0.872***	0.812***	0.934***	0.873***	0.929***	0.595**	0.791***
SPAD	0.814***	0.929***	0.960***	0.994***	0.647***	0.581**	0.776***	0.926***	1.000	0.941***	–0.109^{ns}	0.956***	0.931***	0.917***	0.923***	0.938***	0.903***	0.586**	0.805***
NDVI	0.673***	0.823***	0.881***	0.952***	0.751***	0.564**	0.688***	0.917***	0.941***	1.000	0.104^{ns}	0.991***	0.986***	0.934***	0.969***	0.927***	0.946***	0.794***	0.889***
Total Proline Content	–0.136^{ns}	–0.181^{ns}	–0.052^{ns}	–0.069^{ns}	–0.120^{ns}	0.269^{ns}	–0.125^{ns}	0.055^{ns}	–0.109^{ns}	0.104^{ns}	1.000	0.084^{ns}	0.031^{ns}	0.109^{ns}	0.176^{ns}	0.043^{ns}	–0.109^{ns}	0.263^{ns}	0.144^{ns}
Shoot N Content	0.689***	0.837***	0.891***	0.961***	0.701***	0.568**	0.693***	0.920***	0.956***	0.991***	0.084^{ns}	1.000	0.979***	0.937***	0.984***	0.989***	0.955***	0.781***	0.886***
Root N Content	0.625**	0.814***	0.831***	0.944***	0.790***	0.514*	0.689***	0.872***	0.931***	0.986***	0.031^{ns}	0.979***	1.000	0.910***	0.969***	0.904***	0.938***	0.826***	0.913***
Shoot P Content	0.838***	0.923***	0.889***	0.925***	0.593**	0.771***	0.667***	0.812***	0.917***	0.934***	0.109^{ns}	0.937***	0.910***	1.000	0.882***	0.919***	0.903***	0.653***	0.778***
Root P Content	0.807***	0.743***	0.846***	0.902***	0.692***	0.518**	0.690***	0.934***	0.923***	0.969***	0.176^{ns}	0.984***	0.969***	0.882***	1.000	0.943***	0.912***	0.817***	0.926***
Seed Yield per Plant	0.634***	0.819***	0.833***	0.951***	0.804***	0.529**	0.698***	0.873***	0.938***	0.927***	0.043^{ns}	0.989***	0.904***	0.919***	0.943***	1.000	0.939***	0.828***	0.903***
Biological Yield per Plant	0.814***	0.930***	0.962***	0.994***	0.649***	0.581**	0.776***	0.929***	0.903***	0.946***	–0.109^{ns}	0.955***	0.938***	0.903***	0.912***	0.939***	1.000	0.598**	0.809***
HI	0.169^{ns}	0.409*	0.434*	0.638***	0.836***	0.293^{ns}	0.427*	0.595**	0.586**	0.794***	0.263^{ns}	0.781***	0.826***	0.653***	0.817***	0.828***	0.598**	1.000	0.846***
Fiber Yield per Plant	0.674**	0.648***	0.683***	0.821***	0.799***	0.520**	0.811***	0.791***	0.805***	0.889***	0.144^{ns}	0.886***	0.913***	0.778***	0.926***	0.903***	0.809***	0.846***	1.000

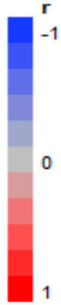


Figure 5. Correlation coefficients heatmap between evaluated traits. ns: No significant effect; *, ** and ***: Significant at 5%, 1% and 0.1% probability levels, respectively. AMF: Arbuscular mycorrhizal fungi; SPAD: Soil plant analysis development; NDVI: Normalized difference vegetation index; N: Nitrogen, and; P: Phosphorus.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023

doi:10.20944/preprints202309.0345.v1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

In the same manner with the previous growth parameters, the values of shoot and root dry biomass had a negative response to the increase in salt levels and exhibited a positive performance with AMF inoculation (Figure 1C and 1D). Various previous studies have demonstrated that AMF can alleviate the negative effects of salinity and improve plant growth by improving growth of shoots and roots [45,46,48–51,58,59]. In a study conducted by Jerbi et al. [51], it was found that AM fungi inoculation significantly increased the plant biomass at various salinity levels. As well, Klinsukon et al. [58] confirmed that AMF colonization affects root dry weight at all salt stress levels compared to non-inoculated plants. The result will be an increase in the number of extraradical hyphae and an increase in the surface area of the mycelia network of the mycorrhiza thereby contributing to the increase in the biomass of roots [58,59].

The root to shoot ration was not affected by either the different salt levels or the AMF inoculation. At this point, it is worth noting that the plants treated with lower salinity levels, as well as the AMF-inoculated plants, showed slightly higher values (Figure 1E). In particular, the application of AMF increased the root to shoot ratio in inoculated plants, revealing that AMF-inoculated plants divided more biomass to the roots than to the shoots. An increased root to shoot ratio is generally thought to enhance the source to sink ratio for water and nutrients and could therefore prove advantageous under salinity conditions [60,61].

All the above-mentioned findings show a substantial positive relationship between AMF root colonization and plant growth characteristics including the plant height, leaf area per plant, shoot and root dry biomass, and root to shoot ratio (Figure 5). AMF root colonization percentage was negatively affected by salinity (Figure 1F). The presence of NaCl in soil generally reduces the colonization of AMF roots by inhibiting their activity [58,62]. In cases in which AMF was inoculated, the colonization rate was higher, resulting in a more intensive symbiotic relationship between AMF and the host plant. AMF colonization was also observed in the non-inoculated plants, suggesting that AMF populations capable of colonizing flax plants were already present in the control soils. As a result of the above-mentioned mechanism, root system reinforcement is promoted and, consequently, plant growth is enhanced [63].

The leaf relative water content (RWC) was also significantly affected by salinity and AMF inoculation. Although salinity decreased, mycorrhizal plants increased RWC (Figure 2A). Several factors contribute to the higher RWC in leaves of AMF-inoculated plants: (a) AMF enhance soil-plant hydraulic conductivity at low water potential, (b) the AMF can reduce the osmotic potential of plants when they are experiencing water deficits by increasing levels of organic osmolytes, (c) AMF-inoculated plants exhibit higher stomatal conductance, and (d) AMF hyphae improve water relation [58,64–66].

Chlorophyll content in leaves, a significant physiological parameter of plant photosynthetic capacity, was significantly influenced by both salinity and AMF inoculation. There was a significant reduction in leaf total chlorophyll content due to increasing salt levels (Figure 2B), possibly as the result of repression of specific enzymes of the photosynthesis system as well as reduced nutrient uptake such as nitrogen (N) and magnesium (Mg) for chlorophyll biosynthesis. A significant increase in leaf total chlorophyll content was observed following AMF inoculation. As a result, the plants were able to take up more nutrients and have a lower Na content, which resulted in a higher capacity for photosynthetic respiration [58,67]. Specifically, AMF enhances photosynthesis by increasing Rubisco activity, electron transport rates, adenosine triphosphate (ATP) synthesis, and even the ratio of ATP to ADP (adenosine diphosphate) in leaves by increasing the mass fractions of N and P in leaves [68,69].

The soil plants analysis development (SPAD) value represents the relative content of chlorophyll in plants. According to the results of SPAD value, a decrease in chlorophyll content was observed as a result of salt stress. The presence of AMF compounds inoculants increases the SPAD value of flax plants (Figure 2C), thereby promoting their photosynthesis. AMF inoculation has been shown in previous studies to increase the rate of photosynthesis in plants [70,71]. Using AMF-inoculated flax may improve photosynthetic capacity by increasing the capacity for exchanging gas and absorbing water, thereby reducing salt stress-induced toxicity [70].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

Non-destructive optical chlorophyll meters are often utilized to quantify leaf chlorophyll content [72]. The proportion of transmitted red light and near-infrared light released by a red and a near-infrared LED, accordingly, through a leaf is used to determine the relative chlorophyll content [73]. Considering that chlorophyll absorbs red light effectively, the amount of red light transmitted through a leaf is proportional to its chlorophyll content [72,74]. As chlorophylls absorb very little near-infrared light, near-infrared light can be used as a measure of the absorption spectrum associated with non-chlorophylls [73,74]. In remote sensing, natural difference vegetation index (NDVI) sensors equipped with red and near-infrared light detectors are commonly employed to measure vegetation coverage [74]. NDVI sensors are capable of detecting red and near-infrared light from solar radiation, and they can therefore be used to determine the chlorophyll content in leaves by measuring the transmitted red and near-infrared light [72]. NDVI values, like the previous measurements assessing chlorophyll content directly (total chlorophyll content) or indirectly (SPAD index), demonstrated a negative reaction to an increase in NaCl levels and a positive response to AMF inoculation (Figure 2D). In addition, all the above-mentioned about total chlorophyll content, SPAD, and NDVI values are also confirmed by the positive and significant correlations of total chlorophyll content with SPAD ($r = 0.926$, $p < 0.001$), total chlorophyll content with NDVI ($r = 0.917$, $p < 0.001$) and SPAD with NDVI ($r = 0.941$, $p < 0.001$) (Figure 5).

A surplus of salt reduces soil osmotic potential, limiting plants from absorbing soil water resulting in physiological drought in plants. In plant cells, a substantial number of osmoregulatory metabolites, such as proline, are generated and stored; these metabolites can reduce intracellular osmotic potential and enable appropriate water uptake and use from plant species. This approach is utilized by plants to cope with physiological drought produced by salt stress [75]. This physiological alteration ensures that plants take up and utilize water normally, reducing the physiological drought induced by salt stress. Furthermore, it has been proposed that proline in plants performs a number of crucial roles, including scavenging reactive oxygen species and stabilizing proteins and cell membrane structures, as well [76,77]. The present study reported that during salt stress at 50 mM, 100 mM, and 150 mM NaCl, the proline content in flax increased considerably. Inoculation with AMF enhanced the proline content of flax plants even more (Figure 2E). The results demonstrated that inoculating flax with AMF could stimulate proline accumulation under salt stress, lower cellular osmotic potential, and improve flax plant salt resistance. Nevertheless, the contribution of AMF in the accumulation of proline in plants is not constant: a few investigations found higher proline content in AMF-inoculated plants under stress, while other research demonstrated lower proline content [18,50,51,77].

Nitrogen (N) is an essential component of several molecules, including amino acids, amides, polyamines, and proteins, all of which play a role in plant salt tolerance via diverse methods. Appropriate N metabolism management is critical for plant tolerance to salt. Nevertheless, depending on the level and length of salt stress, the plant species, the stage of plant growth, and the amount, type, and shape of N in the rhizosphere, the interaction among salinity and N metabolism is an extremely intricate network [14]. The mechanism of absorbing N by plants is primarily impeded in saline environment likely because of the diametrically opposed impact of salt ions with nitrate (NO_3^-) and ammonium (NH_4^+) disruption in the N ions transferring into root xylem, decreased transpiration rate, reduced water absorption owing to osmotic changes in the root zone, destruction in root structure of membranes, and less N demand due to the decreased plant growth rate [78]. In the saline environment, chloride (Cl^-) constitutes the major anion, and NO_3^- uptake is known to compete with that of Cl^-. At higher chloride concentrations, this interaction results in lower N uptake and content in plant tissues and decreased plant development [53,78]. Since Cl^- competes against NO_3^- uptake, sodium (Na^+) competes against NH_4^+ uptake at the plasma membrane. Salt-induced membrane protein disruption, which affects plasma membrane integrity, impacts NO_3^- and NH_4^+ uptake [78]. Since nitrate reductase constitutes a substrate-inducible enzyme, this competition leads to reduced NO_3^- flow from soil to roots [79].

AMF colonization in saline-grown host plants has been demonstrated to enhance nutrient absorption and maintain ionic balance. In fact, it has been established that AMF hyphae can deliver

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

up to 25% of the plant's nitrogen [80]. The higher nitrate reductase activity in mycorrhizal plants is ascribed to AMF-facilitated membrane stability maintenance [79,81]. Furthermore, AMF colonization influences the concentration and profile of polyamines in the plant, thereby helping in the maintenance of ion homeostasis in plant cells by increasing nutrient and water uptake [66,82]. In this study, salt stress significantly reduced the N content in shoots and roots of flax plant, while inoculation with the AMF inoculant improved flax N content status (Figure 3A and 3B). Similar results were recorded in the salt tolerance tests of AMF-inoculated *Euonymus maackii*, *Lallemantia iberica,* and *Leymus chinensis* plants conducted by Li et al. [41], Heydari and Pirzad [83] and Cao et al. [84], respectively.

In all treatments of the present study, AMF colonization increased phosphorus (P) contents in shoots and roots (Figure 3C and 3D). The current analysis demonstrated that the AMF effect on plant P nutrition was greater in plants inoculated with AMF, likely as a result of a lower level of nutrient mobility under salinity conditions. Under a wide range of salinity conditions, AMFs are able to enhance the amount of P in plant tissues significantly across all salinity levels. A number of researchers have concluded that the main mechanism of improvement in plant P concentration in mycorrhizal plants under salt stress is the improvement of plant P concentration [80,82]. Based on a previous study conducted by Frosi et al. [71], P is capable of being transported into the root through protoplasmic circulation by AMF, thereby reducing the transport resistance and increasing its speed [80]. Although other studies have demonstrated that mycorrhizal plants grow better under salt stress than non-AMF-inoculated plants, even if their P levels are similar under unstressed conditions [41,85].

Mycorrhizal contribution to nutrient (N and P) contents in shoots and roots of flax plants was higher in AMF-inoculated plants than in non-mycorrhizal plants under salt stress, explaining the better growth performance and biomass accumulation of AMF-inoculated plants under salinity. Thus, this suggests that the increased salinity tolerance of plants can be attributed to improved uptake and concentration of N and P in plant tissues. It is evident that AMF contributes to the plant's biomass and nutrient content in a positive manner. This was also confirmed by the positive and significant correlations of shoot dry weight with shoot N content ($r = 0.891$, $p < 0.001$), root N content ($r = 0.831$, $p < 0.001$), shoot P content ($r = 0.889$, $p < 0.001$) and root P content ($r = 0.846$, $p < 0.001$) (Figure 5).

Saline solutions have a direct effect on cell proliferation; nevertheless, the specific mechanism through which this arises is unknown [55]. Based on the biophysical model of the elongation of cells, the rate of cell elongation can be modulated or controlled by changes in any of a number of variables (cell wall extensibility, turgor pressure, and yield threshold). The yield threshold is the turgor pressure value below which there is no irreversible expansion of the cell wall. Because increased salt content reduces the osmotic potential of the soil solution, it is commonly assumed that root zone salinity affects growth through lowering cell turgor. Growth inhibition produced by a quick increase in external solute concentrations is very definitely mediated by unexpected decreases in turgor pressure [86]. Many yield components reduced as plant height and leaf area and number decreased, resulting in reduced seed and biological yield.

In the present experiment, salinity reduced the seed and biological yield, with a greater effect at higher salinity levels, while AMF-inoculated plants resulted in higher values of these yield parameters than non-inoculated plants (Figure 4A and 4B). A number of investigations on various plants have demonstrated that AMF supports the plant under salt stress conditions by improving photosynthetic ability, nutrient absorption, protection against antioxidants, and osmolyte accumulation, leading to enhanced plant growth and tolerance [55,87]. Specifically, mycorrhization increased biomass and seed yield in maize [48], wheat [49], chickpea [50], hulless barley [51] and *Euonymus maackii* [41] under salinity stress.

As for the harvest index (HI), it was not affected by AMF inoculation or the various salt level regimes. The AMF-inoculated plants displayed marginally higher values at all salt levels (Table 2). Overall, the range of HI values was between 0.292 and 0.358. Various researchers have reported similar results [88–90] when studying the development of flax plants under salinity conditions. Finally, in regard to the fiber yield, this was presented a similar trend as seed and biological yield,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

with salinity had a negative impact on this trait, especially under high salinity levels. Salinity adversely impacts stem fiber yield as a result of the reduction in plant height, allowing the plants to produce inferior fibers [88]. This is also supported by the considerable positive and significant association between stem fiber yield and plant height ($r = 0.674$, $p < 0.01$) (Figure 5).

5. Conclusions

Based on the findings of the current study and their assessment, it is evident that salt stress caused negative physiological effects, including limited growth, reduced photosynthesis, and decreased nutrients (N and P) content in shoots and roots of flax plants. In addition, mycorrhizal association improved the salt tolerance of the plants by increasing chlorophyll content, enhancing shoot and root nutrients content, and consequently yield parameters, such as seed, biological and stem fiber yield, particularly at moderate salt concentrations (50 and 100 mM NaCl). As a result of using AMF, flax plants grown under salt stress exhibited tolerance, suggesting that AMF could be applied in saline environments to maintain ecological stability.

Author Contributions: Conceptualization, I.K., P.S., I.R., A.M. and D.B.; methodology, I.K., P.S., I.R., A.M., A.K. and D.B.; validation, I.K., P.S., I.R., A.M., A.K. and D.B.; formal analysis, I.K., P.S., I.R., A.M., A.K. and D.B.; investigation, I.K., P.S., I.R., A.M. and D.B.; resources, I.K., P.S., I.R., A.M. and D.B.; writing—original draft preparation, I.K., P.S., I.R. and D.B.; writing—review and editing, I.K., I.R. and D.B.; supervision, I.K. and D.B.. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jhala, A.J.; Hall, L.M. Flax (*Linum usitatissimum* L.): Current uses and future applications. *AJBAS* **2010**, *4*, 4304–4312.
2. Kiryluk, A.; Kostecka, J. Pro-environmental and health-promoting grounds for restitution of flax (*Linum usitatissimum* L.) cultivation. *J. Ecol. Eng.* **2020**, *21*, 99–107.
3. Bilalis, D.; Roussis, I.; Cheimona, N.; Kakabouki, I.; Travlos, I. Organic agriculture and innovative feed crops. In *Agricultural Research Updates*; Gorawala, P., Mandhatri, S., Eds.; Nova Science Publishers Inc.: New York, NY, USA, 2018; Volume 23, pp. 55–100.
4. Debnath, S. Flax fiber extraction to textiles and sustainability: A holistic approach. In *Sustainable Fashion and Textiles in Latin America*, 1st ed.; Gardetti, M.A., Larios-Francia, R.P., Eds.; Springer: Singapore, 2021; pp. 73–85.
5. Stavropoulos, P.; Mavroeidis, A.; Papadopoulos, G.; Roussis, I.; Bilalis, D.; Kakabouki, I. On the path towards a "greener" EU: A mini review on flax (*Linum usitatissimum* L.) as a case study. *Plants* **2023**, *12*, 1102.
6. Vaisey-Genser, M.; Morris, D.H. Introduction: History of the cultivation and uses of flaxseed. In *Flax*, 1st ed.; Muir, A.D., Westcott, N.D., Eds.; CRC Press: Boca Raton, FL, USA, 2003; pp. 13–33.
7. Cloutier, S. Linseed: Overview. In *Encyclopedia of Food Grains*, 1st ed.; Wrigley, C., Corke, H., Seetharaman, K., Faubion, J., Eds.; Elsevier Ltd.: Amsterdam, The Netherlands, 2016; Volume 1, pp. 259–264.
8. Adorian, T.J.; Pianesso, D.; Bender, A.B.B.; Speroni, C.S.; Mombach, P.I.; Kowalski, É.A.; da Silva, L.P. Fractionation of linseed and obtaining ingredients rich in protein and fibers: Alternatives for animal feed. *J. Sci. Food Agric.* **2021**, *102*, 1514–1521.
9. Tang, Z.X.; Shi, L.E.; Wang, X.M.; Dai, G.W.; Cheng, L.A.; Wan, Z.X.; He, H.; Wu, Q.; Wang, Y.B.; Jin, X.Y.; Ying, R.F.; Huang L.H. Whole flaxseed-based products and their health benefits. *Food Sci. Technol. Res.* **2020**, *26*, 561–578.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

10. Kolodziejczyk, P.; Ozimek, L.; Kozłowska, J. The application of flax and hemp seeds in food, animal feed and cosmetics production. In *Handbook of Natural Fibers*, 1st ed.; Kozłowski, R.M., Ed.; Woodhead Publishing: Sawston, Cambridge, UK, 2012; Volume 2, pp. 329–366.
11. Begum, H.; Alam, A.K.M.M.; Chowdhury, M.J.A.; Hossain, M.I. Genetic divergence in linseed (*Linum usitatissimum* L.). *Int. J. Sustain. Crop Prod.* **2007**, *2*, 4–6.
12. Topnikova, E.V.; Pirogova, E.N.; Danilova, E.S. Quality assessment of linseed oil. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *1*, 012096.
13. Munns, R.; Gilliham, M. Salinity tolerance of crops – what is the cost? *N. Phytol.* **2015**, *208*, 668–673.
14. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681.
15. Hanin, M.; Ebel, C.; Ngom, M.; Laplaze, L.; Masmoudi, K. New insights on plant salt tolerance mechanisms and their potential use for breeding. *Front. Plant Sci.* **2016**, *7*, 1787.
16. Dastogeer, K.M.G.; Zahan, M.I.; Tahjib-Ul-Arif, M.; Akter, M.A.; Okazaki, S. Plant salinity tolerance conferred by arbuscular mycorrhizal fungi and associated mechanisms: A meta-analysis. *Front. Plant Sci.* **2020**, *11*, 588550.
17. Niu, X.; Bressan, R.A.; Hasegawa, P.M.; Pardo, J.M. Ion homeostasis in NaCl stress environments. *Plant Physiol.* **1995**, *109*, 735-742.
18. Evelin, H.; Giri, B.; Kapoor, R. Contribution of Glomus intraradices inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. *Mycorrhiza* **2012**, *22*, 203– 217.
19. Liu, M.Y.; Li, Q.S.; Ding, W.Y.; Dong, L.W.; Deng, M.; Chen, J.H.; Tian, X.; Hashem, A.; Al-Arjani, A.F.; Alenazi, M.M.; Abd-Allah, E.F.; Wu, Q.S. Arbuscular mycorrhizal fungi inoculation impacts expression of aquaporins and salt overly sensitive genes and enhances tolerance of salt stress in tomato. *Chem. Biol. Technol. Agric.* **2023**, *10*, 5.
20. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*. Academic Press: New York, NY, USA, 2010.
21. Wu, N.; Li, Z.; Wu, F.; Tang, M. Comparative photochemistry activity and antioxidant responses in male and female *Populus cathayana* cuttings inoculated with arbuscular mycorrhizal fungi under salt. *Sci. Rep.* **2016**, *6*, 37663.
22. Caruso, C.; Maucieri, C.; Berruti, A.; Borin, M.; Barbera, A. Responses of different *Panicum miliaceum* L. genotypes to saline and water stress in a Marginal Mediterranean environment. *Agronomy* **2018**, *8*, 8.
23. Xiao, X.; Liao, X.; Yan, Q.; Xie, Y.; Chen, J.; Liang, G.; Chen, M.; Xiao, S.; Chen, Y.; Liu, J. Arbuscular mycorrhizal fungi improve the growth, water status, and nutrient uptake of *Cinnamomum migao* and the soil nutrient stoichiometry under drought stress and recovery. *J. Fungi* **2023**, *9*, 321.
24. Guo, Y.; Gao, P.; Li, F.; Duan, T. Effects of AM fungi and grass endophytes on perennial ryegrass *Bipolaris sorokiniana* leaf spot disease under limited soil nutrients. *Eur. J. Plant Pathol.* **2019**, *154*, 659–671.
25. Van Der Heijden, M.G.A.; Martin, F.M.; Selosse, M.-A.; Sanders, I.R. Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytol.* **2015**, *205*, 1406–1423.
26. Smith, S.E.; Facelli, E.; Pope, S.; Smith, F.A. Plant performance in stressful environments: Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* **2009**, *326*, 3–20.
27. Smith, S.E.; Jakobsen, I.; Grønlund, M.; Smith, F.A. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol.* **2011**, *156*, 1050–1057.
28. del Mar Alguacil, M.; Lozano, Z.; Campoy, M.J.; Roldán, A. Phosphorus fertilization management modifies the biodiversity of am fungi in a tropical savanna forage system. *Soil Biol. Biochem.* **2010**, *42*, 1114–1122.
29. Kaur, S.; Suseela, V. Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. *Metabolites* **2020**, *10*, 335.
30. Püschel, D.; Bitterlich, M.; Rydlová, J.; Jansa, J. Drought accentuates the role of mycorrhiza in phosphorus uptake. *Soil Biol. Biochem.* **2021**, *157*, 108243.
31. Bowles, T.M.; Jackson, L.E.; Cavagnaro, T.R. Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes. *Glob. Chang. Biol.* **2017**, *24*, e171–e182.
32. Konvalinková, T.; Püschel, D.; Rezácová, V.; Gryndlerová, H.; Jansa, J. Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. *Plant Soil* **2017**, *419*, 319–333.
33. Liu, H.; Chen, W.; Wu, M.; Wu, R.; Zhou, Y.; Gao, Y.; Ren, A. Arbuscular mycorrhizal fungus inoculation reduces the drought-resistance advantage of endophyte-infected versus endophyte-free *Leymus chinensis*. *Mycorrhiza* **2017**, *27*, 791–799.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

34. Giovannetti, M.; Mosse, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* **1980**, *84*, 489–500.
35. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161.
36. Yamasaki, S.; Dillenburg, L.R. Measurements of leaf relative water content in *Araucaria angustifolia*. *R. Bras. Fisiol. Veg.* **1999**, *11*, 69–75.
37. Arnon, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1–15.
38. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207.
39. Khan, M.N.; Siddiqui, M.H.; Mohammad, F.; Khan, M.M.A.; Naeem, M. Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. *World J. Agric. Sci.* **2007**, *3*, 685–695.
40. Wu, N.; Li, Z.; Wu, F.; Tang, M. Microenvironment and microbial community in the rhizosphere of dioecious *Populus cathayana* at Chaka Salt Lake. *J. Soils Sediments* **2019**, *19*, 2740–2751.
41. Li, Z.; Wu, N.; Meng, S.; Wu, F.; Liu, T. Arbuscular mycorrhizal fungi (AMF) enhance the tolerance of *Euonymus maackii* Rupr. at a moderate level of salinity. *PLoS ONE* **2020**, *15*, e0231497.
42. Porcel, R.; Aroca, R.; Azcón, R.; Ruiz-Lozano, J.M. Regulation of cation transporter genes by the arbuscular mycorrhizal symbiosis in rice plants subjected to salinity suggests improved salt tolerance due to reduced Na^+ root-to-shoot distribution. *Mycorrhiza* **2016**, *26*, 673–684.
43. Bagheri, V.; Shamshiri, M.H.; Alaei, H.; Salehi, H. The role of inoculum identity for growth, photosynthesis, and chlorophyll fluorescence of zinnia plants by arbuscular mycorrhizal fungi under varying water regimes. *Photosynthetica* **2019**, *57*, 409–419.
44. Ashraf, M.; Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216.
45. Mathur, N.; Singh, J.; Bohra, S.; Bohra, A.; Vyas, A. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. *Am. J. Plant Physiol.* **2006**, *1*, 210–213.
46. Jamil, M.; Rehman, S.U.; Lee, K.J.; Kim, J.M.; Rha, H.K. Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Sci. Agric.* **2007**, *64*, 111–118.
47. Kapoor, K.; Srivastana, A. Assessment of salinity tolerance of *Vigna mungo* var. Pu-19 using *ex vitro* and *in vitro* methods. *Asian J. Biotechnol.* **2010**, *2*, 73–85.
48. Begum, N.; Ahanger, M.A.; Su, Y.; Lei, Y.; Mustafa, N.S.A.; Ahmad, P.; Zhang, L. Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. *Plants* **2019**, *8*, 579.
49. Bernardo, L.; Carletti, P.; Badeck, F.W.; 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.* **2019**, *137*, 203–212.
50. Hashem, A.; Kumar, A.; Al-Dbass, A.M.; Alqarawi, A.A.; Al-Arjani, B.F.; Singh, G.; Farooq, M.; Abd-Allah, E.F. Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. *Saudi J Biol Sci* **2019**, *26*, 614–624.
51. Jerbi, M.; Labidi, S.; Laruelle, F.; Tisserant, B.; Dalpé, Y.; Lounès-Hadj Sahraoui, A.; Ben Jeddi, F. Contribution of native and exotic arbuscular mycorrhizal fungi in improving the physiological and biochemical response of hulless barley (*Hordeum vulgare* ssp. *nudum* L.) to drought. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 2187–2204.
52. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681.
53. Bilalis, D.J.; Roussis, I.; Kakabouki, I.; Karydogianni, S. Effects of salinity and arbuscular mycorrhizal fungi (AMF) on root growth development and productivity of chia (*Salvia hispanica* L.), a promising salt-tolerant crop, under Mediterranean conditions. In *Handbook of Halophytes*; Grigore, M.N., Ed.; Springer: Cham, Switzerland, 2020.
54. Wu, Q.S.; Zou, Y.N.; Abd Allah, E.F. Mycorrhizal association and ROS in plants. In *Oxidative Damage to Plants*; Ahmad, P., Ed.; Elsevier: New York, NY, USA, 2014; pp. 453–475.
55. Borde, M.; Dudhane, M.; Kulkarni, M. Role of arbuscular mycorrhizal fungi (AMF) in salinity tolerance and growth response in plants under salt stress conditions. In *Mycorrhiza-Eco-Physiology, Secondary Metabolites. Nanomaterials*; Varma, A., Prasad, R., Tuteja, N., Eds.; Springer: Cham, Switzerland, 2017; pp. 71–86.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

56. Bheemareddy, V.S.; Lakshman, H.C. Effect of salt and acid stress on *Triticum aestivum* inoculated with *Glomus fasciculatum*. *J. Anim. Plant Sci.* **2011**, *7*, 945–956.
57. Chaves, M.M.; Flexas, J.; Pinheiro, C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* **2009**, *103*, 551–560.
58. Klinsukon, C.; Lumyong, S.; Kuyper, T.W.; Boonlue, S. Colonization by arbuscular mycorrhizal fungi improves salinity tolerance of eucalyptus (*Eucalyptus camaldulensis*) seedlings. *Sci. Rep.* **2021**, *11*, 4362.
59. da Silva, H.F.O.; Tavares, O.C.H.; da Silva, L.S.; Zonta, E.; da Silva, E.M.R.; Júnior, O.J.S.; Nobre, C.P.; Berbara, R.L.L.; García, A.C. Arbuscular mycorrhizal fungi and humic substances increased the salinity tolerance of rice plants. *Biocatal. Agric. Biotechnol.* **2022**, *44*, 102472.
60. Albacete, A.; Ghanem, M.E.; Martínez-Andújar, C.; Acosta, M.; Sánchez-Bravo, J.; Martinez, V.; Lutts, S.; Dodd, I.C.; Pérez-Alfocea, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *J. Exp. Bot.* **2008**, *59*, 4119–4131.
61. Vázquez-Glaría,A.; Eichler-Löbermann, B.; Loiret, F.G.; Ortega, E.; Kavka, M. Root-system architectures of two Cuban rice cultivars with salt stress at early development stages. *Plants* **2021**, *10*, 1194.
62. Juniper, S.; Abbott, L.K. Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. *Mycorrhiza* **2006**, *16*, 371–379.
63. Nacoon, S.; Ekprasert, J.; Riddech, N.; Mongkolthanaruk, W.; Jogloy, S.; Vorasoot, N.; Cooper, J.; Boonlue, S. Growth enhancement of sunchoke by arbuscular mycorrhizal fungi under drought condition. *Rhizosphere* **2021**, *17*, 100308.
64. Augé, R.M. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **2001**, *11*, 3–42.
65. Kapoor, R.; Sharma, D.; Bhatnagar, A.K. Arbuscular mycorrhizae in micropropagation systems and their potential applications. *Sci. Hortic.* **2008**, *116*, 227–239.
66. 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* **2008**, *18*, 287–296.
67. Giri, B.; Kapoor, R.; Mukerji, K.G. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. *Biol. Fertil. Soils* **2003**, *38*, 170–175.
68. Kaschuk, G.; Kuyper, T.W.; Leffelaar, P.A.; Hungria, M.; Giller, K.E. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol. Biochem.* **2009**, *41*, 1233–1244.
69. Bechtaoui, N.; Rabiu, M.K.; Raklami, A.; Oufdou, K.; Hafidi, M.; Jemo, M. Phosphate-dependent regulation of growth and stresses management in plants. *Front. Plant Sci.* **2021**, *12*, 679916.
70. Chang, W.; Sui, X.; Fan, X.X.; Jia, T.T.; Song, F.Q. Arbuscular mycorrhizal symbiosis modulates antioxidant response and ion distribution in salt-stressed *Elaeagnus angustifolia* seedlings. *Front Microbiol.* **2018**, *9*, 652.
71. Frosi, G.; Barros, V.A.; Oliveira, M.T.; Santos, M.; Ramos, D.G.; Maia, L.C.; Santos, M.G.J.T.P. Arbuscular mycorrhizal fungi and foliar phosphorus inorganic supply alleviate salt stress effects in physiological attributes, but only arbuscular mycorrhizal fungi increase biomass in woody species of a semiarid environment. *Tree Physiol* **2018**, *38*, 25–36.
72. Ferrarezi, R.; Geiger, T.C.; Greenidge, J.; Dennery, S.; Weiss, S.A.; Vieira. G.S. Microirrigation equipment for okra cultivation in the U.S. Virgin Islands. *HortScience* **2020**, *55*, 1045–1052.
73. Monje, O.A.; Bugbee B. Inherent limitations of nondestructive chlorophyll meters: A comparison of two types of meters. *HortScience* **1992**, *27*, 69–71.
74. Wang, Y.; Dunn, B.L.; Arnall, D.B.; Mao, P. Use of an active canopy sensor and SPAD chlorophyll meter to quantify geranium nitrogen status. *HortScience* **2012**, *47*, 45–50.
75. El Moukhtari, A.; Cabassa-Hourton, C.; Farissi, M.; Savouré, A. How does proline treatment promote salt stress tolerance during crop plant development? *Front. Plant Sci.* **2020**, *11*, 1127.
76. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J. Plant salt-tolerance mechanisms. *Trends Plant Sci.* **2014**, *19*, 371–379.
77. Zhang, B.; Shi, F.; Zheng, X.; Pan, H.; Wen, Y.; Song, F. Effects of AMF compound inoculants on growth, ion homeostasis, and salt tolerance-related gene expression in *Oryza sativa* L. under salt treatments. *Rice* **2023**, *16*, 18.
78. Ashraf, M.; Shahzad, S.M.; Imtiaz, M.; Rizwan, M.S. Salinity effects on nitrogen metabolism in plants – Focusing on the activities of nitrogen metabolizing enzymes: A review. *J. Plant Nutr.* **2018**, *41*, 1065–1108.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2023 doi:10.20944/preprints202309.0345.v1

79. Hoff, T.; Stummann, B.M.; Henningsen, K.W. Structure, function and regulation of nitrate reductase in higher plants. *Physiol. Plant.* **1992**, *84*, 616–624.
80. Marschner, H.; Dell, B. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* **1994**, *159*, 89–102.
81. Talaat, N.B.; Shawky, B.T. Influence of arbuscular mycorrhizae on yield, nutrients, organic solutes, and antioxidant enzymes of two wheat cultivars under salt stress. *J. Plant Nutr. Soil Sci.* **2011**, *174*, 283–291.
82. Sheng, M.; Tang, M.; Zhang, F.; Huang, Y. Influence of arbuscular mycorrhiza on organic solutes in maize leaves under salt stress. *Mycorrhiza* **2011**, *21*, 423–430.
83. Heydari S, Pirzad A. 2020. Mycorrhizal fungi and *Thiobacillus* co-inoculation improve the physiological indices of *Lallemantia iberica* under salinity stress. *Curr. Microbiol*. **2020**, *77*, 2523–2534.
84. Cao, Y.; Wu, X.; Zhukova, A.; Tang, Z.; Weng, Y.; Li, Z.; Yang, Y. Arbuscular mycorrhizal fungi (AMF) species and abundance exhibit different effects on saline-alkaline tolerance in *Leymus chinensis*, *J. Plant Interact.* **2020**, *15*, 266–279.
85. Poss, J.A.; Pond, E.; Menge, J. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. *Plant Soil.* **1985**, *88*, 307–309.
86. Volkamar, K.M.; Hu, Y.; Steppuhn, H. Physiological responses of plants to salinity: A review. *Can. J. Plant Sci*. **1998**, *78*, 19–27.
87. Hameed, A.; Egamberdieva, D.; Abd Allah, E F.; Hashem, A.; Kumar, A.; Ahmad, P. Salinity stress and arbuscular mycorrhizal symbiosis in plants. In Use of microbes for the alleviation of soil stresses; Miransari, M., Ed.; Springer: New York, NY, USA, 2014; Volume 1, pp. 139–159.
88. Dubey, S.; Bhargava, A.; Fuentes, F.; Shukla, S.; Srivastava, S. Effect of salinity stress on yield and quality parameters in flax (*Linum usitatissimum* L.). *Not. Bot. Horti Agrobot. Cluj Napoca* **2020**, *48*, 954–966.
89. Nasir Khan, M.; Siddiqui, M.H.; Mohammad, F.; Masroor, M.; Khan, A.; Naeem, M. Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. *World J. Agric. Sci.* **2007**, *3*, 685–695.
90. Abdullah; Mahmood, A.; Bibi, S.; Naqve, M.; Javaid, M.M.; Zia, M.A.; Jabbar, A.; Ud-Din, W.; Attia, K.A.; Khan, N.; Al-Doss, A.A.; Fiaz, S. Physiological, biochemical, and yield responses of linseed (*Linum usitatissimum* L.) in α-tocopherol-mediated alleviation of salinity stress. *Front. Plant Sci.* **2022**, *13*, 867172.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.