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Nitrogen Availability Drives Mycorrhizal Effects on Wheat Growth, Nitrogen Uptake and Recovery under Salt Stress

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Abstract: The arbuscular mycorrhizal (AM) symbiosis is generally considered effective in improving salt tolerance in plants; however, the advantages it offers can vary greatly depending on the context in which it occurs; furthermore, the mechanisms underlying these responses are still unclear. A study was conducted to investigate the role of nitrogen (N) availability on the effectiveness of AM symbiosis in durum wheat (*Triticum durum* Desf.) plants grown under salt stress. Plants were grown in pots in the absence or in presence of salt stress (soil electrical conductivity of 1.50 and 13.00 dS m^{-1}, respectively), with or without AM fungi inoculation (*Rhizophagus irregularis* and *Funneliformis mosseae*), varying the N dose supplied (0 or 80 mg N per pot). Results indicate that AM symbiosis can alleviate the detrimental effects of salt stress on the growth of durum wheat only when plants are grown under sufficient N availability in soil; in such conditions mycorrhizal symbiosis determined an improvement of leaf traits (leaf area, SLA, stability of plasma membranes and SPAD), N uptake, N fertilizer recovery and water use efficiency. On the contrary, when wheat plants were grown in conditions of N deficiency, the mycorrhizal symbiosis had no effect (under salt stress) or even depressive effect (under unstressed condition) on plant growth and N uptake, highlighting how, in some cases, competition for nutrients between plants and AM can arise. This study suggests that N availability in the soil can drive the effects of AM symbiosis in assisting the plant with containing saline stress.

Keywords: arbuscular mycorrhizal fungi; salt stress mitigation; plant growth; nitrogen alleviates salt stress

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1. Introduction

Soil salinization is one of the major threats to soil degradation and agroecosystems productivity. It affects an estimated area of about one billion hectares across more than 100 countries [1], in particular in arid and semi-arid environments where the high evaporative rate increases the possibility of salt accumulation in the soil profile and therefore can lead to soil salinization [2]. Plants growing in salinized soils can experience several biochemical and physiological disturbances affecting all plant phases, from seed germination to vegetative and reproductive development [3]. In fact, the increment of toxic ions concentration in salinized soils, mainly Na^+ and Cl^-, can alter plant enzymes activity, synthesis of proteins, respiration and photosynthesis, reduce cell membrane integrity, determine nutritional imbalances, etc. Moreover, soil salinization decreases the soil osmotic potential, which decreases the plant water availability and limits plant transpiration. Therefore, salinization can expose plants to ions excess and water deficiency at the same time [4,5].

Depending on their level of salt tolerance, plant species have evolved various molecular, physiological, and biochemical mechanisms to cope with the adverse effects of salinization [6]. For instance, plants growing in salinized soils have shown an altered expression of genes involved in the transport of water and nutrients and in their compartmentation [7,8],

an increase in the activity of antioxidant enzymes such as ascorbate peroxide, superoxide dismutase, etc. [9], and an accumulation of proline and phenolic compounds [10,11]. Additionally, plants can increase their tolerance to salinity by establishing relationships with a wide range of soil microorganisms that naturally colonize their roots. In fact, several microorganisms can stimulate molecular, physiological, and biochemical processes in plants that are effective at mitigating the negative effects of soil salinity [4,12,13]. Among others, arbuscular mycorrhizal (AM) fungi are a phylum of soil fungi that establish symbiosis with two-thirds of terrestrial plants including major crops such as wheat [14]. Several authors have shown that AM fungi can markedly reduce the adverse effects of soil salinity on plant growth through the following ways: (i) regulation in the expression of genes related to water and nutrients uptake and transport [8]; (ii) increment of the activities of antioxidant enzymes [15]; (iii) maintenance of membrane integrity and ion balance through selective absorption, ions compartmentation, and regulation of K^+/Na^+ ratio [16,17]; (iv) and not least increment in the uptake of water and mineral nutrients such as phosphorus, nitrogen (N), calcium, and potassium [8,18,19]. This latter aspect, and in particular the positive effect that AM fungi can have on plant N nutrition during salinity, can play a key role in overcoming this abiotic stressor [20,21]. Besides contributing to crop yield and quality, nitrogen plays a crucial role in many biochemical and physiological processes that mitigate saline stress, such as the synthesis of photosynthetic pigments [22], photosynthesis regulation, reactive oxygen species scavenging, and antioxidant and osmolyte metabolism [23]. However, the role of AM fungi in N plant nutrition is controversial [24,25]. Mycorrhization has been shown to positively influence plant N uptake, since plants can explore a greater volume of soil through AM fungi, increasing their chances of intercepting nutrients [26,27]. It was also highlighted that mycorrhizal symbiosis enhances the plant's competitiveness against other soil microorganisms for inorganic N [28]. Moreover, AM fungi may promote organic matter mineralization processes, thereby increasing plant nitrogen uptake, by influencing microbial communities and soil aggregation status [29,30].On the other hand, AM fungi have a notable N demand for their own metabolism [31] and when the soil is N-deficient they can even compete with the host plant for soil N [32,33].

To our knowledge, no studies have been conducted to determine whether the availability of nitrogen in the substrate influences the response of mycorrhization on the growth of plants grown under salinity stress. Expanding knowledge on the context in which mycorrhizae are able to perform their potential is certainly also useful in order to make optimal use of mycorrhizal technology to mitigate the negative effects of salinity on crops. To contribute to the filling of this knowledge gap, we carried out a pot experiment to investigate the effects of AM fungi inoculation on saline stress mitigation in durum wheat (*Triticum durum* Desf.) grown in the presence or absence of N fertilization. Our hypothesis is that enhanced production of mycorrhizal plants in saline environment is strictly related to mycorrhizal-mediated N uptake.

2. Materials and Methods

2.1. Experimental Design and Plant Management

The durum wheat plants were grown in pots in a wire house under a transparent plastic roof with open sides (pots were protected from the rain) at the Pietranera farm (Lima Mancuso Foundation; S. Stefano Q., AG, Italy; 37°53′ N, 13°51′ E; 162 m a.s.l.).

The treatments were: (i) Salinity stress (no stress/stress; soil electrical conductivity of 1.50 and 13.00 dS m^{-1}, respectively); (ii) AM fungal inoculation, with plants grown in the presence (+myc) or absence (−myc) of AM fungi; and (iii) Nitrogen fertilization: addition of 0 or 80 mg N per pot (0 N/+N).

A complete randomized factorial design with six replicates was adopted. Each pot (diameter 130 mm, height 175 mm) was filled with 1800 g of a mixture of silica sand (Gras Calce Srl, Trezzo sull'Adda, Italy), whose concentration of N (Kjeldahl) and P (Olsen) were, respectively, 0.11 g kg^{-1} and 7.44 mg kg^{-1}, and of agricultural soil (1:1 by weight). The agricultural soil was taken from a well-structured clayey soil (Vertic Haploxerept; layer

0–30 cm) that derived from the gypsum-sulphur series; the soil had the following properties: clay 267 g kg^{-1}, silt 247 g kg^{-1}, and sand 486 g kg^{-1}; pH 8.0; total carbon (C) 6.3 g kg^{-1}; total N 0.86 g kg^{-1}; available P (Olsen) 40.1 mg kg^{-1}; exchangeable K_2O 135 mg kg^{-1}; saturated electrical conductivity (EC) (25 °C) 1.70 dS m^{-1}. Therefore, the resulting mixture was poor in N and sufficient supplied with P and K. The mixture (soil and sand) was sterilized as follows: three cycles of humidification, (24 h at room temperature and 24 h at 130 °C). Furthermore, to avoid contamination, all the pots and seeds were previously sterilized by immersion in a 2.5% sodium hypochlorite solution for 4 min. The native soil microflora was extracted from an unsterilized soil sample by suspending the soil in distilled water in a ratio of 1:4 (*W*/*W*), stirring for 20 min (140 oscillations per minute), then filtering with filter paper (11 µm mesh) to remove the spores of AM fungi present in the native microbial community of the soil.

In the +myc treatments, the inoculation of AM fungi was performed with the addition of 1.5 g per pot of a commercial inoculum (AEGIS IRRIGA, Italpollina SpA, Rivoli Veronese, Italy) consisting of a mixture of 2 species of AM fungi (*Rhizophagus irregularis* and *Funneliformis mosseae*), equally present at a density of 700 spores g^{-1}. The commercial inoculum also contained 1×10^7 g^{-1} rhizosphere bacteria. To isolate the effects of AM fungi, the bacterial community of the inoculum was extracted using the same protocol reported above; the bacterial community of the inoculum was added to the −myc treatments at sowing. The inoculation was split into two stages: two-thirds (1 g per pot) were added during the pot filling by mixing with the substrate and the remaining third (0.5 g per pot) was added at the sowing to the top layer (0–5 cm). Following the same procedure, in the −myc treatment, 1.5 g of previously sterilized commercial inoculum was added. Furthermore, to establish an equal bacterial community in both +myc and −myc treatments, immediately after sowing the native microbial community of the soil, extracted as described above, was reintroduced into all pots adding 80 mL per pot of soil filtrate.

In the N fertilized treatments (+N), 80 mg of N per pot was applied 7 days after plant emergence as ammonium sulphate ($[^{15}NH_4]_2SO_4$) with an isotopic enrichment of 10%. No other fertilization was carried out.

Eighteen durum wheat seeds (cv. Anco Marzio), previously surface sterilized with 4% H_2O_2 for 3 min, were sown in each pot on 10 March 2021. Ten days after the emergence, the plants were thinned to obtain six seedlings per pot. To prevent osmotic shock, the salt was added gradually over a week, starting from the 15th day after emergence by distributing a total of 0.5 L of NaCl solution (20 g L^{-1}) in each pot. This brought the electrical conductivity of the saturated soil extract to 1.50 and 13.00 dS m^{-1} in the treatments without and with salt stress, respectively. Then, the pots were irrigated with distilled water until they were cut. During the experiment, irrigation was carried out every 2–4 days; for each pot, sufficient amounts of water were added to allow the total replenishment of the water lost due to evapotranspiration. Evapotranspiration losses were determined considering the weight variations in the pots measured daily. During the experiment, the amount of water available never dropped below 60% of its maximum value and water losses by percolation were never observed. In Figure 1, daily temperatures during the trial period and dates in which the main cultivation technique interventions were carried out (sowing, thinning, irrigation, fertilization and harvesting) are reported.

2.2. Measurements, Analytical Methods and Calculations

The plants of all pots were harvested 49 days after emergence. On the harvest day, before biomass was sampled, the chlorophyll contents of leaves were determined using a hand-held chlorophyll meter (SPAD-502, Konica Minolta, Osaka, Japan), averaging readings from ten full expanded leaves of plants randomly selected in each pot. The aboveground biomass was cut at the ground level; biomass was immediately separated into botanical fractions (stems, green leaves, and senescent and dry leaves) and weighed. A fresh sample of the leaf fraction was used to determine the leaf area with an area meter (LI-3100C; LiCOR, Lincoln, NE, USA). Furthermore, a sample of green fully expanded leaves (about 400 mg)

was taken from each pot to determine the membrane stability index (MSI). The leaf material was divided in two sets of 200 mg each. The first set was heated at 40 °C for 30 min in a water bath (10 cm^3); then the electrical conductivity bridge (C1) was measured. The second set was boiled at 100 °C for 10 min (in 10 cm^3 of water) before measuring the electrical conductivity bridge (C2). MSI was calculated according to the formula by [34]:

$$MSI = 1 - \frac{C1}{C2} \times 100$$

The stems and leaves fractions were then oven-dried at 70 °C to a constant weight to determine the dry matter content.

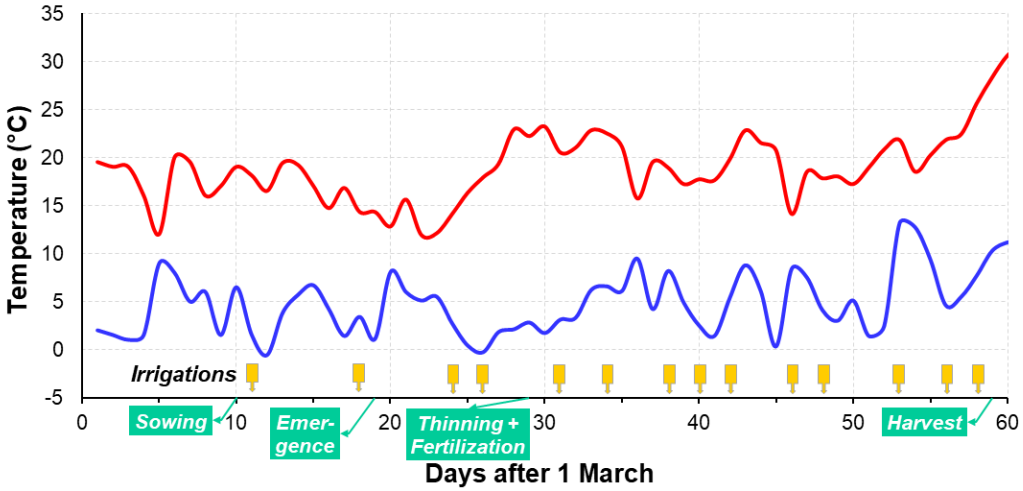

Figure 1. Daily minimum (blue line) and maximum (red line) temperatures during the trial period. The arrows indicate the dates of irrigation. Dates of sowing, emergence, thinning and N fertilization, and harvest are also indicated.

To extract the roots, we carefully removed the substrate with abundant washing in water. Then the roots were oven-dried at 40 °C until constant weight and the dry weight was recorded. We extracted a root subsample which was cleared with 10% KOH, stained with 0.05% trypan blue, using the Phillips and Hayman method [35], and used to quantify the percentage of AM fungi infection using the method developed by McGonigle et al. [36].

Both above- and belowground biomass fractions were ground to a fine powder, gathered in a single sample (mixing 30% of the total shoot weight and 30% of the total root weight), and analyzed for the concentration of total N with the Dumas method (DuMaster D-480; Büchi Labortechnik, Flawil, Switzerland) and for ^{15}N content with an elemental analyzer (NA1500; Carlo Erba, Milan, Italy) paired with a mass spectrophotometer (Isoprime, Cheadle, UK).

The total N uptake was obtained by multiplying the N content of the biomass by the amount of biomass of each pot.

The ^{15}N concentration was used to determine the amount ($^{15}Nrec$) and percentage ($\%^{15}Nrec$) of N recovered from the fertilizer, respectively, with Equations (1) and (2):

$$^{15}Nrec = N_{t\times} \frac{\text{atom\% } ^{15}Nfp \text{ excess}}{\text{atom\% } ^{15}Nfert \text{ excess}} \quad (1)$$

$$\%^{15}Nrec = \frac{^{15}Nrec}{f} \times 100 \quad (2)$$

where N_t is N content (g pot^{-1}) in the biomass, atom% ^{15}Nfp excess is the ^{15}N isotopic excess (atom% ^{15}N—0.3663) in the fertilized plant, atom% $^{15}Nfert$ is the ^{15}N isotopic excess in the fertilizer, and f is the amount of fertilizer (g pot^{-1}).

The total biomass production (shoots and roots) and the total water consumption ($water_{cons}$) were used to calculate the water use efficiency (WUE) as follows:

$$WUE = \frac{biomass}{water_{cons}} \quad (3)$$

The total water consumption ($water_{cons}$) was calculated as the sum of water applied during the experiment.

2.3. Statistical Analysis

All data were subjected to statistical analysis using R software [37] using a generalized least square models in the "nlme" package [38], with the implemented varIdDent() function to account for the heterogeneity of variance. Model residuals were checked for heteroscedasticity and normal distribution. Within each treatment (stressed/non-stressed and fertilized/non-fertilized) the responses of the inoculated treatments (+myc) were compared with the respective non−mycorrhized treatments (−myc) using the "dabestr" package [39] to calculate effect sizes as unpaired mean differences and generate 95% confidence intervals (CI). This approach has been used for the growing recognition of the limitation of using only the "*p*-value statistics" approach and avoiding dichotomous cuts [39,40].

3. Results

Uninoculated plants showed insignificant mycorrhizal colonization levels (always <1% of root length colonized). In +myc treatment, characteristic structures of AM fungi were observed in the roots after inoculation with different levels of mycorrhization with the treatments studied (Figure S1; the interaction 'stress x fertilization' had $p = 0.0278$; Table S1). In particular, in the non-fertilized treatment the percentage of mycorrhization was about 21% both in the plants subjected to saline stress and in those not stressed (Figure S2); in the fertilized theses (+N treatment) the percentage of mycorrhization was decidedly higher in the stressed treatment compared to the not stressed treatment (23.6 and 14.2%, respectively). As expected, the shoot biomass increased with N fertilization (+25%, on average) and was drastically reduced in the salt-stressed treatments compared to the non-stressed treatment (−55%; Figure 2). The mycorrhizal symbiosis improved shoot dry mass (DM) production in fertilized treatments (+15%, on average), particularly when saline stress was present (+41%). In contrast, in the 0 N treatments, mycorrhization either did not affect shoot biomass (saline-stressed plants) or resulted in a depressive effect (unstressed plants; −14%).

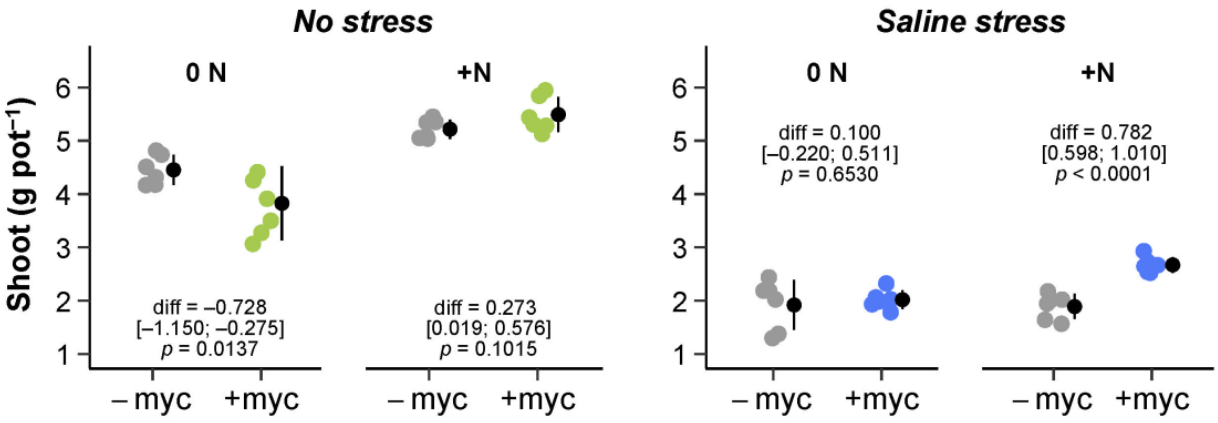

Figure 2. Shoot dry weight (g per pot) of durum wheat to varying treatments: no-stress or saline stress condition; N fertilization treatments (0 N, +N); absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc). Black circles represent means, with whiskers representing ± SE ($n = 6$). Differences (diff) between +myc and −myc means within the same fertilization treatment and the same saline treatment, estimated 95% confidence intervals of mean difference (in brackets), and *p* values are reported inside the plots.

The mycorrhizal symbiosis resulted in positive effects on the leaf area of the plants grown in saline stress conditions with more evident effects in the fertilized treatment (Table 1). On the contrary, in the unstressed treatments, the symbiosis caused a reduction in the leaf area, particularly in the non-fertilized treatment. The Specific Leaf Area (SLA) was drastically reduced due to the effect of saline stress (−18%, on average) and the mycorrhizal symbiosis appeared able to mitigate this effect both in 0 N and +N treatments.

Soil salinization decreased markedly the membrane stability index (MSI) values compared to the non-stressed condition (−15%, on average; Table 1). Mycorrhizal symbiosis had no effect on this parameter when the plants were grown under non-stressed conditions; on the contrary, under salinity stress the MSI values were higher in +myc compared to −myc treatment (+9%, on average), particularly in +N treatment (+13%).

Both saline stress and N fertilization increased SPAD values (+15.4% with $p = 0.0001$, and +12.9% with $p = 0.0039$, respectively); mycorrhizal symbiosis did not affect this trait.

The saline stress caused a marked decrease in the root biomass (−72%, on average; Figure 3). In general, the mycorrhizal symbiosis stimulated a greater root growth, but the effects were relevant only when the plants were saline-stressed both in 0 N treatment (+29%) and in +N (+55%).

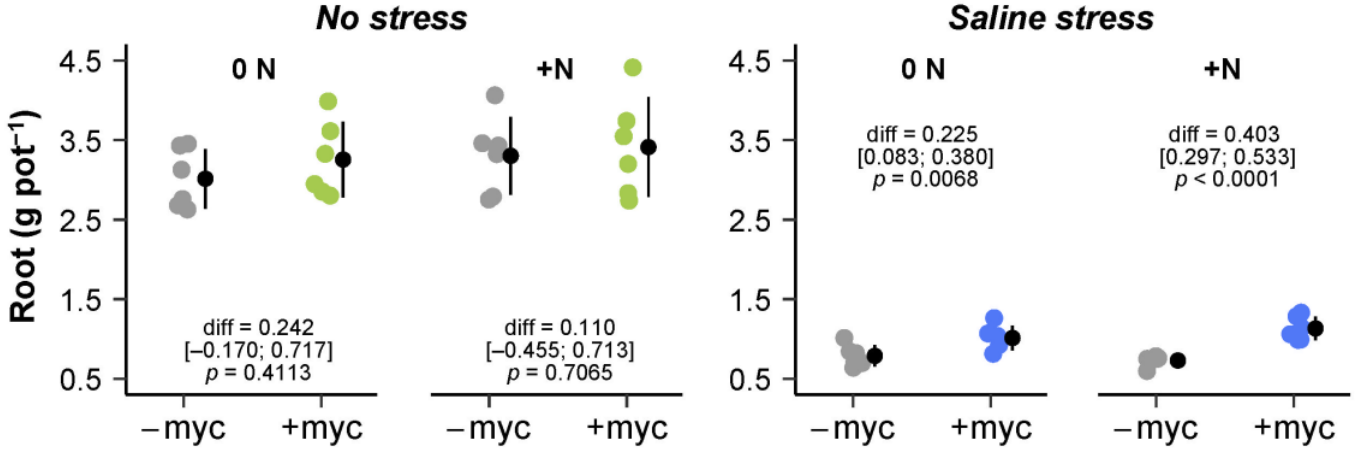

Figure 3. Root dry weight (g per pot) of durum wheat to varying treatments: no-stress or saline stress condition; N fertilization treatments (0 N, +N); absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc). Black circles represent means, with whiskers representing ± SE ($n = 6$). Differences (diff) between +myc and −myc means within the same fertilization treatment and the same saline treatment, estimated 95% confidence intervals of mean difference (in brackets), and p values are reported inside the plots.

The N concentration of the total biomass (shoots and roots), on average, was 2.27% in the stressed treatment and 1.21% in the not stressed treatment (Figure 4). The mycorrhization caused a considerable reduction in N concentration only when the plants were stressed and fertilized (−20%), while it did not have substantial effects in all other cases.

The effects of mycorrhizal symbiosis on the total N uptake (shoots and roots) varied markedly according to the applied treatment (Figure 5). In detail, mycorrhization in the control treatment (not stressed plants) had a positive effect in +N treatment (+7%) and a negative effect in 0 N treatment (−6%). In the saline treatments, mycorrhizal symbiosis did not determine relevant effects in 0 N treatment, while it induced marked increases in +N treatment (+16%).

Table 1. Percentage of green leaves on the total biomass, leaf area, specific leaf area, Membrane Stability Index (MSI), and SPAD values to varying treatments: no-stress or saline stress condition; N fertilization treatments (0 N, +N); absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc).

Response Variable	No Stress				Saline Stress			
	0 N		+N		0 N		+N	
	−myc	+myc	−myc	+myc	−myc	+myc	−myc	+myc
Green leaves (%)	22.93 (2.40)	22.10 (2.86)	24.42 (2.08)	23.12 (2.60)	20.27 (1.49)	18.60 (1.79)	22.77 (0.85)	21.25 (1.38)
Diff and estimated 95% CIs	−0.83 [−3.8; 1.68]		−1.27 [−3.68; 1.18]		−1.66 [−3.29; 0.11]		−1.52 [−2.9; −0.53]	
p value	0.5703		0.3787		0.0556		0.0493	
Leaf Area (cm^2 pot^{-1})	225.5 (23.1)	199.6 (34.8)	300.4 (17.7)	286.6 (17.3)	69.6 (21.2)	75.7 (5.8)	75.3 (11.6)	113.5 (6.8)
Diff and estimated 95% CIs	−25.9 [−58.6; 3.2]		−13.7 [−30.5; 5.7]		6.14 [−7.4; 25.7]		38.2 [29.3; 48.9]	
p value	0.0797		0.3392		0.4210		0.0001	
Specific Leaf Area (cm^2 g^{-1})	221.4 (9.8)	240.1 (35.9)	236.8 (14.6)	227.4 (16.0)	176.5 (13.8)	203.5 (19.8)	174.9 (11.3)	201.9 (27.0)
Diff and estimated 95% CIs	18.7 [−9.2; 44.1]		−9.4 [−25.8; 6.0]		27.0 [8.3; 44.0]		26.9 [4.7; 47.7]	
p value	0.1212		0.4284		0.0285		0.0290	
MSI	80.71 (2.82)	80.01 (3.63)	82.20 (4.36)	79.47 (4.80)	65.90 (0.51)	69.62 (3.09)	66.32 (5.24)	75.09 (3.76)
Diff and estimated 95% CIs	−0.699 [−4.22; 2.47]		−2.73 [−7.02; 2.29]		3.72 [1.81; 6.63]		5.76 [1.62; 11.40]	
p value	0.7173		0.3269		0.0156		0.0435	
SPAD values	37.93 (1.94)	38.33 (2.60)	43.15 (1.11)	43.05 (3.55)	45.20 (3.02)	42.92 (2.94)	50.32 (4.46)	49.08 (2.76)
Diff and estimated 95% CIs	0.39 [−1.68; 3.12]		−0.07 [−3.60; 2.16]		−2.29 [−5.09; 1.22]		−1.23 [−4.12; 4.19]	
p value	0.8156		0.9535		0.1877		0.4733	

Data are means ($n = 6$) ± SD in parenthesis. Unpaired mean difference (diff) in +myc minus −myc, estimated confidential intervals of mean difference (CIs), and *p* values within each N level in absence or presence of saline stress are reported in the row below each response variable.

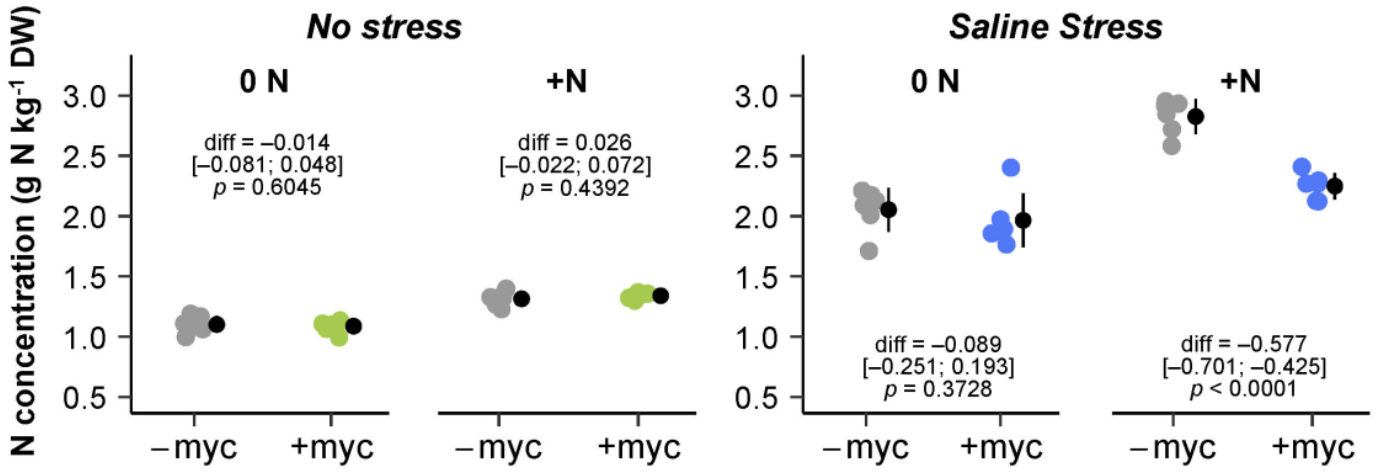

Figure 4. N concentration (g N kg^{-1} of dry weight) of durum wheat total biomass to varying treatments: no-stress or saline stress condition; N fertilization treatments (0 N, +N); absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc). Black circles represent means, with whiskers representing ± SE (n = 6). Differences (diff) between +myc and −myc means within the same fertilization treatment and the same saline treatment, estimated 95% confidence intervals of mean difference (in brackets), and p values are reported inside the plots.

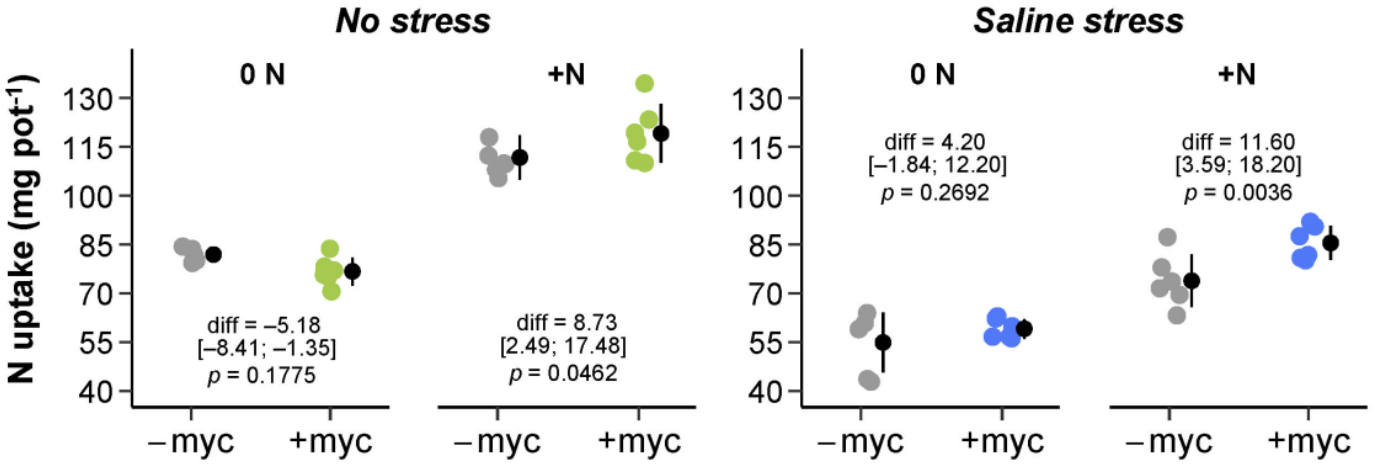

Figure 5. N uptake (mg N per pot) of durum wheat to varying treatments: no-stress or saline stress condition; N fertilization treatments (0 N, +N); absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc). Black circles represent means, with whiskers representing ± SE (n = 6). Differences (diff) between +myc and −myc means within the same fertilization treatment and the same saline treatment, estimated 95% confidence intervals of mean difference (in brackets), and p values are reported inside the plots.

In the control plants (not subjected to saline stress), the N derived from the fertilizer (Nrec) was 43.3% of the total N uptaken and was not affected by mycorrhization (Figure 6). In plants subjected to saline stress, Nrec was on average equal to 29.3% (with a reduction of about 32% compared to the non-stressed treatment); in this case, the mycorrhization determined a considerable increase in the recovered fertilizer (33.8% compared to 24.7% of −myc).

As expected, the saline stress markedly reduced Water Use Efficiency (WUE) compared to the unstressed control (on average, 3.08 and 1.52 g DM per litre, respectively, for no stress and stress treatment; Figure 7). The mycorrhization has determined relevant effects on this parameter only for plants subjected to stress and fertilized (+45%); in all other conditions, no changes were observed on the WUE.

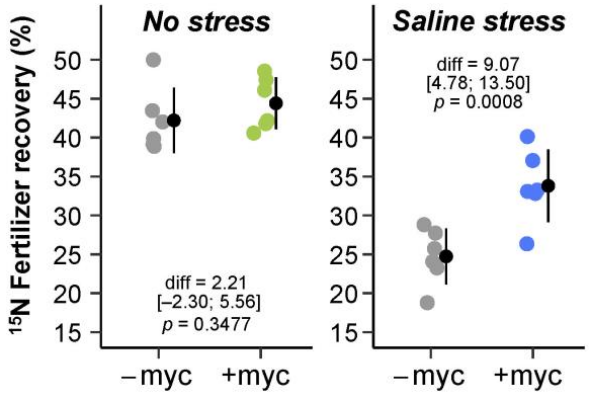

Figure 6. ^{15}N fertilizer recovery (%) of durum wheat to varying treatments: no-stress or saline stress condition; absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc). Black circles represent means, with whiskers representing ± SE (n = 6). Differences (diff) between +myc and −myc means within the same saline treatment, estimated 95% confidence intervals of mean difference (in brackets), and p values are reported inside the plots.

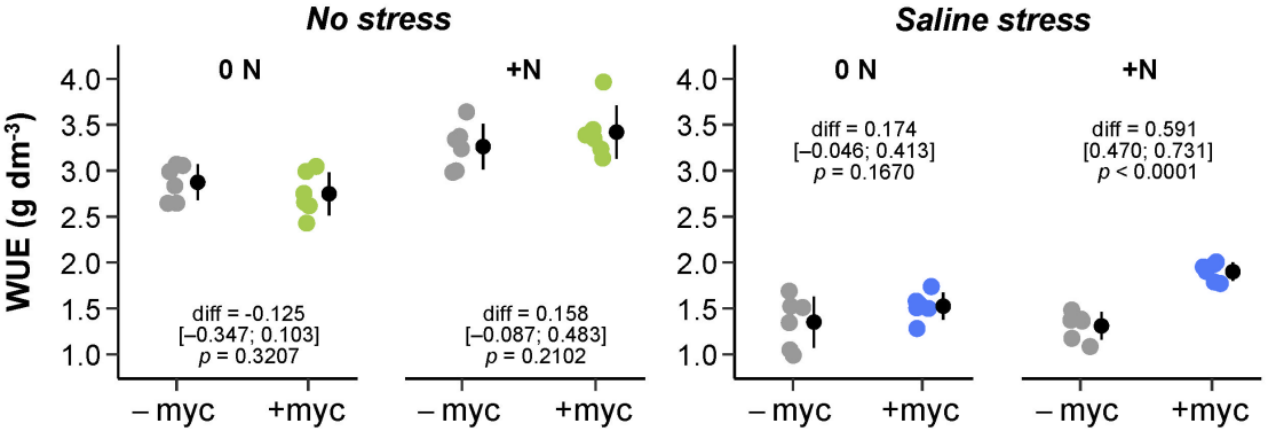

Figure 7. Water use efficiency (WUE; g DW per dm^3 H_2O utilized) of durum wheat to varying treatments: no-stress or saline stress condition; N fertilization treatments (0 N, +N); absence or presence of arbuscular mycorrhizal fungal inoculum (−myc, +myc). Black circles represent means, with whiskers representing ± SE (n = 6). Differences between +myc and −myc means within the same fertilization treatment and the same saline treatment, estimated 95% confidence intervals of mean difference (in brackets), and p values are reported inside the plots.

4. Discussion

As expected, the application of saline stress, with the modalities and intensity used, resulted in marked negative effects on aboveground and belowground plant growth, as well as on leaf traits (leaf area, SLA, stability of plasma membranes and SPAD), N uptake and water use efficiency. After all, it is known that salinity leads to osmotic stress, which negatively affects plant growth, inhibiting cell expansion and division with decreased stomatal opening and transpiration [41–43].

Surprisingly, we observed a consistent increase in N concentration in the plant tissues due to salinity. This is in contrast to what has been observed in various studies [16,44,45]. Evidently, saline stress depressed plant growth much more than N uptake. In other terms, under salt stress conditions, it lacked the dilution effect that was instead observed in the unstressed treatments, where biomass accumulation increased, compared to the salt stress conditions, to a much greater extent than N uptake. On the other hand, it has sometimes been observed that, due to the effect of salinity, the N concentration in plant tissues increases or remains unchanged [46–48]. These conflicting results highlight how the effects of salinity can vary with the context in which it occurs. Among the various factors potentially involved

in orienting the type of response, it is possible to include: cultivated species, intensity of stress, plant development phase when stress occurs, availability and form of N in the soil (NH_4^+-N/NO_3^--N ratios), availability of other elements (P, Ca, K, etc.) [49–52].

Nitrogen fertilization improved plant growth (above- and belowground), N uptake, and WUE both in plants not exposed to saline stress and in stressed plants, although the advantages appear modest in the latter case. Ahanger et al. [23] highlighted how the increase in N availability in the substrate mitigates the negative effects of salinity through modulations in the metabolism of antioxidants, osmolytes and metabolites, preventing the effects of salinity on the functioning of the photosynthetic apparatus and thus reducing the oxidative damage.

When plants were grown in conditions of marked N deficiency and in the absence of saline stress, the mycorrhizal symbiosis penalized the shoot growth and the uptake of N; this confirms the results of other research conducted in N limiting conditions [33,53,54], which showed that AM fungi efficiently use the limited amount of soil N for their own growth with negative repercussions on the plant growth, suggesting that in these conditions a competition is established between AM fungi and plants for N. On the contrary, under sufficient N availability, AM fungi increased shoot and plant N uptake; therefore, overall, our results confirm that the effects of AM fungi on plant performance are driven by the availability of N in the soil. Given that we used ammonium sulphate as N fertilizer and some authors (e.g., [55]) have shown that sulphur (S) can stimulate the development of AM fungi and plants, it could be argued that the benefits of fertilizer addition could also be partly linked to the increase in the availability of S in the soil. However, we believe this is unlikely to have occurred in our experiment, given that the soil used, which derived from the gypsum sulphur series, was itself rich in S and that further addition of S would be ineffective in such conditions. The mycorrhizal symbiosis appeared able to mitigate the salinity stress only when plants were grown under sufficient N availability in soil. This result certainly represents a growth in knowledge about the agronomic, ecological, and environmental role that this symbiosis plays in saline environments. Salinity condition reduces plant N uptake by immobilizing N, both as nitrate and ammonium, and influencing different aspects of N metabolism [56]. Garg and Chandel [57] highlighted that AM colonization, improving N uptake and assimilation under salt stress, helps to mitigate the toxic effects of Na^+ via regulating its uptake and indirectly helps the host plant to preserve chlorophyll concentration. In addition, the salt destroys the membrane proteins by changing the stability and integrity of the membrane itself as highlighted in this research and by [58], and so reducing the uptake of NO_3 and NH_4. This results in a reduced flow of NO_3 from the soil to the roots with marked consequences on the activity of the nitrate reductase (NR), which is a substrate-susceptible enzyme. The increase in NR activity observed in mycorrhized plants grown under salt stress [44,59] can be attributed to various factors which, moreover, can act jointly: (1) increase in Phosphorus uptake, which is necessary for the functioning of the enzyme; (2) regulation of the genes involved in NR activity; (3) increase in the flow of nitrates (the substrate of the enzyme) favoured by mycorrhizal symbiosis. Obviously, the latter factor could not have occurred in the conditions of N deficiency in the soil, and this explains why no advantages due to mycorrhization were observed in this condition. Overall, this study clearly shows how AM Fungi colonization helps the plant to overcome saline stress by increasing the N uptake and the N fertilizer recovery (determined by the isotopic dilution method).

However, under salt stress, the concentration of N in the tissues was lower in mycorrhized plants than in non−mycorrhized ones, confirming what was observed by Zhu et al. [17]. This may indicate an N dilution effect due to an extra increase in C accumulation and growth in mycorrhizal plants. Therefore, according to Talaat and Shawky [38], one of the most important mechanisms through which AM fungi alleviate the negative effects of salt stress is the enhanced metabolism of C and N.

It is interesting to highlight how the mycorrhizal symbiosis has increased the water use efficiency of plants grown in conditions of saline stress, but only when the plants

were grown in conditions of adequate N availability, highlighting once again how the effects of mycorrhization are N driven oriented. Several reports show an increase in plant WUE by the AM symbiosis under osmotic stress conditions [60–62]. This is related to the ability of mycorrhized plants to accumulate solutes (making possible an adjustment of the osmotic potential), an increase in stomatal conductance, an increase in transpiration and a greater photosynthetic efficiency [63–65] but also by leaf morphological traits as specific leaf weight or leaf area ratio [66], parameters that were favourably influenced by mycorrhization in this research.

5. Conclusions

Based on our data we conclude that arbuscular mycorrhizal symbiosis can have a key ecosystemic role in guaranteeing and supporting the growth of the plants grown under salt stress. However, this beneficial effect has materialized only when plants are grown under sufficient N soil availability; on the contrary, when wheat plants were grown in conditions of N deficiency, the mycorrhizal symbiosis had no effect in mitigating the damages induced by salinity on plant growth. Therefore, the results clearly show how the nitrogen availability is able to guide mycorrhizal effects on wheat growth under salt stress. All of this certainly could have practical implications, especially in arid and semi-arid environments where the problems linked to salinity are becoming increasingly pressing. It is certainly important to deepen our understanding of the mechanisms underlying the advantages offered by mycorrhiza in mitigating salinity effects; this would allow improvement in the role that this symbiosis plays from an agronomic, ecological, and environmental viewpoint and maximize the benefits that this symbiosis can potentially offer in saline environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12112823/s1, Table S1: ANOVA results for the studied traits. Figure S1: Durum wheat root colonized by arbuscular mycorrhizal fungi. The bar indicates 100 μm. Figure S2: Arbuscular mycorrhizal fungi root colonization of durum wheat in the two fertilization treatments (0 N and +N) and in presence or absence of saline stress. Vertical bars represent standard error (N = 6).

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