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AMF Inoculation Enhances Growth and Improves the Nutrient Uptake Rates of Transplanted, Salt-Stressed Tomato Seedlings

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Abstract: The study aimed to investigate the effects of commercially available AMF inoculate (*Glomus* sp. mixture) on the growth and the nutrient acquisition in tomato (*Solanumlycopersicum* L.) plants directly after transplanting and under different levels of salinity. Inoculated (AMF+) and non-inoculated (AMF−) tomato plants were subjected to three levels of NaCl salinity (0, 50, and 100 mM· NaCl). Seven days after transplanting, plants were analyzed for dry matter and RGR of whole plants and root systems. Leaf tissue was analyzed for mineral concentration before and after transplanting; leaf nutrient content and relative uptake rates (RUR) were calculated. AMF inoculation did not affect plant dry matter or RGR under fresh water-irrigation. The growth rate of AMF−plants did significantly decline under both moderate (77%) and severe (61%) salt stress compared to the fresh water-irrigated controls, while the decline was much less (88% and 75%,respectively)and statistically non-significant in salt-stressed AMF+ plants. Interestingly, root system dry matter of AMF+ plants (0.098 g plant^{-1}) remained significantly greater under severe soil salinity compared to non-inoculated seedlings (0.082 g plant^{-1}). The relative uptake rates of N, P, Mg, Ca, Mn, and Fe were enhanced in inoculated tomato seedlings and remained higher under (moderate) salt stress compared to AMF− plants This study suggests that inoculation with commercial AMF during nursery establishment contributes to alleviation of salt stress by maintaining a favorable nutrient profile. Therefore, nursery inoculation seems to be a viable solution to attenuate the effects of increasing soil salinity levels, especially in greenhouses with low natural abundance of AMF spores.

Keywords: AMF inoculation; growth; nutrient uptake; salinity; seedling; tomato

1. Introduction

Soil salinity is a common and very severe environmental stressor in agriculture [1,2], affecting almost every aspect of plant physiology and biochemistry [3]. Actually, about 2% of land farmed by dry-land agriculture, and >20% of irrigated land have already been damaged by excess soil salinity [4]. Soil salinization is dramatically exacerbated by irrigation [5], which "imports" large quantities of new ions to the soil and/or relocates them to surface soil layers, *i.e.*, the rooting zone, by evaporation [6]. Soil salinization is predicted to intensify in the decades to come [5], especially under protected cultivation where natural leaching of excess salts by rain water is absent. Additionally, erroneous fertilization schemes contribute to salt accumulation in plant rooting zone and rapid degradation of soil chemical and physical properties. In particular, Na^+ can promote

dispersion of soil aggregates to break down; the increasing bulk density will make the soil more compact and decrease total porosity, thereby hampering soil aeration and as a result, plants in saline soils not only suffer from high Na^+ levels, but are also affected by some degree of hypoxia [5].

Tomatoes (*Solanum lycopersicum* L.) are among the most widely produced and consumed vegetables worldwide. The worldwide production has been estimated at 100 million t $year^{-1}$ with a total production area of about 4.2 million ha [7]. Although considered moderately tolerant to salt stress, tomato fruit yield decreases by about 10% for each unit of soil EC above a threshold value of 2.5 $dS \cdot m^{-1}$ [8]. Additionally to the development of salt-tolerant cultivars [1], three major cultivation techniques have so far proved useful to attenuate the effects of excess soil salinity: (i) subjecting seedlings to water- or NaCl-stress can facilitate the adaptation of salt-stressed adult plants; (ii) mist application improves vegetative growth and yield of salt-stressed tomato plants grown under Mediterranean conditions; and (iii) grafting tomato cultivars onto appropriate rootstocks can reduce the effects of salinity [3].

Arbuscular mycorrhizal fungi (AMF) have been frequently reported to improve crop plants' tolerance to stressful abiotic environments such as saline soils. Although AMF can themselves be negatively affected by soil salinity [9,10], several studies report improved growth and performance of mycorrhizal plants under salt stress [9,11–16]. The symbiosis of plants with AMF often results in increased nutrient uptake, accumulation of osmoregulatory compounds, an increase in photosynthetic rates, and an decrease in root respiration and water use, suggesting that salt stress alleviation by AMF results from a combination of effects ranging from nutritional to molecular levels [11,17,18].

Unfortunately, modern cultivation techniques have resulted in progressively reduced AM fungal diversity and frequency in agricultural soils and potting substrates, an effect that is believed to be related to tillage methods, the use of mineral fertilizers and nursery substrate sterilization among other factors [19]. In consequence, the external application of mycorrhizal spores has been practiced, adding AMF inoculum either to seedlings' growing medium or into the planting hole at time of transplanting. Because of this, two main agronomic benefits are expected: superior growth of seedlings in the nursery and improved performance of mature plants following planting in the field [20]. The effects of excessive salinity on plant growth and vitality involve: reduction in the osmotic potential of the soil solution causing physiological drought, nutrient imbalance caused by reduced nutrient uptake and/or transport to the shoot, and direct cell toxicity of excessive Na or Cl ion concentrations [21]. The salinity effect on plants can be described as an two-phase growth response [22]. In the first phase the excess salt ion concentration outside the roots is lowering the osmotic potential of the soil [22], making it harder for the plants to extract water and causing water stress. An immediate response to this effect, which also mitigates ion flux to the shoot, is stomatal closure [5]. The second, ion specific, phase corresponds to the excess accumulation of salt ions in tissues, especially the leaf blade. The consequences of Na^+ or Cl^- build-up in the cell walls are often catastrophic and include dehydration and oxidative stress [22,23], finally causing leaf dieback. If the rate of leaf dieback is faster than the rate of leaf expansion, then the amount of reserve carbohydrate per plant will be reduced in proportion to the reduction in leaf area [24]. The main acclimatization strategy for glycophytes to excess soil salinity levels is to control ion flux into root xylem and as the result, restrict also nutrient ion movement to the shoot [25]. Furthermore, several ions such as P become rather unavailable—P precipitates with Ca^{2+}, Mg^{2+} and Zn^{2+} ions in saline soil [26].

AMF inoculation has been reported to increase the P content in plants through the enhanced uptake by the hyphae [27]. This suggests that AMF increased P uptake may reduce the negative effect of NaCl in plants. Similar, AMF-inoculated plants have been reported to enhance K^+ ions under NaCl stress [28], increasing plants' ability to cope with ROS and to regulate water balance. Thus, many previous studies have addressed AMF effects on growth, yield, and concentration/uptake rate of different mineral elements over several weeks or the entire plant life [11,17,19]. However, available information regarding the temporal development of stress amelioration by AMF is still very limited.

An increased sensitivity towards stress in the seedling stage as compared to adult plants, resulting in greater growth reductions compared with stress onset during later developmental stages, has been previously reported for tomato and other plant species, e.g., under drought [29], ozone [30] and salinity [31–34]. Basic questions such as how fast Na^+ ions reach the photosynthetic organs of tomato transplants and what are the immediate effects of substrate salinity on plant nutrition after transplanting remain largely unknown. Subsequently, in this study we examined the early effect of salt stress on growth and leaf chemical composition on vegetable seedlings performance immediately after transplanting. Since seedling establishment depends largely on the supply of water and nutrients, there are several practices in commercial production aiming to enhance growth and improve water and nutrient acquisition by the root system of recently transplanted seedlings. Commonly they engage the extensive use of different chemical compounds, which acts as root growth enhancers, or salinity alleviators. Instead, it will be of great interest to identify and employ environmental friendly alternatives such as the pre-inoculation of vegetable seedlings with AM fungal spores in the growing substrate. Accordingly, the objective of this study is to assess the efficiency of commercial AMF inoculum addition to the germination substrate (i) to enhance the growth rate; and (ii) to improve the nutrient status of recently transplanted tomato seedlings under normal and saline conditions. Considering the high significance of a fast and successful establishment of transplanted seedlings on further plant performance, the period of study was specifically limited to the immediate stand establishment period only.

2. Material and Methods

2.1. Plant Material and Experimental Set-Up

The experiment was conducted during April 2013, in a plastic greenhouse located in Tirana, Albania (41°23′27″ N, 19°39′18″ E). Graded seeds of the commercial tomato (*Solanum lycopersicum* L.) cultivar "Syta F1" were sown in styrofoam transplant trays (30 cm^3 volume of wells) filled either with (i) vermiculite (Agra-Vermiculite, Pull Rhenen B.V., The Netherlands) and 10% (vol/vol) crushed, expanded clay particles coating AM-fungal spores (~200 spores g^{-1}; mixture of *Glomus intraradices*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus geosporum*, and *Glomus clarum*; AMF+); or (ii) vermiculite with crushed, spore-free expanded clay particles (10% vol/vol; AMF–). The clay particles with/without AMF spores were supplied by BioSym B.V. (Hengelo, Netherlands) and homogenously mixed with the vermiculite before sowing; both substrate types were saturated with a nutrient solution containing 1 g·L^{-1} Terraflex T (N, P_2O_5, K_2O; 18%, 7%, 25% + trace elements; ICL Fertilizers, Grobbendonk, Belgium) fertilizer. After sowing, plants were irrigated with tap water as needed.

At DAS (day after sowing) 30, tomato seedlings were separately transplanted into 200 cm^3 plastic pots (7 cm high, 5.5 cm wide) filled with nutrient-saturated (1 g· L^{-1}, Terraflex T) vermiculite. Three different levels of salt-stress (0, 50 and 100 mM· NaCl) were established by the addition of different amounts of sodium chloride (NaCl) to the nutrient solution. Non-inoculated (AMF–) and inoculated (AMF+) seedlings were equally distributed to the three salinity treatments. Thus, a full factorial design with the factors arbuscular mycorrhizal fungi (AMF− or AMF+) and salinity (0, 50 or 100 mM· NaCl) resulting in six treatments, *i.e.*, Control (AMF−, 0 mM· NaCl), moderate (AMF−, 50 mM· NaCl) and severe salt stress without mycorrhizal inoculation (AMF–, 100 mM· NaCl), and mycorrhizal only (AMF+, 0 mM· NaCl), and moderate (AMF+, 50 mM· NaCl) and severe salt stress with mycorrhizal inoculation (AMF+, 100 mM· NaCl) was established. Plants were watered during DAS 30–37 with tap water as needed; leaching was avoided.

2.2. Plant Sampling and Measurements

At DAS 30 and 37, 10 plants of each treatment were selected randomly and harvested. Roots were gently washed free of adhering vermiculate particles, and plants were dissected and separated into roots, stems and leaves. The experimental period after transplanting was restricted to 7 days to focus

on immediate effects of salinity on transplants—further avoiding changes of plant developmental stage from vegetative to flowering. Quantification of AMF infection rate of roots at DAS 30 and 37 failed due to technical difficulties; however, due to the use of clay particles without spores as control (see above) other confounding influences can be excluded. The plant organs were subsequently dried (65 °C, 48 h) and weighted separately to an accuracy of 0.001 g (TP 303; Denver Instruments GmbH, Göttingen, Germany). Based on the determined dry matter, the relative growth rate (RGR) of the whole plant (RGR_{Plant}) and the root system (RGR_{Root}) was calculated for each treatment between DAS 30 and 37 [35,36]. Both parameters were used as indicators to assess the growth rate of AMF− and AMF+ tomato seedlings immediately after transplanting under different NaCl treatments. Root dry matter (DM_{Root}) and aboveground dry matter (DM_{Shoot}) were used to calculate root:shoot ratios (R:S).

Leaf material was ground with a planetary mill (Pulverisette5; Fritsch, Idar-Oberstein, Germany) for mineral analysis. Total C and N contents in leaves were measured in inoculated (AMF+) and not inoculated (AMF–) seedlings one week after transplanting (DAS 37) with the elemental analyzer TruSpec CN (Leco, St. Joseph, MI, USA) according to the Austrian ÖNORM L 1080 protocol; C:N ratios were calculated.

Nutrient contents of leaves (K, Ca, Mg, Mn, P, Na, Fe, Al and S) at DAS 30 and DAS 37 were analyzed after acid hydrolysis in nitrohydrochloric acid by ICP-OES (Optima 8300; Perkin Elmer, Waltham, USA); see ÖNORM L 1085 and L 1202 for technical details. The mineral concentrations ($mg \cdot g^{-1}$ dry matter) were measured on 10 samples per each organ and treatment. All chemical analyzes were conducted at the Institute of Forest Ecology, University of Natural Resources and Life Sciences Vienna, Austria.

The content (total amount) of each nutrient in the leaves of each plant (mg $plant^{-1}$) was calculated as the product of leaf dry matter and nutrient concentration, multiplying leaf elemental concentration with the respective leaf dry matter. Based on this, an index of relative uptake rate (RUR, Equation(1)) was calculated according to Aprile *et al.* [37] and used to estimate the daily rate of nutrient accumulation(mg per mg of existing nutrient element per each day).

$$RUR = \frac{\ln(Cn) - \ln(Cn-1)}{(tn) - (tn-1)} \quad (1)$$

where Cn and $Cn-1$ are the accumulated leaf nutrients per plant at two sequential times (tn and $tn-1$, respectively) with n ranging between 1 (DAS 30) and 7 (DAS 37).

2.3. Statistics

A factorial arrangement of six treatments in randomized complete block design was used with 10 replicates each. Residuals of all variables were tested for homogeneity of variances and normality using the tests after Levene and Shapiro–Wilk, respectively. Differences in DM, RGR, nutrient concentrations and nutrient uptake, elemental ratios and RUR of specific elements were tested between by two way ANOVA, using the PC program StatPlus 2009 (AnalystSoft Inc., Walnut, CA, USA). Each significant ANOVA result ($p < 0.05$) was followed by an LSD tests at $p < 0.05$ as *post-hoc* test to compare pair wise means within and among treatments. Values given throughout the text are means ± SD.

3. Results

3.1. Growth

Under abundant fresh water and nutrient supply during the nursery period (until DAS 30), no differences were found regarding plant growth parameters of inoculated and non-mycorrhizal tomato seedlings. After transplanting, salinity stress immediately and significantly affected the relative growth rate and dry matter accumulation (DAS 30–37; Table 1). The relative growth rate of plants (RGR_{Plant}) and the relative growth rate of roots (RGR_{Root}) of transplanted seedlings (DAS 30–37)

were significantly reduced due to increasing salinity (Figure 1A,B). However, in inoculated (AMF+) plants, RGR_{Plant} was significantly reduced under severe salt stress (100 mM· NaCl) only, while in non-inoculated (AMF−) plants both moderate (50 mM· NaCl) and severe salinity caused a significant reduction of RGR_{Plant}. Under severe soil salinity, the RGR_{Plant} of AMF+ plants remained almost equal to that of AMF− plants grown under 50 mM· NaCl (Figure 1A). No significant reduction was found regarding the relative growth rate of AMF+ roots (RGR_{Root}) under both moderate or severe salinity, while the RGR_{Root} of AMF– plants was significantly reduced under severe salinity (Figure 1B).

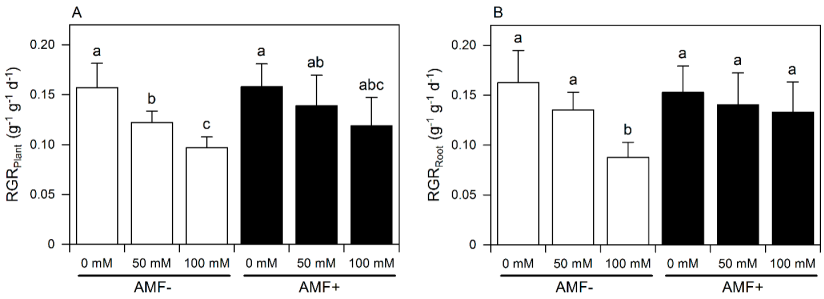

Figure 1. Relative growth rates of the whole plant (RGR_{Plant}) (**A**) and of roots only (RGR_{Root}) (**B**) between DAS 30 and DAS 37 of transplanted tomato seedlings without arbuscular mycorrhizal fungi (AMF−, white bars) and with AM fungi inoculation (AMF+, black bars) under three levels of salinity (0, 50, and 100 mM· NaCl). Different letters indicate significant differences within parameters (Fisher LSD test, $p < 0.05$; mean + SD, $n = 10$).

Table 1. Two-way ANOVA results including cross effects on growth parameters (DM_{Plant}; DM_{Root}; root shoot ratio (R:S); and RGR_{Plant}, RGR_{Root}) of inoculated (AMF+) and non-inoculated (AMF−) tomato seedlings one week after transplanting (DAS 37) into pots with three different levels of soil salinity (0, 50, and 100 mM· NaCl). p values are given; significant ($p < 0.05$) values are highlighted in bold.

Sources of Variation	DM_{Plant}	DM_{Root}	R:S	RGR_{Plant}	RGR_{Root}
AMF	0.855	0.205	0.234	0.053	0.079
Salinity	**0.000**	**0.000**	0.182	**0.000**	**0.000**
AMF × salinity	0.263	**0.010**	0.190	0.408	0.147

Subsequent to above, dry matter (DM) of the whole plant (DM_{Plant}), and/or of the roots (DM_{Root}), was significantly affected by salinity (Table 1, Figure 2). A significant decrease of DM_{Plant} was found in non-inoculated (AMF−) seedlings under both moderate and severe salinity while in salt-stressed AMF+ plants the reduction in dry matter under salinity was not significant (Figure 2). DM_{Plant} of AMF+ seedlings was slightly (not significantly) smaller compared to AMF− seedlings under non-saline condition (0 mM· NaCl), they maintained non-significantly different DM_{Plant} values under moderate and severe salinity (Figure 2). Increasing salinity generally resulted in less DM_{Root} in both AMF+ and AMF− seedling; however, root biomass was only significantly reduced in severely salt-stressed AMF− plants (Figure 2). Neither salinity nor the inoculation with mycorrhizal symbionts influenced the root to shoot ratio (R:S) of tomato seedlings (Table 1).

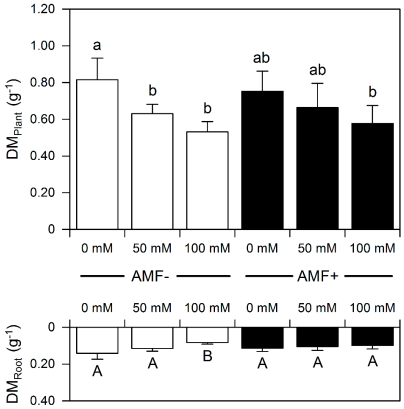

Figure 2. Whole plant dry matter (DM_{Plant}) (**top**) and root dry matter (DM_{Root}) (**bottom**) of transplanted seedlings without arbuscular mycorrhizal fungi (AMF−, white bars) and with AM fungi inoculation (AMF+, black bars) under three levels of salinity (0, 50, and 100 mM· NaCl) at DAS 37. Different lower case letters indicate significant differences within DM_{Plant}, different capital letters indicate significant differences within DM_{Root} (Fisher LSD test, $p < 0.05$; mean + SD, $n = 10$).

3.2. C and N Content and C:N Ratio

N and C concentrations were significantly higher in leaves of inoculated tomato seedlings under non-saline conditions than in leave of AMF− plants. Salt stress significantly affected the N and C contents in leaves of transplanted seedlings; in both AMF+ and AMF− seedlings, N and C concentrations significantly declined with increasing salinity (Tables 2 and 3). However, under moderate salinity leaf N and C concentrations remained higher in AMF+ seedlings compared to non-inoculated seedlings. While fresh water-irrigated plants had similar C:N ratios with or without inoculation, C:N ratios gradually increased with increasing salinity (Table 3). The respective values of AMF+ plants were generally higher compared to AMF− plants, but differences were significant only under severe salt stress.

Table 2. Two-way ANOVA results including cross effects on C and N concentration and C:N ratio of inoculated (AMF+) and non-inoculated (AMF−) tomato seedlings one week after transplanting (DAS 37) into pots with three different levels of soil salinity (0, 50, and 100 mM· NaCl). p values are given; significant ($p < 0.05$) values are highlighted in bold.

Sources of Variation	N	C	C:N
AMF	0.053	**0.031**	**0.004**
Salinity	**0.000**	**0.000**	**0.000**
AMF × salinity	**0.046**	0.271	**0.000**

Table 3. C and N concentrations and C:N ratio of transplanted seedlings at DAS 37 in six treatments with/without arbuscular mycorrhizal fungi (AMF+/AMF−) and under three levels of salinity (0, 50 and 100 mM· NaCl). Different letters (a, b, c, d) indicate significant differences within parameters (Fisher LSD test, $p < 0.05$; mean ± SE, $n = 10$).

Factors		N (mg g^{-1})	C (mg g^{-1})	C:N
AMF−	0 mM· NaCl	1.75 ± 0.33^{b}	18.7 ± 2.96^{b}	10.7 ± 0.64^{c}
	50 mM· NaCl	1.05 ± 0.25^{d}	12.5 ± 2.26^{c}	11.2 ± 1.14^{b}
	100 mM· NaCl	1.09 ± 0.12^{d}	12.2 ± 1.12^{c}	12.1 ± 0.54^{b}
AMF+	0 mM· NaCl	2.06 ± 0.23^{a}	22.4 ± 2.84^{a}	10.8 ± 0.35^{c}
	50 mM· NaCl	1.35 ± 0.11^{c}	16.1 ± 1.71^{b}	11.8 ± 0.40^{b}
	100 mM· NaCl	0.95 ± 0.14^{d}	12.9 ± 1.83^{c}	13.5 ± 0.24^{a}

The relative uptake rate (RUR) of N of AMF+ plants was significantly higher than AMF− plants (Tables 4 and 5). N relative uptake rate of both; AMF+ and AMF− plants was gradually and significantly reduced due to raised salinity, but it always remained higher in AMF+ plants (Table 5). It was significantly higher than AMF− plants at non saline and moderate saline conditions and even, under severe salinity N relative uptake rate of AMF+ plant was at the same statistical group with AMF− plants under no saline condition (Table 5).

3.3. Leaf Nutrient Concentration and Relative Uptake Rate (RUR)

Salt stress resulted in lower content of most tested elements in leaves of tomato seedlings, shortly after transplanting (DAS 30−37; Tables S1 and S2). Na concentration and total content in tomato leaf tissue significantly increased under salinity stress (Table S2). Inoculation with AMF spores led to significantly higher contents of K, Ca, Mg, P and Fe ions in tomato leaf tissue under non-saline conditions, as well as significantly higher Ca and Fe ion contents under moderate salinity (Tables S1 and S2). Significant reductions in mineral contents of leaves were observed between non saline and moderately saline conditions for most elements, while severe salinity caused a significant further reduction of K and Ca (Table S2). No significant influence of AMF inoculation was found regarding the leaf content of most of elements under severe salt stress.

Table 4. Two way ANOVA results on nutrient relative uptake rate (RUR) in the leaves of inoculated (AMF+) and not inoculated (AMF−) tomato seedlings one week after transplanting (DAS 37) into pots with three different levels of soil salinity (0, 50, 100 mM· NaCl). p values are given; significant ($p < 0.05$) values are highlighted in bold.

Sources of Variation	N	K	Ca	Mg	P	Na	Mn	Fe	Al	S
AMF	**0.000**	0.375	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	0.865	**0.000**	0.446
Salinity	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
AMF × salinity	**0.028**	**0.015**	0.130	0.557	**0.037**	0.108	0.557	0.058	0.408	0.196

Table 5. Relative uptake rates(RUR; mg·mg^{-1}· day^{-1}, μg·μg^{-1}· day^{-1}) of several nutrients in the leaves of inoculated (AMF+) and not inoculated (AMF−) tomato seedlings one week after transplanting (DAS 37) into pots with three different levels of soil salinity (0, 50, 100 mM· NaCl). Different letters (a, b, c, d) indicate significant differences within nutrient element (Fisher LSD test, $p < 0.05$; mean ± SE, $n = 10$).

Factors		N (mg· mg^{-1}· day^{-1})	K (mg·mg^{-1}· day^{-1})	Ca (mg· mg^{-1}· day^{-1})	Mg (mg· mg^{-1}· day^{-1})	P (μg·μg^{-1}· day^{-1})	Na (μg·μg^{-1}· day^{-1})	Mn (μg·μg^{-1}· day^{-1})	Fe (μg·μg^{-1}· day^{-1})	Al (μg·μg^{-1}· day^{-1})	S (μg·μg^{-1}· day^{-1})
AMF−	0 mM	0.153 ± 0.02 ^{b,c}	0.179 ± 0.03 ^{b}	0.151 ± 0.03 ^{b}	0.147 ± 0.03 ^{b}	0.064 ± 0.03 ^{b}	0.206 ± 0.00 ^{d}	0.155 ± 0.03 ^{b}	0.150 ± 0.02 ^{c}	0.139 ± 0.00 ^{a}	0.167 ± 0.03 ^{a}
	50 mM	0.078 ± 0.03 ^{c}	0.142 ± 0.03 ^{b}	0.089 ± 0.03 ^{c}	0.110 ± 0.03 ^{c}	0.037 ± 0.01 ^{c}	0.348 ± 0.00 ^{b}	0.110 ± 0.03 ^{c}	0.063 ± 0.03 ^{d}	0.060 ± 0.00 ^{b}	0.063 ± 0.03 ^{b}
	100 mM	0.087 ± 0.01 ^{c}	0.135 ± 0.02 ^{c}	0.094 ± 0.00 ^{c}	0.100 ± 0.00 ^{c}	0.021 ± 0.01 ^{c}	0.293 ± 0.00 ^{c}	0.102 ± 0.01 ^{c}	0.076 ± 0.01 ^{d}	0.025 ± 0.00 ^{c}	0.070 ± 0.01 ^{b}
AMF+	0 mM	0.220 ± 0.01 ^{a}	0.223 ± 0.02 ^{a}	0.205 ± 0.02 ^{a}	0.201 ± 0.02 ^{a}	0.130 ± 0.02 ^{a}	0.271 ± 0.00 ^{c}	0.201 ± 0.03 ^{a}	0.160 ± 0.02 ^{a}	0.074 ± 0.00 ^{b}	0.173 ± 0.02 ^{a}
	50 mM	0.160 ± 0.01 ^{b}	0.156 ± 0.02 ^{b}	0.140 ± 0.02 ^{b}	0.153 ± 0.01 ^{b}	0.055 ± 0.01 ^{b}	0.381 ± 0.00 ^{a}	0.145 ± 0.03 ^{b}	0.082 ± 0.02 ^{b}	0.033 ± 0.00 ^{c}	0.074 ± 0.02 ^{b}
	100 mM	0.109 ± 0.02 ^{c}	0.104 ± 0.02 ^{c}	0.103 ± 0.02 ^{c}	0.130 ± 0.02 ^{c}	0.045 ± 0.02 ^{b}	0.373 ± 0.00 ^{a}	0.118 ± 0.02 ^{c}	0.042 ± 0.02 ^{e}	0.040 ± 0.00 ^{c}	0.038 ± 0.01^{c}

In fresh water-irrigated tomato seedlings, inoculation with AMF significantly enhanced the relative uptake rates (RUR) of N, K, Ca, Mg, P, Na, Mn and Fe ions between DAS 30 and DAS 37, and significantly reduced the RUR of Al ions (Table 5). Beside Na ions, salt stress significantly reduced RUR of all nutrients in tomato leaves (Tables 4 and 5). Except for K and Al ions, no significant differences were found between AMF− seedlings' mineral RUR under moderate and severe salinity (Table 5), while in AMF+ seedlings, the RUR of N, K, Ca, Mg, Mn, Fe and S ions were further reduced under severe salinity compared to moderate salt stress. Compared to AMF− tomato seedlings, inoculated (AMF+) seedlings possessed significantly greater RUR of N, Ca, Mg, P, Na, Mn and Fe ions under moderate salinity, no effects were found regarding K and S, and the RUR of Al ions decreased (Tables 4 and 5). RUR of N, K, Ca, Mg, Mn, and Al ions did not differ significantly between AMF+ and AMF− plants under severe salinity, while the RUR of P and Na ions were significantly higher and the RUR of Fe and S ions were significantly lower in severely stressed AMF+ plants respectively.

4. Discussion

The capacity of a transplant to overcome transplant shock and become established in a field environment following transplanting depends on the water and nutrient uptake capacity of the roots, and the capacity of the preexisting root system to rapidly (re-)generate new lateral, basal, or adventitious roots [38]. Because, similar to many crops, tomato is most sensitive to salt stress during early plant developmental stages [39], successful establishment of tomato seedlings is especially critical under excess soil salinity. While the fast growth of root system is essential to ensure successful transplanting of young seedlings, high salinity was reported to significantly reduce the root length and root dry matter of tomato plants [9]. Similar to previous reports for other vegetable crops [40,41], this study found that soil salinity reduced the growth rate and thus the dry matter production of young (30 DAS) tomato seedlings of the variety Syta F_1 within the first seven days after transplanting. We observed that salt stress had not significantly reduced RGR_{Plant} in AM-inoculated tomato plants immediately after transplanting while in non-inoculated AMF− plants the growth rate was significantly lower. Our results are in accordance with previous studies on other vegetable crops [14,17,42] and tomato itself [16,43–45]. Considering the differences between AMF+ and AMF− plants regarding RGR_{Plant} changes within seven days after transplanting into saline substrate, differences in plant dry matter are expected to become larger over time.

Because reduced growth under salinity is partially caused by ion imbalances [46,47] and/or non-availability of nutrient ions due to their competition with major ions (Na^+ and Cl^-) in the soil [48], the sustained growth of AMF+ plants under salinity is partially based on improved uptake of nutrients and maintaining favorable ionic ratios [48]. Beside optimized fertigation, improved plant nutrition can be reached either by enhanced nutrient uptake by mycorrhizal hyphae or changes on plant allometry, *i.e.*, relatively larger root systems (under the premise of a stable physiology) compared to the shoot. Interestingly, RGR_{Roots} and root biomass of AMF+ seedlings were significantly less affected, *i.e.*, remained higher, under severe soil salinity than those of non-inoculated tomato plants. Our results corroborates with the findings of Abeer and coauthors (2015) [9] whose also reported a smaller decrease of root dry matter in AMF+ tomato plants compared with non-inoculated plants under severe salinity conditions.

Previously, Ruiz-Lozano and Azcón [27], found that one mechanism of *Glomus* sp. -induced salt stress amelioration is stimulating root growth of the host plant. However, possible effects of AMF on root biomasses under moderate salt stress and/or root: shoot (R:S) ratios were not visible, possible due to the limited growth period after transplanting. Overall, we have not found any significant difference in root to shoot ratios between transplanted AMF+ and AMF− seedlings with or without salt stress, while Abdel Latef and He Chaoxing [11] found AM fungi's effect on dry matter accumulation in tomato was more pronounced on aboveground biomass rather than on root biomass and thus changed the R:S ratio. Further experiments, possible under different nutrient supply and including different varieties, are needed to clarify these findings. However, since RGR_{Roots} and

root biomass of inoculated (AMF+) plants remains more stable under most saline soil conditions, nursery inoculation with arbuscular mycorrhizae spores will benefit stand establishment of the tested tomato variety.

Salinity influences total nitrogen (N) uptake [48] and as well as interferes at different stages of its metabolism [46]. N and C concentrations and C:N ratios are positively correlated with RGR [49,50]. In addition, previous studies have reported that improved N nutrition may help to reduce the toxic effects of Na ions by reducing its uptake and this may indirectly help in maintaining the chlorophyll content of the plant [21]. Because AM fungi can function as facilitator for N uptake [48], a higher leaf N content was found in AMF+ transplanted tomato seedlings and consequently higher or at least more stable growth rates (RGR_{Root} and RGR_{Plant}, respectively) were achieved in the first seven days after transplanting.

Commonly, the effects of AM fungi on plant nutrient acquisition are discussed based on the differences of nutrient concentration in plant tissues. Anyway, we found the relative uptake rate of nutrient elements (RUR) a better tool to distinguish the differences among different treatments over a short period, when the nutrient concentration could be largely influenced by the dilution effect of fast growth in young plants. Thus, this study found that N relative uptake rate (RUR) values of AMF+ plants under both non-saline and moderate saline conditions were higher in AMF+ plants than in non-inoculated seedlings. Though we were not able to quantify the presence of AM fungi in AMF+ plants, we found that in both inoculated and non-inoculated plants, RUR of N ions was gradually and significantly reduced by increasing soil salinity levels. Mycorrhizal symbionts are generally more important for the uptake of rather immobile nutrients, while N, especially as nitrate, is transported within the soil solution. Because salt-stressed plant regularly reduce transpiration, the reduction in N uptake is likely related to reduced water uptake with increasing salinity levels.

Inoculation with AM fungal spores significantly affected the nutrient concentrations and relative uptake rate (RUR; daily rate of nutrient accumulation per existing nutrient element) of many ions (Tables S1, S2, Tables 4 and 5) under both fresh water and saline conditions after transplanting. The raise of salinity in the transplanting substrate led to increased sodium (Na^+) content (Table S2) in the leaves of transplanted tomato seedlings. Al-Karaki [44] and Evelin and co-authors [48] found lower Na concentrations in leaves of AMF free plants, concluding that AMF induce a regulatory effect on uptake and/or translocation of Na to the aerial parts. Similar, we found significantly less Na content in AMF+ tomato seedlings under moderately saline conditions (Table S2); however, no significant differences were found under severe soil salinity. Furthermore, the respective Na RUR values in the studied AMF+ tomato seedlings are even significantly higher than those of AMF− seedlings under both salinity levels (Table 5). While early studies occasionally reported increased Na ion uptake by AMF symbionts [51], our results are a reminder that it remains an open question if AM symbionts commonly reduce excess Na ion uptake and/or translocation to leaves under saline conditions and in most species and varieties or if an dilution effect by greater growth is responsible for lower Na ion concentrations in less-salt affected tissues [43,44].

However, while exposure of plants to salinity for a short period of time (DAS 30–37) significantly reduced RUR of almost all nutrient elements beside Na ions, AMF+ plants were able to retain significant higher RUR of Ca, Mg, Mn and Fe ions under moderate salt stress and of Phosphorus (P) under both moderate and severe salinity (Table 5). It has been reported that extensive hyphal network and the higher hyphae affinity and a lower threshold concentration for absorption than in plant roots are the most important mechanism of higher P uptake by mycorrhized plants, but it is yet unclear how AMF improve the uptake of, e.g., Ca and Mg ions [48]. Earlier studies found that the improved growth of mycorrhizal plants in saline conditions is highly related to mycorrhizal-mediated enhancement of host plant P nutrition [13,17]. While P is an integral component of several key plant structure compounds, improved P uptake by AMF in salt-stressed plants may also contribute to vacuolar membrane integrity and facilitate the compartmentalization of Na ions [52]. Also, an enhanced Ca ion RUR and content in AMF+ plants can reduce the toxic effects of NaCl, presumably

by facilitating higher K/Na ion selectivity, and thus enhancing overall plant metabolic activity [53]. In this study, however, Ca ion RUR was only higher in moderately salt-stressed tomato transplants. Surprisingly, inoculation with AMF spores (AMF+) did only modify the Potassium (K) ion RUR and content in tomato leaves under non-saline conditions but not under salinity. K ions plays a key role in plant metabolism; under excess soil salinity, Na ions compete with K for binding sites essential for various cellular functions but cannot replace the function [54] and Na-induced K ion deficiency has been implicated in growth and yield reductions of various crops, including tomato [55].Thus, in accordance with our results, numerous studies on a wide variety of crops have shown that K ion concentration in plant tissue declines as soil salinity increases. However, although previous studies frequently reported enhanced K uptake by mycorrhizal symbionts [21], we did not find this effect for unknown reasons (theoretical by e.g., uptake competition between K and NH_4 ions).

Some studies attributed salt stress effects to a decreased Mg ion absorption and thus detrimental effects on photosynthetic capacity [56]; indeed, the Mg ion concentration was reduced by salt stress in our experiment but not modified by AMF inoculation. In contrast, Evelin *et al.* [48] found that Mg ion uptake was not influenced by NaCl salinity in *Trigonella* sp. and Giri and colleagues [57] reported higher Mg ion uptake in mycorrhized *Acacia* sp. plants. Further studies on host or arbuscular mycorrhizal species effects on Mg ion uptake under saline soil conditions are thus urgently needed, taking especially Ca ion concentration in the soil solutions as a cofactor into account [58]. In saline soils, the solubility and mobility of Cu, Fe, and Zn ions decreases further [47]. As available Cu, Zn, and Fe levels decrease with increase in salinity, a depletion zone is formed around the roots. In our experiment, the uptake of Fe ions is indeed limited under salinity but AMF+ plants retained a higher Fe ion RUR under moderate salinity. Similar to our results, NaCl-induced Mn deficiency has been previously observed in tomato (Alam *et al.*, 1989, cited after Grattan and Grieve) [47]). However, other studies with tomato suggested that salinity either had no effect or increased Mn in leaf or shoot tissue [59,60]. Further studies are needed to clarify these finding and relate them to experimental conditions and/or tomato/AMF variety/species. Interestingly and scarcely studied, the Al ion RUR was lower in our mycorrhizal tomato seedlings; while this was not the focus of our study, this might have implications for Al plant nutrition in rather acid saline soils [61]. Similar, very little attention is given to salinity's influence on Sulfur (S) uptake and accumulation in crops in general. In our study we found reduced S contents and RUR under NaCl salinity, independent from AM inoculation. Previously, Mor and Manchanda [62] reported reduced S content in salt-stressed pea shoots.

5. Conclusions

This study addresses the performance of instantly transplanted tomato seedlings of the cultivar "Syta F1". It provides novel information, namely that nursery inoculation with commercially available AM-fungal spores (mixtures of *Glomus* sp.) is useful to reduce the detrimental effects of excess soil salinity on tomato seedlings directly after transplanting. Among likely mechanisms are the higher root biomass of inoculated seedlings under severe salinity and the facilitated nutrient uptake of several elements under moderate and, for P, severe soil salinity. Because of the relative low price of inoculate and easy storage and addition to nursery substrate, inoculation with AMF seems to be a viable solution to attenuate the effects of excess soil salinity levels after transplanting, especially in greenhouse soils with a low natural abundance of AMF spores. However, additional studies, covering all developmental stages and testing more varieties under diversified soil conditions are needed to further validate the usefulness of commercial AM inoculate to ameliorate salt stress in commercial tomato production. Previously it has been found that species diversity and species number in AMF communities are significantly higher in soils from organic farms, while being reduced by wastewater pollution, excess fertilization and tillage [63,64]. While other studies conclusively found that AM infection rates are lower under salinity, more research is needed to determine the availability and vitality of natural AM mycorrhizal spores in salt-affected agricultural soils—comparing AM infection

rates of crops under actual and in inoculate-amended soils. In any case, changes in soil properties must be considered keenly in future management practices in order to prevent a decrement of native AMF population—maintaining sustainable horticultural production systems.

Supplementary Materials: Table S1: Two way ANOVA results on nutrient amount in the leaves of inoculated (AMF+) and not inoculated (AMF−) tomato seedlings one week after transplanting (DAS 37) into pots with three different levels of soil salinity (0, 50, 100 mM· NaCl). Table S2: Total nutrient amount (mg; µg) in the leaves of inoculated (AMF+) and not inoculated (AMF−) tomato seedlings one week after transplanting (DAS 37) into pots with three different levels of soil salinity (0, 50, 100 mM· NaCl).

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