Albanian j. agric. sci. 2015;14 (1): 25-30 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

The Effects of Endogenous Mycorrhiza (*Glomus spp*.) on Stand Establishment Rate and Yield of Open Field Tomato Crop

ANGJELINA VUKSANI[1], GLENDA SALLAKU[1], ASTRIT BALLIU[1]*

[1]Department of Horticulture and Landscape Architecture, Agricultural University of Tirana, Tirana, Albania.

Abstract

Graded seeds of a tomato commercial cultivar (Suta F_1) were sown in plugged (30 cm^3) foam trays filled with vermiculate. For the half of seeds the substrate was homogenously mixed (10% vol/vol) with broken expanded clay biologically activated by immobilized propagules of naturally occurring AM-fungi (*Glomus intradadices, Glomus etunicatum, Glomus mosseae, Glomus geosporum, Glomus clarum*), supplied by BioSym B.V. For the next half, it was homogenously mixed with free clay particles of the same size with the previous one (10% vol/vol). Thirty days after sowing, tomato seedlings were transplanted in to 200 cm^3 plastic pots filled with vermiculite. Either AMF non pre inoculated seedlings (non AMF), or AMF pre inoculated (AMF), were equally divided in three subgroups based on the level of salinity applied in the nutrient solution (0, 50 and 100 mM sodium chlorate). On the day of transplanting and 7 days after transplanting, 10 plants were randomly selected for each experimental plot. For each of them, the dry weight of root (RW), leaf (LA), steam (SW) and the whole plant (W) was determined and based on that, the relative growth rates (RGR) and root relative growth rate (RRGR) were calculated and used as the parameters assessing the stand establishment rate of tomato seedlings after transplanting. Additionally, stomata conductance and leaf temperature were measured to assess AMF effects on physiological performance of transplanted seedlings. In order to assess the effect of AM fungi pre inoculation on total yield, parallel with pot transplanting, a field experiment was conducted according to a three replication randomized block design. It was proved that the production of open field tomato can be enhanced by the pre inoculation of AM fungi to tomato transplants at the nursery stage. Due to the pre inoculation of AM fungi, the tomato seedlings can subsequently benefit a faster stand establishment rate and higher vigor which is further reflected to a prolonged harvesting period and higher yield.

Keywords: AMF, transplanting, growth rate, cumulative yield.

1. Introduction

Salinity, drought, extreme temperatures and other abiotic stress conditions can greatly reduce plant stand establishment, immediately after transplanting, thus reducing yield in high value vegetable cropping systems.

In addition to stress-adaptative mechanisms developed by plants, arbuscular mycorrhizal fungi have been shown to improve plant tolerance to abiotic environmental factors. Though arbuscular mycorrhizal fungi (AMF) can be themselves negatively affected by salinity or other abiotic stress factors [7], many reports show improved growth and performance of mycorrhizal plants under abiotic stress conditions [13]. The symbiosis of plants with AM fungi often results in increased nutrient uptake, accumulation of osmoregulator compounds, and increase in photosynthetic rate and water use efficiency [13].

In practice, it should be kept in mind that mycorrhizal plants may profit from other benefits than improved nutrition, for example increased drought tolerance or disease resistance [12]. Depending on the nutritional context AM can result in strong promotion of plant growth or in a reduction in qualitative and/or quantitative traits. A plausible explanation for neutral or negative growth effects could be that AM confer a benefit other than growth promotion, e.g. a qualitative benefit, or that the benefit is not evident under the respective experimental conditions [12]. Depending on the nutritional context AM can result in strong promotion of plant growth or in a reduction and thus

*Corresponding author: Astrit Balliu; E-mail: aballiu@ubt.edu.al
(Accepted for publication March 25, 2015)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

the outcome of AM symbiosis is highly context-dependent [12].

Modern agricultural practice has in many instances resulted in progressively reduced AM fungal diversity and frequency, an effect that is believed to be related to tillage methods and to the use of mineral fertilizers. There are reports shown that in a given AM system, nutrient supply has a strong influence not only on plant growth and nutrient content, but also on AM colonization. In order to minimize this risk, the external application of mycorrhiza in growing plant has been practiced by adding mycorrhizal fungi inoculum to planting hole at time of transplanting. The difficulty in producing a large amount of inoculum of AM fungi for agricultural practices might be a less problem in horticultural crops, where inoculation can take place in seedlings or cutting beds, over a relatively small surface area (Al-Karaki). Due to that, two main benefits are expected: superior stronger growth in the nursery and improved performance after planting in field [6]. Depending on the nutritional context AM can result in strong promotion of plant growth or in a reduction.

It was reported before that the addition of a mixture of Glomus species has significantly improved the stand establishment rate of grafted cucumber seedlings and its subsequent yield under protected cultivation conditions [3, 4]. The question was whether a similar effect could be received for other vegetable crops, under open field growing system. Therefore, the objective of the present study was to assess the symbiotic efficiency of a mixture of pre inoculated AM fungi on the stand establishment rate and yield of tomato transplants on common commercial open field conditions.

2. Materials and Methods

The experiment was conducted during the year 2013, in a plastic greenhouse of a commercial nursery, located in Tirana, Albania. Graded seeds of a tomato commercial cultivar (Suta F_1) were sown in plugged (30 cm^3) foam trays filled with vermiculate. For the half of seeds the substrate was homogenously mixed (10% vol/vol) with broken expanded clay biologically activated by immobilized propagules of naturally occurring AM-fungi (*Glomus intradadices*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus geosporum*, *Glomus clarum*), supplied by BioSym B.V. For the next half, it was homogenously mixed with free clay particles of the same size with the previous one (10% vol/vol). Each case, before sowing the mixed substrate was saturated with a nutrient solution contains 1 g L^{-1} Terraflex T (18, 7, 25 +TE). Following, only tap water was periodically added to adequately fill up plant's water demand

Thirty days after sowing, tomato seedlings were transplanted in to 200 cm^3 plastic pots filled with vermiculite. The substrate was saturated with the same nutrient solution (1 gr L^{-1} Terraflex T), where different amounts of sodium chlorate were added. Following that, either AMF non pre inoculated seedlings (non AMF), or AMF pre inoculated (AMF), were equally divided in three subgroups based on the level of salinity applied in the nutrient solution (0, 50 and 100 mM sodium chlorate). During the next 7 days, equal amounts of tap water were added when necessary, taking care not to drain it out of pots.

On the transplanting day, as well as 7 days after transplanting, 10 plants were randomly selected for each experimental plot. After the harvest, the roots were carefully washed out from the substrate by using a soft water jet and 2 mm sieves and afterwards, all parts of harvested plants were dried at 70°C for 24 h to determine the dry weight of root (RW), leaf (LA), steam (SW) and the whole plant (W). Based on that data the relative growth rates (RGR) was computed according to methods described by [9 and 10]. The root relative growth rate (RRGR) of each experimental plot was also calculated as the slope of linear regression of ln root dry mass on time. Both, RGR and RRGR were used as the parameters assessing the stand establishment rate of seedlings after transplanting. Additionally, two successive measurements of stomata conductance and leaf temperature were conducted in the course of pot transplanting by a SC-1 portable porometer (Degagan Devices), to assess AMF effects on physiological performance of transplanted seedlings.

In order to estimate the effects of mycorrhiza applications on tomato yield, parallel with pot transplanting, a field experiment was conducted in Vora village (41°23′38″N 19°39′16″E) near to Tirana, according to a three replication randomized block design. Twenty randomly selected seedlings were transplanted for each replication. The planting density was settled at 2.8 plants per square meter (0.9 m x 0.4 m), with two rows for each replication and a total of 7.2 square meters for each experimental unit. The common commercial growing practices were applied during the whole growing period.

Plant height (cm), number of newly formed leaves per plant were successively recorded during the whole growth period, every 10 day to 10 fixed plants for each experimental unit, and the harvested yield at full fruit ripening stage was also recorded per each replication. One way, or two way statistical analyses of variance, was conducted by BIOMstat 4.10 for different measured parameter to test the differences between non AMF and AMF seedlings. The least significant differences test (LSD) at 5% significance level was performed to test the differences between different treatments.

3. Results and Discussions

As it was expected, the raise of salinity in pot substrate has significantly reduced the overall plant dry matter, and slowed down the stand establishment rate of transplanted plants. All growth parameters, W, RRGR and RGR were significantly reduced due to raised salinity (Table 1). Anyway, it is largely reported the mycorrhizal plants grew better than non mycorrhizal plants [11, 13].

We found a trend of higher dry matter production (W) of AMF tomato transplants in 7 initial days after transplanting, but yet, not statistically significant (Table 1), and similar results were reported by other authors [2] for tomato seedlings. At the same time, the pre inoculation of mychorrhiza fungus in the growing substrate has slightly improved the relative growth rate (RGR) of transplanted seedlings. The same trend was observed regarding root relative growth rate (RRGR), but again, the differences found due to AMF pre inoculation were not statistically significant. That might be a consequence of very short time of growth we analyzed (7 initial days after transplanting). Still, considering the significant differences exist between AMF and non AMF plants regarding the respective relative growth rates (Table 1), we assume that in a longer time span the differences in plant dry matter will become significant.

The increased salinity has also sharply decreased the stomata conductance of pot transplanted seedlings, while a steady and significant increase was recorded for leaf temperature. Again, AM fungi have significantly improved the physiological performance of transplanted seedlings. Obviously, there was a clear advantage of AMF group regarding estimated physiological parameters (stomata conductance and leaf temperature) under non saline conditions. The AMF plants kept the advantage over increased salinity levels for both, stomata conductance and leaf temperature, even having similar values with the non infested plants under non saline conditions (Table 1).

Since as it is explained above, AMF transplants showed a significantly higher RGR, versus non AM plants, the pre inoculation of AM fungi will especially contribute to a faster stand establishment rate (RRGR) of transplanted seedlings. The effects were more visible under saline conditions, and seem to have contributed to the significant differences between non AMF and AMF plants. Anyway, the primary purpose of nursery inoculation is not to promote plant growth at this stage of production, but to establish AM fungi on plant roots so that mycorrhizae will be efficiently transformed to the field [2]. The pre inoculation of transplants prior to transplanting could bypasses the potential inhibitory effects different abiotic stresses could have on AM fungal spore germination. The pre infested plants can have also other benefits from mycorrhizae colonization [14] such as increased gas exchange (increased photosynthetic rate, transpiration, stomata conductance and water use efficiency).

Therefore, after field transplanting, some positive effects of mychorrhiza pre inoculation were expected to be demonstrated on the growth parameters and harvested yield. Indeed, we found that the influence of AM fungi after field transplanting was highly positive, promoting a more vigorous growth of plants and providing a higher yield. The AMF pre infested plants have recorded a significantly higher stem length (137.1 cm) versus non AMF plants (123.5 cm). Since the length of internodes between non AMF and AMF plants were not statistically different (Table 2), the higher stem length of AMF plants was achieved due to significantly higher internode (leaves) numbers. Such as, the higher leaf number in AMF plants (22 versus 19 of non AMF plants) is an indication of their higher growth rate.

Table 1. Dry matter (W), relative growth rate (RGR), root relative growth rate (RRGR), stomata conductance, and leaf temperature of transplanted seedlings according to AM fungi presence, salinity and combinations of AM application with salinity. Different letters within the same column indicate significant difference at P<0.05.

Factors		W	RGR	RRGR	Stomata conductance	Leaf temperature
Mychorrhiza	Free (F)	0.62	0.126	0.133	192 b	31.2 a
	Infested (I)	0.65	0.133	0.136	280 a	29.1 b
Salinity	0 mM	0.76 a	0.155 a	0.150 a	375 a	29.1 c
	50 mM	0.62 b	0.126 ab	0.141 ab	182 b	30.3 b
	100 mM	0.52 b	0.107 b	0.112 b	151 b	31.0 a
Interaction mychorrhiza x salinity	F x 0 mM	0.76 a	0.148 a	0.154a	303 b	30.9 a
	F x 50 mM	0.60 ab	0.116 b	0.130ab	134 c	31.1 a
	F x 100 mM	0.50 bc	0.090 c	0.114b	139 c	31.5 a
	I x 0 mM	0.76 a	0.160 a	0.147a	447 a	27.3 c
	I x 50 mM	0.65 ab	0.135 ab	0.152a	230 bc	29.4 b
	I x 100 mM	0.54 bc	0.111 b	0.109b	162 c	30.5 a

As AM fungi develop a dense hyphal network that extends far into the soil, this extra radical mycelium provides the plant with water and nutrients that would otherwise remain inaccessible to roots [1, 5, 8]. Thus, higher yields are expected by AMF plants, especially under adverse soil conditions. Also, we found that total harvested yield by AMF plants was significantly higher compared to non AMF plants (Table 2). Similar results were reported by other authors [3] comparing non AMF and AMF cucumber plants grown in plastic greenhouses.

The cumulative yields of both, non AMF and AMF plants were best fitted by polynomial equations. As it clearly can be seen by figure 2, though started at an almost identical point, the cumulative yield of AMF plants has gradually become significantly higher compared to non AMF plants. That because, as it can be judged by the respective trend lines, the curvature component of the respective equation for AMF plants was much smaller versus non AMF plants (0.004 versus 0.006). This is the indication of a longer productive life of AMF plants.

Therefore, we assume that AM fungi have contributed to higher yield of AMF plants by maintaining their vigor alongside with fruit ripening stage when plants abilities are (especially under adverse growing conditions) often deteriorated.

Table 2. Stem height, leaf number, internode length and total yield of open field transplanted tomato seedlings according to AM fungi pre inoculation.

Source of variation	**Stem height (cm)**	**Leaf number (no.)**	**Internode length (cm)**	**Yield (kg/variant)**
Non AMF plants	123.5	19	6.14	13.9
AMF plants	137.1	22	6.44	17.4
Pr > F	0.0025	0.0003	0.0674	0.0245

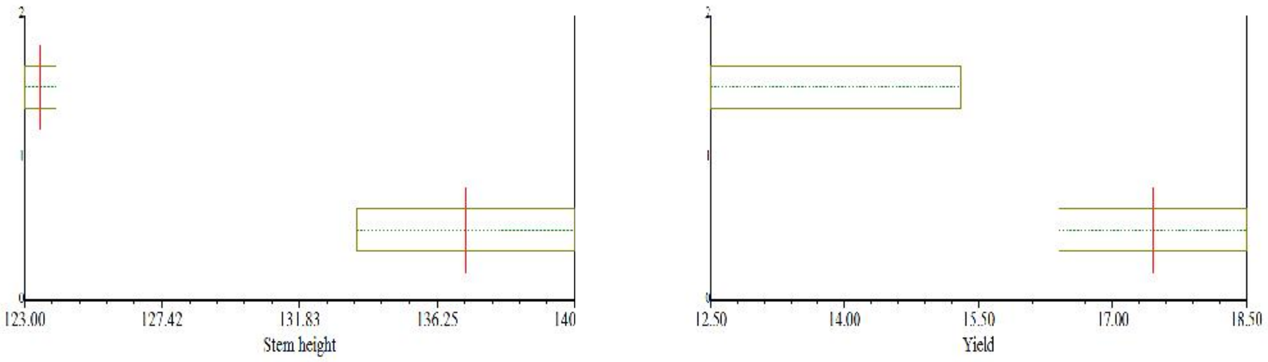

Figure 1. Mean values of stem height (right) and yield (left) of non AMF (first bar in respective graphs) and AMF (second bars in the respective bars) tomato plants. Lack of overlapping between respective bars indicate significant difference at P<0.05.

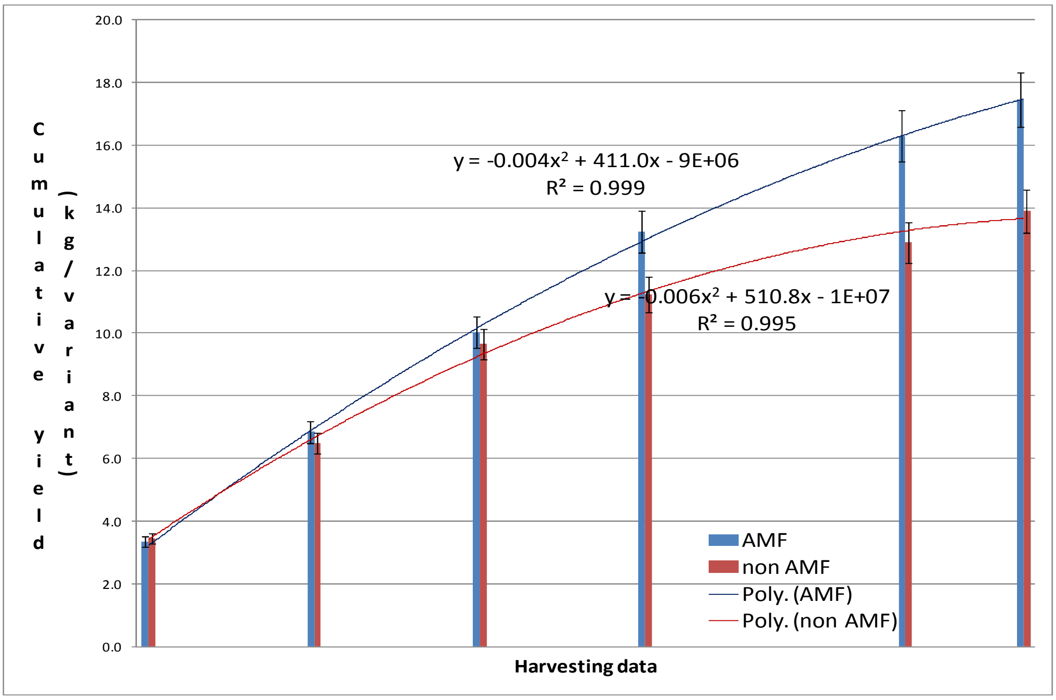

Figure 2. The cumulative yield of non AMF and AMF pre inoculated plants, and the respective polynomial equations of cumulative yield versus harvesting period.

4. Conclusions

The production of open field tomato can be enhanced by the pre inoculation of AM fungi to tomato transplants at the nursery stage. Due to the pre inoculation of AM fungi, the tomato seedlings can subsequently benefit a faster stand establishment rate and higher vigor which is further reflected to a prolonged harvesting period and higher yield.

5. References

1. Aggarwal A, Kadian N, Neetu K, Tanwar A, Gupta KK: **Arbuscular mycorrhizal symbiosis and alleviation of salinity stress.** *J. Appl. & Nat. Sci* 2012, **4** (1):144-155.
2. Al-Karaki GN: **Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water** 2006, *Sci. Hort*:**109**:1–7

3. Babaj I, Sallaku G, Krasniqi P, Kaciu S, Goranson H, Rewald B, Balliu A: **The effect of grafting methods and endogeneous mycorrhiza application on chemical composition and stand establishment rate of grafted cucumber (cucumis sativim L) seedlings.** 1st Annual Conference of COST Action 1204-ROOTOPOWER Workshop. Murcia/Spain 2013, pg. 48

4. Babaj I, Sallaku G, Balliu A: **The effects of endogenous mycorrhiza (Glomus spp.) on plant growth and yield of grafted cucumber (Cucumis sativum L.) under common commercial greenhouse conditions**. *Albanian J. Agric. Sc*. 2014, **13**(2):24-28

5. Balzergue C, Chabaud M, Barker D.G, Bécard G, Rochange SF: **High phosphate reduces host ability to develop arbuscular mycorrhizal symbiosis without affecting root calcium spiking responses to the fungus.** *Frontiers in Plant Science* 2013, Volume **4**. Article 426

6. Giananazzi S, Schuepp H, Barea JM, Haselwandter K: **Mycorrhizal Technology in Agriculture: From Genes to Bioproducts.** Birkhauser. Basel, Switzerland. 2001.

7. Jahromi F, Aroca R, Porcel R, Ruiz-Lozano J. M: **Influence of Salinity on the In Vitro Development of Glomus intraradices and on the In Vivo Physiological and Molecular Responses of Mycorrhizal Lettuce Plants**. *Microbial Ecology* 2008, **55:**45-53.

8. Jansa J, Bukovská P, Gryndler M: **Mycorrhizal hyphae as ecological niche for highly specialized hypersymbionts–or just soil free-riders?** *Frontiers in Plant Science.* 2013.Volume **4**. Article 134

9. Hoffmann A.B, Poorter H: **Avoiding Bias in calculations of relative growth rate**. *Annals of Botany* 2002, **80**:37-42.

10. Hunt R, Causton DR, Shipley B, Askew AP: **A modern tool for classical plant growth analysis**. *Annals of Botany* **2002**, **90**:485-488.

11. Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL, Cullu MA: **The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity**. *Sci. Hort.* 2009, **121**:1–6.

12. Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D: **Phosphorus and Nitrogen Regulate Arbuscular Mycorrhizal Symbiosis in Petunia hybrida.** *PLoS ONE* 2014, 9(3):e90841. doi:10.1371/journal.pone.0090841

13. Porcel R, Aroca R, Ruiz-Lozano JM: **Salinity stress alleviation using arbuscular mycorrhizal fungi. A review.** *Agron. Sustain. Dev*. 2012, **32**:181–200

14. Ruiz-Lozano JM, Azcón R: **Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal Glomus sp. from saline soils and Glomus deserticola under salinity**. *Mycorrhiza* 2000, **10**:137–143.