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Fertilization and Nutrient Use Efficiency in Mediterranean Environments

**Editors: Dimitris Bouranis, Silvia Haneklaus,
Styliani Chorianopoulou, Jie Li, Luit De Kok, Ewald Schnug, Lanzhou Ji**



Proceedings



**November 3-4, 2020
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Programme

28th International Symposium of CIEC

Programme

Tuesday, November 3, 2020	
09.00 - 9.30	Registration
09.30 - 10.00	Opening ceremony
10.00 - 11.00	Oral presentations - Fertilizer technology Chair: Silvia Haneklaus, Ioannis Massas
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10.20 – 10.40	Traditional nitrogen fertilizers compared to control release urea technology effect, on nitrogen use efficiency in bread wheat (<i>Triticum aestivum</i> L.), maize (<i>Zea mays</i> L.) and cotton (<i>Gossypium hirsutum</i> L.) in Balkan region Vasilis Tsambardoukas, <u>Thanasis Rosoglou</u>
10.40 – 11.00	Preliminary assessment of N stabilizer N-Lock™ with Optinyte™ technology (nitrapyrin) applied with urea fertilizers in cotton (<i>Gossypium hirsutum</i> L.) agrosystem at Imathia, Greece <u>Georgios Giannopoulos</u> , Georgios Zanakis, Lars Elsgaard, Nick Barbayiannis
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11.30 – 12.50	Oral presentations - Soil quality and amelioration Chair: Ewald Schnug, Petros Roussos
11.30 – 11.50	Silent alienation of soils through microplastics in the anthropocene <u>Xijuan Chen</u> , Elke Bloem, Jie Zhuang, Ewald Schnug
11.50 – 12.10	Is acidification a suitable method to limit ammonia losses from slurry? <u>Silvia H. Haneklaus</u> , Martin Kaupenjohann, Ewald Schnug
12.10 – 12.30	The nutritional profiles of fields cultivated with <i>Aloe barbadensis</i> crops in Neapolis, Laconia, Greece, and their impact on leaf sulfur status <u>Mary Perouli</u> , Artemios Chatziartemiou, Styliani N. Chorianopoulou, Dimitris L. Bouranis
12.30 – 12.50	Glycine betaine, <i>Bacillus amyloliquefaciens</i> IT45 and zeolite-bentonite mixture as ameliorating agents against salt stress in strawberry Ntanos Efstathios, Assimakopoulou Anna, Dionisios Gasparatos, Nikoleta-Kleio Denaxa, Kosta Anna, <u>Roussos A. Petros</u>
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14.00 – 14.20	<p>Sustainable phosphorus management depends on safer phosphate fertilizers: mitigation of heavy metal contamination</p> <p><u>Liankai Zhang</u>, Yajie Sun, Bernd G. Lottermoser, Roland Bol, Miyuki Maekawa, Heike Windmann, Silvia H. Haneklaus, Ewald Schnug</p>
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14.40 – 15.00	<p>NUTRISENSE: A novel software operating as an internet application to support plant nutrition and fertilization via nutrient solutions in greenhouse crops grown hydroponically</p> <p><u>Dimitrios Savvas</u></p>
15.00 – 15.20	<p>Effect of biostimulants on yield performance of two durum wheat cultivars</p> <p><u>Vasilis Koutsougeras</u>, Panayiota Papastylianou</p>
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16.00 – 17.20	<p>Oral presentations - Foliar applications</p> <p>Chair: Mario Malagoli, Thomas Sotiropoulos</p>
16.00 – 16.20	<p>Effects of silicon, potassium and calcium applications on kiwi fruit quality characteristics and nutrient concentration</p> <p>Ntanos Efstathios, Tsafouros Athanasios, Denaxa Nikoleta-Kleio, Kosta Anna, Assimakopoulou Anna, <u>Roussos A. Petros</u></p>
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10.20 – 10.40	Sulfate assimilation in C₄ plants Silke Gerlich, Anna Koprivova, Ivan Zenzen, Parisa Rahimzadeh Karvansara, Timothy O. Jobe, <u>Stanislav Kopriva</u>
10.40 – 11.00	Impact of sulfur nutrition on the expression and activity of Group 1 sulfate transporters in developing Brassica pekinensis seedlings Dharmendra H. Prajapati, Ties Ausma, Tahereh A. Aghajanzadeh, <u>Luit J. De Kok</u>
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Colonization with arbuscular mycorrhizal fungi (AMF) enhances growth and mineral acquisition of tomato (*Solanum lycopersicum* L.) plants under normal and drought stress conditions

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Plants are sessile and sensitive organisms that are exposed to a diverse range of biotic (fungi, bacteria, nematodes, insects, viruses and viroids) and abiotic (drought, heat, cold, salinity, heavy metals) stresses. Arbuscular mycorrhizal fungi (AMF) are soilborne microorganisms that represent a monophyletic fungal lineage (*Glomeromycota*) and establish a mutualistic symbiotic association with most land plants. Besides enhancing plant growth and nutrient uptake this relationship is known to improve plant performance under water restrictions. In order to assess drought tolerance of mycorrhizal tomato plants (*Solanum lycopersicum* L. cv. EVIA F1) grown in a sand-vermiculite medium, a greenhouse experiment was carried out with two different mycorrhizal strains applied singly at two irrigation regimes (70% of water holding capacity as control and 30% of WHC inducing severe stress). Plants were inoculated and transplanted at the stage of 4 true leaves and drought stress regimes were initiated after two weeks for a time-interval of four weeks. Mycorrhizal colonization generally enhanced plant vegetative growth, both under normal and reduced irrigation. Shoot dry matter yield, photosynthesis and leaf area were higher in mycorrhizal than in nonmycorrhizal plants. Total accumulation of nutrients was higher in AMF inoculated than in uninoculated plants under both control and drought stress conditions. Moreover, premature flowering in response to water stress has been noticed only in the case of all inoculated plants. This study suggests that inoculation with AMF contributed to alleviation of water stress by improving plant fitness and maintaining a favorable nutrient profile. We conclude that endomycorrhizal colonization can mitigate the adverse limitations of water stress on treated tomato plants, restoring most of the key growth parameters to levels similar or close to those in unstressed plants.

Keywords: AMF; microorganisms; tomato; drought stress; nutrients.

Acknowledgment: This work is part of the project “Mixed microbial inocula for vegetable production in the Western Peloponnese – application to soil, propagating material, hydroponics, enhanced growth substrates - **MIMIN** (MIS:5029903)” funded under the framework of the single RTDI state aid action "RESEARCH - CREATE - INNOVATE".

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Colonization with arbuscular mycorrhizal fungi enhances growth and mineral acquisition by tomato plants under normal and salinity stress conditions

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Abstract

Plants are exposed to a diverse range of biotic and abiotic stress. Arbuscular mycorrhizal fungi (AMF) establish a mutualistic symbiotic association with most land plants aiding them to alleviate stress. A greenhouse experiment was carried out to assess salinity tolerance of mycorrhizal vs non-mycorrhizal tomato plants grown in a sand-vermiculite medium, under normal and salinity stress conditions. Mycorrhizal colonization, by two different AMF inocula generally enhanced plant vegetative growth, both under normal and salinity conditions. Shoot fresh biomass, relative water content, leaf area and leaf P concentration were higher in mycorrhizal compared to non-mycorrhizal plants. Application of the inocula alleviated salinity effects and led to growth and nutrient uptake values comparable to those of control non-inoculated plants grown under no salt stress. Interestingly, inoculated plants showed premature flowering in response to salinity. We conclude that inoculation with AMF may generally enhance plant growth and performance, and can mitigate the adverse effects of salinity stress on inoculated tomato plants, restoring most of the key growth characteristics values to levels similar, or close, to those observed for non-stressed plants.

Keywords: Arbuscular mycorrhizal fungi; microorganisms; tomato; salinity stress; plant nutrition.

Introduction

As a consequence of millions of years of competition, coexistence and coevolution, the plant-microbe “interactome” covers a spectrum of associations between plants and microorganisms that range along a broad continuum, from strong mutualism to parasitism [1]. Under natural conditions, plants frequently interact with microbes, which directly mediate plant responses to environmental adversities. Among beneficial microbes, arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms, which can form a symbiotic association with most terrestrial plants [2,3]. A growing body of literature shows that these root symbionts offer an array of benefits to host plants [3] ranging from plant growth and nutritional enhancement [4,5], to tolerance against various stress conditions such as drought, salinity, heavy metals and extreme temperatures.

Salinity is a major environmental constraint in crop production worldwide. The deleterious effects of salt stress are associated with (i) high osmotic potential of soil solution (water stress), (ii) nutritional imbalance, (iii) specific ion effects (salt stress), (iv) detrimental effects on soil structure or (v) a combination of these factors [6,7]. All effects previously mentioned lead to negative direct and indirect pleiotropic effects on plant growth and performance at the physiological, biochemical and molecular level. Although sessile, plants have evolved to be

highly flexible systems, implementing many adaptive strategies to improve their fitness in a changing environment. In this context, plants exhibit growth plasticity, accumulation of compatible osmolytes to maintain cell turgor and prevent ultrastructural damage, ion homeostasis, regulation of water uptake and enhanced water use efficiency through stomatal adjustments, antioxidant mechanisms (enzymes and molecules) to negate the harmful effects of the excess ROS production and induction of phytohormones [6,8,9]. The inoculation of crops with AMF could be embedded into an integrated strategy for increasing crop resilience against adverse environmental conditions. A number of authors have recognized that direct changes in plant physiology aided by AMF colonization and the enhanced soil exploitation ability conveyed by the extraradical mycelia of mycorrhizal plant roots. These can support a range mechanisms in plants to manage salt stress through: (i) enhancing nutrient acquisition and maintaining ionic homeostasis, (ii) improving water uptake and sustaining osmotic equilibrium in plants, (iii) inducing antioxidant systems to prevent damage by ROS, (iv) protecting the photosynthetic apparatus and enhancing photosynthetic efficiency and (v) modifying the hormonal responses to minimize salt effects on growth and development [8–10].

In the present study we evaluated the effect of the association of two AMF strains (*Funneliformis mosseae* and *Rhizophagus intraradices*) with tomato seedlings under normal and salinity stress conditions on plant growth and performance. We focused on characteristics of plant biomass, nutrition, stomatal activity and water status and we show that mycorrhizal colonization generally enhanced plant performance, both under normal and salt stress conditions.

Materials and Methods

In this experiment, two AMF strains were used, a *Rhizophagus intraradices* (DAOM 197198) and a *Funneliformis mosseae* strain. *F. mosseae* was isolated from a certified organic farm in Greece, whereas *R. intraradices* DAOM 197198 was purchased from Agronutrition (Labège, France). The inocula consisted of the potting medium containing colonized *Z. mays* roots, hyphae, and spores.

Sterilized seeds of the commercial tomato (*Lycopersicon esculentum* L.) cultivar “EVIA F1” were sown in 50 ml QP Standard plastic pot trays filled with sterile Klamann-TS2 peat medium (Klamann-Deilmann, Geeste, Germany) and allowed to germinate in darkness. At 27 DAS (Days after sowing), thirty tomato plants were transplanted to pots (1.4 L) filled with 2:1 v/v sand : vermiculite medium, at the stage of 3 true leaves. During transplantation, 10 g of each AMF inoculum (almost 60 spores plus hyphae) were added per plant to form the inoculation treatments (10 + 10 plants) while another 10 plants were supplied with an autoclaved form of the same inoculum. Until the stage of 4 true leaves, plants were irrigated with water and fertilized with modified Hoagland nutrient solution [11]. For a time-interval of one month, plants were subjected to two salt concentrations by adding (or not adding) NaCl to the irrigation water. This resulted in substrate EC values of 1.4 dS/m (control without salt stress) and 6.5 dS/m (salt stress). The experiment was conducted in a glasshouse (latitude 37.98° N, longitude 23.70° E) under controlled conditions of 25–30°C and 60–80% RH, during summer 2019.

At harvest (69 DAS) plant growth was determined by measuring total length of shoot, total number of leaves and shoot and root fresh and dry weights (destructive sampling). Leaf samples were photographically scanned for the determination of leaf area (LA) by image analysis using Gimp (ver. 2.10.20, GIMP Development Team). Roots were washed free of soil, and a subsample was used to estimate AM fungal colonization with trypan blue stain [12].

Mycorrhizal colonization was estimated on slides according to McGonigle et al. [13]. Net photosynthetic rate (PN), stomatal conductance (gs), transpiration rate (E) of tomato plant leaves were recorded with a Li-6400, portable photosynthesis system (LiCor Bioscience Inc., Lincoln, NE, USA). Leaf relative water content (RWC) was measured as described by Sade et al. [14]. Leaf P concentration was determined colorimetrically using the Murphy and Riley method [15] and Ca, Mg, Mn, Zn, Fe and Cu concentrations were determined by atomic absorption spectroscopy. K and Na concentrations were determined by flame photometry.

The experimental design was completely randomized 2×3 factorial, with two levels of salt stress and three levels of inoculation (non-AMF, *R. intraradices* and *F. mosseae*). For all measurements and analyses performed, each plant constituted a single biological replicate. All data were subjected to a two-way analysis of variance (ANOVA) for the determination of the main and interaction effects of salt stress and inoculation by AMF. For multiple comparison of means, Duncan's multiple range test was employed ($\alpha < 0.05$).

Results

AMF inoculation induced a positive effect on many growth traits of stressed and non-stressed plants (Fig.1). However, salinity stress led to significantly stunted growth and, as anticipated, shoot and root fresh and dry weight and leaf area declined regardless of AM status (Fig.1a-c). AMF inoculated plants exhibited significantly increased shoot fresh weights compared to their corresponding controls (Fig 1a). The effect remained also significant on the dry weight level, but only in the case of the non-stressed plants (Fig 1b). On the contrary AMF inoculation did not induce changes on total shoot length, and root fresh and dry weight (data not presented). Furthermore, we recorded a significantly higher leaf area value on salt-affected plants inoculated with AMF compared to their non-inoculated counterpart (Fig. 1c). The inoculation effect was more pronounced in non-stressed plants as a doubling of leaf area was also evident. Net CO₂ assimilation rate (PN) was significantly affected by the inoculation treatment, as a consistent trend for higher rates was noted in all inoculation treatments compared with their corresponding controls (PAMF < 0.05). The rest of the leaf gas exchange parameters were not significantly affected by the experiment treatments. Furthermore, leaf relative water content was significantly affected by both inoculation and salinity treatments. While salinity conditions reduced dramatically the RWC, AMF inoculation had the opposite effect, increasing the RWC regardless of salinity level (Fig 1d).

Due to salinity stress conditions, nonmycorrhizal plants demonstrated poor nutrient acquisition that resulted in lower content in the leaves of the tomato seedlings for most elements. However, not only was leaf P concentration higher in mycorrhizal compared to nonmycorrhizal plants under both normal and high salinity regimes, but, notably, inoculated salt-stressed plants registered P levels similar to non-inoculated non-stressed plants (Fig 2a). Similarly, despite their noticeable decrease in non-inoculated salt stressed plants, leaf Mg and K concentration was enhanced due to inoculation under all salinity levels (data not presented). Salinity conditions reduced Ca leaf concentration in both mycorrhizal and nonmycorrhizal plants significantly, while they increased leaf Na concentration in all treatments (data not presented). As a result, the high salinity regime led to highly decreased leaf Mg:Na and K:Na ratios in the tomato leaves (Fig 2b-c). Inoculated salt-stressed plants maintained an increased leaf K:Na and Mg:Na ratio (apparently due to the improved leaf Mg and K concentration) compared to their non-inoculated counterparts (Fig 2b-c). However they did not reach the leaf K:Na and Mg:Na ratios of the non-stressed plants. Concerning the micronutrients (Fe, Zn, Mn, Cu) no significant differences were marked in all experiment treatments (data not presented)

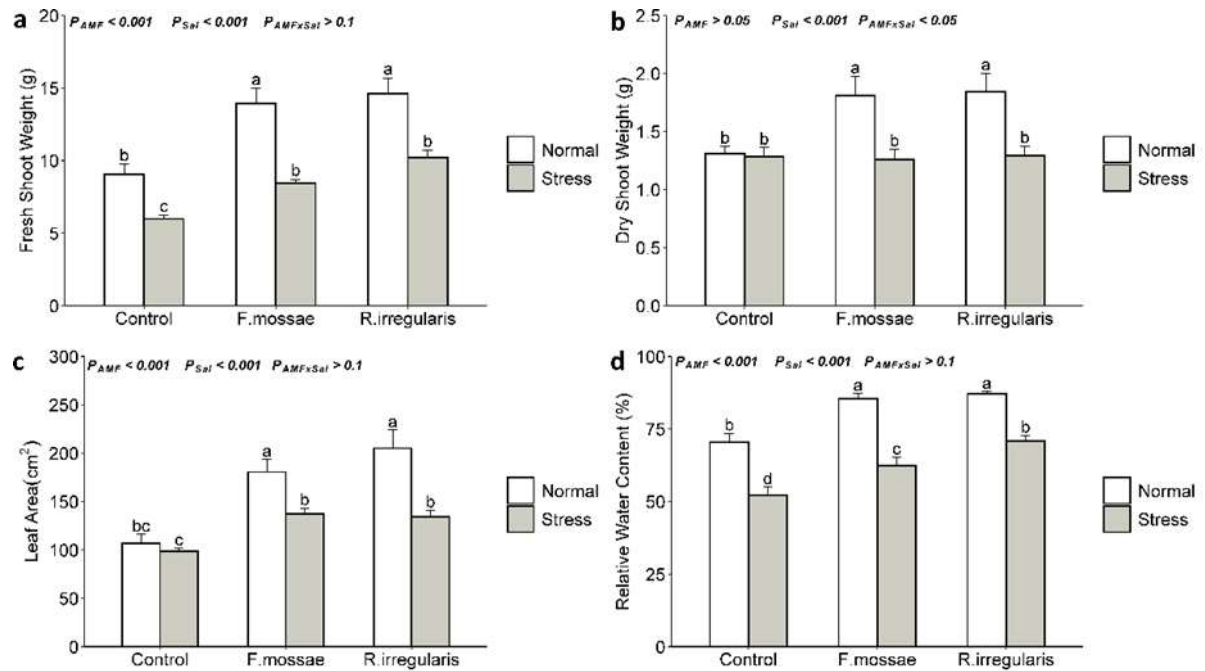


Fig. 1. The effects of salinity stress and inoculation with two AMF strains on (a) Root length Colonization, (b) Leaf Area, (c) Relative Water Content and (d) Leaf P concentration. Lack of shared letters between columns indicate significant differences at $\alpha < 0.05$.

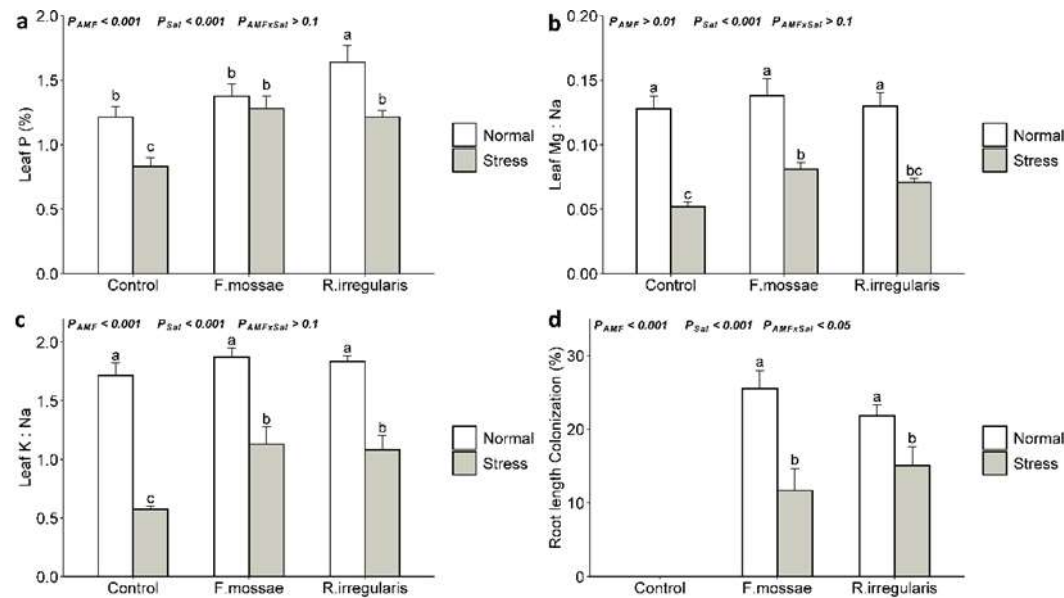


Fig. 2. The effects of salinity stress and inoculation with two AMF species on (a) leaf P, (b) leaf Mg:Na, (c) leaf K:Na and (d) root length colonization. Lack of shared letters between columns indicate significant differences at $\alpha < 0.05$.

Microscope examination confirmed the presence of arbuscules, vesicles and fungal hyphae in all inoculated plants. Higher AMF root colonization was observed in plants grown under control conditions (17-32%) compare to plants grown under saline conditions (4-23%) (Fig 2d). No mycorrhizal structures were observed in the roots of non-inoculated plants.

Discussion

In this study, we observed that the colonization of tomato roots by two strains of AM fungi can alleviate to a great extent growth impairment induced by salinity, via combined effects leading to improved nutritional, physical and cellular status. Regardless of the salinity conditions, above-ground fresh weight increased significantly in all AMF inoculated plants compared with the non-inoculated controls, a result that appears directly linked to the better hydration of the colonized plants tissues. Improved water relations were clearly confirmed by the enhancement of Relative Water Content in the presence of mycorrhizal colonizers in both non-stressed and salt-stressed plants. Increased values of CO₂ assimilation rates under mycorrhizal colonization appear to be related to this. Colonization confers a metabolic response and a relevant metabolic cost to the plant host, as mycorrhizal roots have an increased sink strength due to the presence of the fungus and the higher metabolic activity of arbusculated cells, leading to an increased removal of carbon from shoots altering stomatal behavior and enabling increased photosynthetic activity [16]. The effects of the enhanced photosynthetic rates led to higher above-ground biomass of mycorrhizal non-stressed plants. Mycorrhization also promoted leaf area size regardless of the salinity condition, maintaining the leaf area of water limited inoculated plants to levels similar or close to those in non-stressed non-inoculated plants. Salinity hinders plant growth by increasing osmotic pressure in the soil solution, induces excess availability of Na⁺ and Cl⁻ ions, decreases water potential, and cause nutrient deficiencies or uptake imbalances. Apparently, salinity partly impaired the development of plant root colonization by both fungi. However, the roots of salt treated tomato seedlings were still colonized albeit, at a lower extent. Tomato seedlings grown under high salinity may have a lower affinity for H₂PO₄ (the preferred phosphate ion for plant uptake) [17], leading to salt-induced P deficiency in plants. This, together with root growth and nutrient uptake impediment due to osmotic stress, results in stunted growth of the plant and the older leaves die prematurely [10]. According to our results, AMF can significantly improve P acquisition, critical for improved growth and development of the host plant, while alleviating the detrimental effects of salinity conditions on plant water status. Moreover, mycorrhizal plants have unfailingly shown improved Mg and K nutrition compared to their nonmycorrhizal counterparts under salt stress conditions, eventually maintaining closer to optimal Mg:Na and K:Na ratios that aid them to resist the deleterious effects of salinity [18]. Interestingly, we noticed that among the plants grown under salinity, all inoculated plants began to flower prematurely; however further investigation is needed to elucidate if this effect can have an impact on plant fitness and yield.

Conclusions

Our study revealed that inoculation with arbuscular mycorrhiza fungi can result in improved growth characteristics and nutrient assimilation of tomato plants, especially under salinity stress conditions, apparently engaging various mechanisms to counteract salinity stress. From an agronomic point of view, the use of AMF could provide many benefits in the context of sustainable agriculture; however, their utilization as biofertilizers under field conditions is yet to be fully exploited.

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