

Production and Nutritional Quality of Tomatoes (*Solanum lycopersicum* var. Cerasiforme) Are Improved in the Presence of Biochar and Inoculation with Arbuscular Mycorrhizae

Wilson Castañeda, Marcia Toro*, Andi Solorzano, Doris Zúñiga-Dávila

Laboratorio de Ecología Microbiana y Biotecnología, Departamento de Biología, Facultad de Ciencias, Universidad Nacional Agraria La Molina, Lima, Peru

Email: *mtoro@lamolina.edu.pe, marcia.toro@ciens.ucv.ve

How to cite this paper: Castañeda, W., Toro, M., Solorzano, A. and Zúñiga-Dávila, D. (2020) Production and Nutritional Quality of Tomatoes (*Solanum lycopersicum* var. Cerasiforme) Are Improved in the Presence of Biochar and Inoculation with Arbuscular Mycorrhizae. *American Journal of Plant Sciences*, 11, 426-436.

<https://doi.org/10.4236/ajps.2020.113031>

Received: January 11, 2020

Accepted: March 23, 2020

Published: March 26, 2020

Copyright © 2020 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Tomato is a fruit of great nutritional interest in the basic human diet. The increasing use of agrochemicals to maintain production requires new alternatives to reduce environmental impact. Arbuscular mycorrhizae (AM) are beneficial microorganisms that favor the growth of plants improving their nutrition and development, protecting the plant from biotic and abiotic stresses and favoring the production of bioactive compounds that increase their nutritional value. The use of biochar as soil conditioner is also considered an environmentally friendly resource. A greenhouse experiment was carried out to observe the effect of the use of biochar and AM inoculation on the quality of fruits, yield and polyphenols production of Cherry tomato, *Solanum lycopersicum* var. Cerasiforme. A mixture of rice husk biochar with sterile sand and two inoculums of Glomeromycota native fungi: from a wetland (GWI) and a fallow field (GFI) were used. Control treatments consisted of inoculation with both GWI and GFI in sterile sand. All treatments were irrigated with 50% La Molina® hydroponic solution. After 12 weeks plants were harvested to quantify weight, number and diameters of the fruits, and yield, total polyphenols in the fruit pulp were quantified. In the presence of biochar and the two inoculums, GFI and GWI, fruit production was favored throughout the experiment. The height of the plants was significantly greater in the presence of biochar. Plants grown in biochar and inoculated with GFI had a yield of 8.2 MT/Ha, increasing in 50% this value respect to control with biochar (5.33 MT/Ha). This treatment doubled the number of fruits (59.5)

with respect to the control (32.5). Root colonization by GFI was not affected by the presence of biochar. It is concluded that the combined use of rice husk biochar and Glomeromycota fungal inoculation is recommended for increasing of Cherry tomato yield and improving fruit quality through the production of bioactive compounds.

Keywords

Glomeromycota Fungi, Yield, Polyphenols

1. Introduction

Tomato is a vegetable of nutritional interest and high consumption in the daily diet. In Peru, about 13,796 tons were produced in 2018, in an area of 6000 ha (2.29 ton/ha), a production that was mostly intended for internal consumption [1]. The need to maintain and increase production has led farmers to use increasingly high doses of chemical fertilizers with more frequent applications. The recurrent use of these products must be controlled with alternatives that reduce their impact on the soil, crops and environmental effects. Beneficial microorganisms constitute an option, including growth promoting bacteria, of which there are successful applications in Peru, favoring yield thanks to their ability to promote the plant development [2]. Arbuscular mycorrhizae (AM) are also considered as one of the organisms of importance in agriculture due to their ability to promote nutrition and plant growth by capturing nutrients, mainly P, thanks to the mycelium of Glomeromycota fungi distributed externally to the roots of the plants they colonize symbiotically [3]. Arbuscular mycorrhizae have also proven to be effective in favoring the tolerance to biotic and abiotic stresses of plants and soil structure; they also behave as agents which improve the quality of fruits and/or foods, encouraging the production of bioactive compounds [4] with antioxidant capacities able to enhance their nutritional value [5].

In the agriculture scenario, the ecological impact of fertilization and constant sowing on the soil, degrading it and reducing its fertility must be mitigated. The use of biochar, a carbon-rich substrate produced by pyrolysis [6] from plant biomass used as amendment to induce changes on soil properties, fertility and micro-organisms abundance and/or functionality, including mycorrhizal fungi [7], has become an alternative for sustainable agricultural management. When applied to the soil, it resists decomposition, effectively sequestering the applied carbon and mitigating anthropogenic CO₂ emissions [8], so it is highly recommended as a soil restoring agent [9] [10]. Likewise, several authors refer to the suitability of biochar as support for biofertilizers [11], being this characteristic an added value to the present experimentation. In order to study tomatoes cultivation alternatives promoting an increase in its yield through an environmentally friendly management, an experiment was carried out to test production by

evaluating biochar as part of the substrate together with arbuscular mycorrhizae, its effect on tomatoes yield and on the quality of bioactive tomatoes compounds.

2. Materials and Methods

Substrate description. A biochar derived from rice husk was used, pyrolyzed at 300°C in a retort oven. Its compositional characteristics are the following: strongly alkaline (pH = 8.87), slightly saline (EC = 2.47 dS/m), 62.31% of pyrolytic carbon, N = 0.71%, P₂O₅ = 0.44%, K₂O = 1.62%, CaO = 0.72%, MgO = 0.30%, Na = 0.04%, Fe = 210 ppm, Cu = 4 ppm, Zn = 48 ppm, Mn = 420 ppm, B = 75 ppm, Pb = 3.10 ppm, Cd = 1.95 ppm, Cr = 14.05 ppm, medium humidity retention capacity (65.66%) and moderate CEC (11.20 meq/100 g).

Biochar particles were sieved and those retained between 500 and 2000 µm sieves were selected. 4 kg pots containing a mixture of rice husk biochar and sand in a 1:3 ratio were used. The mixture was sterilized with fluent steam (autoclave) for 1 hour every 24 hours for three days [12].

Greenhouse Experiment

The experiment was installed in the greenhouse of the Laboratory of Microbial Ecology and Biotechnology at La Molina National Agrarian University. Experimental conditions were: 12 h photoperiod, maximum and minimum temperature (32°C and 17°C) and 89% of relative humidity. The experiment was carried out between January and April 2019, lasted 12 weeks.

Biological material. Cherry tomato seeds, *Solanum lycopersicum* var. Cerasiform, were disinfected with 3% sodium hypochlorite (15 minutes) and then rinsed with sterile water. Seeds were placed to germinate in sterile peat trays, watered with sterile water. After 15 days, 2 seedlings with the first pair of leaves were placed in 4 kg pots. The plants were irrigated daily at field capacity, with 50% La Molina® hydroponic solution. The composition of the nutrient solution is as follows (ppm): 190 N, 35 P, 210 pm K, 150 Ca, 45 Mg, 70 S, 1.00 Fe, 0.50 Mn, 0.50 B, 0.15 Zn, 0.10 Cu, 0.05 Mo.

Glomeromycota fungus inoculum. Two inoculums of native Glomeromycota fungi from Peru were used, one from a fallow with *Brachiaria decumbens*, in Pucallpa, Ucayali (Glomeromycota Fallow Inocula-GFI) and another from a wetland with predominance of *Sporobolus* sp. located in Pisco, Ica (Glomeromycota Wetland Inocula-GWI). Both were obtained from samples of rhizospheric soil of the mentioned plants and multiplied in trap pots with *B. decumbens* in sterile sand, irrigated with Long Ashton solution every 15 days [13] In each case, the spores were multiplied in two cycles of 4 months each; rhizospheric samples were taken from the second multiplication, Glomeromycota spores [14] and % root AM colonization [15] were quantified. A similar number of spores of GFI and GWI inoculums were procured to inoculate in each mycorrhized treatment of the experiment. GFI inoculum was formed by the native species mainly by the fungus *R. intraradices* and the genera *Acaulospora*, *Gi-*

gaspora and Archaeospora, of which 3726 spores and rootlets (70% colonization) were added per pot in the base of tomato seedlings. GWI inoculum consisted of *R. intraradices* fungus, of which 3400 spores and rootlets (90% colonization) per pot were applied in the base of transplanted tomato seedlings.

Growth parameters. Height of plants was measured in week 6; chlorophyll content (triplicate) in week 11 with a Konica Minolta brand model SPAD-502 Plus (SPAD units) on three different leaves per plant. In week 12 plants were harvested, shoots were cut and fruits separated. Parameters evaluated for the fruit were: weight of fruits per pot (g/pot) and per treatment. Polar and equatorial diameters were measured and the individual weight of each fruit was determined. To estimate yield in MT/Ha, a planting density of 20,000 plants/Ha, distance of 1 m between rows and 0.5 m between plants were considered.

Quantification of arbuscular mycorrhizae root colonization. From the rhizospheric soil of each mycorrhized plant (treatments T2, T3, T5 and T6) 2 cores (1 inch diameter) were extracted, mixed and a subsample of roots was extracted to clear and stain with trypan blue [16] and quantify mycorrhizal colonization [15]. These determinations were performed in quadruplicate in each treatment.

Determination of polyphenols in the fruit. The methodology according to [17] was applied with modifications for preparing the sample. Collected fruits were washed with distilled water to remove dirt and impurities, and disinfected with alcohol. The fruits of all repetitions per treatment were taken, mixed and 4 ripe fruits without mechanical damage were chosen and ground in mortar with liquid N. The compound obtained was filtered and 25 mL of this liquid was mixed with 25 mL of methanol (analytical grade). The mixture was centrifuged at 350 rpm for 10 minutes. The supernatant was separated in a graduated tube and the volume adjusted to 10 mL with cold methanol. 500 μ L of the test sample and 250 μ L of the Folin-Ciocalteu reagent were taken; 1.25 mL of 7.5% sodium carbonate w/v were added, the mixture was stirred and left for one hour in darkness. After one hour the absorbance was read on a Thermo spectrophotometer, Model Genesis 6 at 755 nm. The value of total polyphenols is expressed as equivalents of Gallic Acid/ 100 g fresh weight (mg GAE/100 g FW).

Brix grade determination. 1 drop was taken from the filtered juice of fruits obtained above and read on a PAL-1 refractometer (ATAGO), the results are expressed in % soluble solids or °Brix.

Statistical analysis. For all measured variables, the analysis of variance was performed with a 95% of confidence using the F test to determine the difference between treatments. Duncan test was applied for means comparisons between treatments, using the statistical software SPSS version 24.

3. Results and Discussion

Fruit production. The formation of fruits from week 5 to week 12 (Table 1) was evaluated, observing an increase in fruits over time. Treatments with biochar, GFI and GWI inoculums showed the greatest significant number of fruits

Table 1. Number of fruits per tomato plant in treatments grown in sand or sand/biochar mixture inoculated with two inoculums of Glomeromycota fungi native from weeks 5 to 12.

Treatment			5	6	7	8	12
			Weeks				
Without biochar	Without mycorrhiza (T1)		0.50a	11.25a	28.00a	34.50a	29.5a
	With mycorrhiza	With GWI (T2)	1.00ab	13.25a	27.75a	35.75a	41.5ab
		With GFI (T3)	1.00ab	12.50a	25.00a	34.50a	43.25b
With biochar	Without mycorrhiza (T4)		3.25ab	15.25ab	29.00a	34.75a	32.5ab
	With mycorrhiza	With GWI (T5)	4.50ab	21.25b	34.75ab	39.00a	37.7ab
		With GFI (T6)	5.00c	28.50c	44.00b	56.25b	59.5c

Values followed by the same letter do not differ significantly ($p > 0.05$), according to the Duncan mean comparison test. Treatments: T1: sand; T2: sand + GWI; T3: sand + GFI; T4: sand + biochar; T5: sand + biochar + GWI; T6: sand + biochar + GFI.

compared to the control of plants grown only in sand (T1) and with biochar (T4). Additionally, treatment with GWI inoculum also presented a higher number of fruits with significant differences with respect to the control without mycorrhiza (T4).

Plant response. Table 2 shows that plants grown with biochar showed significantly greater height than those grown without it. Biochar is known for its ability to improve growth and production of crops (Rawat *et al.* 2019).

It is generally recognized that biochar benefits plant nutrition [9] and its development [8]. Since biochar is derived from plant biomass, it is high in carbon and may contain a range of nutrients suitable for the plant, which may improve yield as shown in Table 2. Some elements that are still in very low proportions can be used by the plant as for example nitrogen, potassium or iron among others. Also, the high moisture retention capacity of biochar could help reducing water loss from the substrate. Physical microstructure may crucially influence the role of biochar on plant nutrient uptake influencing access to mineralized elements by soil solution, microorganisms, and plant roots [18]. The beneficial use of biochar as soil amendment in terms of increased crop yield and improved soil quality has been reported and agrees with our results.

Such beneficial effects are also reflected in the yield obtained by applying biochar and more sharply together with the mycorrhizal inocula (treatments T5 and T6) (Table 2). Some authors indicate variable effects of biochar on mycorrhizal colonization effectiveness, adverse in cases, positive in others [19] [20]. In our case, GFI inoculum was more effective in mycorrhizal colonization when combined with biochar (T5), doubling AM colonization of tomatoes up to 50%, reflected in a 20% increase in yield compared to the control with only biochar (T4). This effect could be related to nutritional functionality of mycorrhizal symbiosis.

Table 2. Height, yield, mycorrhizal colonization (%) and chlorophyll content of tomato plants *S. lycopersicum* var. Cerasiform, grown in the presence or absence of rice husk biochar and inoculated with two inoculums of native Glomeromycota fungi.

Treatments		Height (cm)	Yield g/pot	Yield (Tm/Ha)	AM root colonization (%)	Chlorophyll (SPAD)
Without biochar	Without mycorrhiza (T1)	66.82a	177.22a	3.54a	-	48.17bc
	With mycorrhiza GWI (T2)	68.10a	242ab	4.84ab	25.25a	47.54abc
	GFI (T3)	68.47a	271.67ab	5.43ab	31.75a	44.95ab
With biochar	Without mycorrhiza (T4)	81.35b	266.5ab	5.33ab	-	44.30a
	With mycorrhiza GWI (T5)	82.32b	320.47bc	6.41bc	50.5b	44.07a
	GFI (T6)	84.10b	410.05c	8.2c	31.25a	49.11c

Values followed by the same letter do not differ significantly ($p > 0.05$), according to the Duncan mean comparison test. Treatments: T1: sand; T2: sand + GWI; T3: sand + GFI; T4: sand + biochar; T5: sand + biochar + GWI; T6: sand + biochar + GFI.

In the case of GWI inoculum, AM colonization was similar with and without biochar, however tomato plants in the presence of biochar inoculated with GFI showed the highest yield recorded in the experiment, 54% over the control (T4). The dissimilarity of the colonizing effectiveness of the inoculums may be due to different species of fungi present in each of them, as [21] observed when using consortium inoculums of several Glomeromycota species. The different ability of fungi to take nutrients in the substrate, the interaction of fungi with biochar, the distinct amount of mycelium produced by the fungi of each inoculum reflected in unequal efficiency of taking P [22], along with the dissimilar affinity shown by Glomeromycota species in each inoculum with tomato plants [23], might have influenced the responses observed in our experiment.

Chlorophyll content. Treatment with biochar and GWI (T6) showed the highest chlorophyll content with respect to all treatments, even higher than the content level shown at its control without mycorrhizae (T4). In general, it has been observed that mycorrhizal inoculation improves chlorophyll content of the plant in crops of edible interest [24] [25] and photosynthetic activity [26]. However, chlorophyll content in mycorrhized plants and their fruits may possibly decrease due to the ripening process that favors the degradation of chlorophyll and accumulation of other pigments in the fruits [27] and leaves, effect that may be appreciated with GFI inoculum (T5). The higher content of chlorophyll in the presence of biochar and GWI (T6) could be related to the higher content of chloroplasts in the leaves of inoculated plants, as [28] found in mycorrhized plants.

Fruit number and quality. Regarding fruit number (Table 1 and Table 3), the inoculation with GFI and biochar stands out, obtaining around 50% more fruits than the respective control without mycorrhizae (T4). Likewise, the application of both inoculums was effective in the absence of biochar (T2 and T3),

Table 3. Diameter, weight, brix grades and total polyphenol content (mg GAE/100 g FW) of the fruits of tomato plants *S. lycopersicum* var. Cerasiform, grown in the presence or absence of rice husk biochar and inoculated with two Glomeromycota fungi inocula.

Treatments			Equatorial diameter (cm)	Polar diameter (cm)	Fresh weight /fruit	Number of fruits	Soluble solids (°Brix)	Total polyphenols (mg GAE/100 g FW)
Without mycorrhiza (T1)			23.48	29.2bc	8.82	29.5a	6.17	28.39d
Without biochar	With mycorrhiza	GWI (T2)	23.32	23.48a	9.06	41.5ab	5.27	23.17c
		GFI (T3)	24.36	31.28c	9.94	43.25b	5.92	24.29c
Without mycorrhiza (T4)			24.64	29.34bc	9.42	32.5ab	5.45	22.99b
With biochar	With mycorrhiza	GWI (T5)	24.36	28.06b	9.64	37.7ab	4.5	20.39a
		GFI (T6)	24	29.06bc	9.24	59.5c	6.02	28.21d

Values followed by the same letter do not differ significantly ($p > 0.05$), according to the Duncan mean comparison test. Treatments: T1: sand; T2: sand + GWI; T3: sand + GFI; T4: sand + biochar; T5: sand + biochar + GWI; T6: sand + biochar + GFI.

with up to 45% more fruit content than the control (T1). In relation to fruit characteristics (Table 3), both polar diameter and dry weight/fruit were greater with inoculation of GFI with and without biochar (T3 and T6), although in the last case, the increase was only in trends because these values were in line with controls without MA or biochar (T4 and T1). Mycorrhizal inoculation with and without biochar favored fresh weight per fruit, all the mentioned treatments were greater than the absolute control (T1), thus suggesting a favorable effect of inoculation and/or biochar in this parameter. Our results coincide with [27] who find better development and dimensions of the fruits of *Capsicum annuum* and with [29] who find similar results in strawberry thanks to mycorrhizal inoculation.

Compositional quality of the fruit. Brix grades and polyphenol content. Brix grades were higher only in trends in the absolute control (T1) and Biochar + GFI (T6) treatment. The latter coincides with the results of [30] (Candido *et al.* 2015) who did not observe differences in Brix grades due to mycorrhizal colonization and non mycorrhized plants, but differs from [31] who did find effect on this parameter caused by AM. In our case, neither inoculation with MA nor biochar had an effect on this characteristic of the fruit.

Similarly, the higher content of total polyphenols of the fruits with inoculation of GFI and biochar is remarkable, suggesting that both factors favor the formation of bioactive compounds such as polyphenols in fruits. Our results coincide with [32] who demonstrated that inoculation with AMF enhances both phenolics and antioxidant activity, suggesting a positive influence of AMF inoculation on yield and on nutraceuticals production. Some mechanisms are suggested to explain the accumulation of secondary metabolism compounds in the plant in

response to mycorrhizal symbiosis as an improvement in the nutritional condition of the host, such as alterations in the activity of key-enzymes, activation of metabolic routes, and increase in gene expression [33], some of which may be happening in our case.

However, this result coincides with the highest value of polyphenols recorded for the absolute control treatment (T1), which may be related to the nutritional stress achieved by the plant grown in nutritional deficit conditions (only with 50% nutrient solution), lacking mycorrhizal inoculation and/or developing in a low nutrient substrate [34] [35], influencing plant response with the highest production of antioxidant compounds [36].

The high values of total polyphenols recorded in this work—in the order of 28 mg GAE/100 g fresh weight—are higher than those registered for other tomato varieties different from the variety used in this work, both with and without biochar. [37] recorded 10 mg GAE/100 mg for various tomato varieties or 12 mg GAE/100 mg [38], which illustrates the effectiveness of the use of biochar and mycorrhizae arbuscular nutrition of Cherry tomato (T6). Our data should be compared with other measurements of polyphenols and bioactive substances in Cherry tomato, at present lacking in scientific literature.

Over the past several decades, many researches have pointed out the direct relation between the intake of bioactive compounds present in tomato and a reduced risk of suffering different types of cancer. These bioactive constituents comprise phytochemicals such as carotenoids and polyphenols. The ability of phenolic compounds as reactive oxygen scavengers is the main motivator of its antioxidant properties [38], which highlights the importance of T6 treatment in stimulating the production of polyphenols and the greatest yield of tomato.

4. Conclusions

According to our results, plant height, chlorophyll, fruit number, yield and polyphenol production were improved in tomatoes when inoculated with Glomeromycota fungi and grown with rice husk biochar. Mycorrhizal symbiosis and biochar elicited changes in the levels of total polyphenols. The significant polyphenol production and yield shown by tomato plants inoculated with GFI and biochar points out not only a greater nutritional and antioxidant value but also a defense role against herbivores and phytopathogens. The relationships between AM fungal inoculations, biochar addition and polyphenol production in tomato and other plants is an understudied area. Our findings can be used to improve this understanding as well as the potential of using biochar and AM.

Acknowledgements

Funded by grants 177-215 and 009-2017-FONDECYT from Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (Perú). The authors appreciate Mrs. Auxi Toro collaboration for her support as linguistic advisor.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] INEI (2018) Peru panorama económico departamental. Informe Técnico No. 5. <https://www.Inei.gob.pe>
- [2] Vergani-Boza, I. and Zúñiga-Davila, D. (2018) Effect of Inoculation and Pelleting on the Germination and Growth of Maca (*Lepidium meyenii* W.) *in Vitro* and Greenhouse. *Revista Peruana de Biología*, **25**, 329-334. <https://doi.org/10.15381/rpb.v25i3.14035>
- [3] Barea, J.M. (2015) Future Challenges and Perspectives for Applying Microbial Biotechnology in Sustainable Agriculture Based on a Better Understanding of Plant-Microbiome Interactions. *Journal of Soil Science and Plant Nutrition*, **15**, 261-282. <https://doi.org/10.4067/S0718-95162015005000021>
- [4] Bona, E., Cantamessa, S., Massa, N., Manasser, P., Marsano, F., Copetta, A., Lingua, G., D'Agostino, G., Gamalero, E. and Berta, G. (2016) Arbuscular Mycorrhizal Fungi and Plant Growth-Promoting Pseudomonads Improve Yield, Quality and Nutritional Value of Tomato: A Field Study. *Mycorrhiza*, **27**, 1-11. <https://doi.org/10.1007/s00572-016-0727-y>
- [5] Avio, L., Turrini, A., Giovannetti, M. and Sbrana, C. (2018) Designing the Ideotype Mycorrhizal Symbionts for the Production of Healthy Food. *Frontiers in Plant Science*, **9**, 1089. <https://doi.org/10.3389/fpls.2018.01089>
- [6] Lehmann, J., Rillig, M., Thies, J., Masiello, C.A., Hockaday, W.C. and Crowley, D. (2011) Biochar Effects on Soil Biota: A Review. *Soil Biology and Biochemistry*, **43**, 1812-1820. <https://doi.org/10.1016/j.soilbio.2011.04.022>
- [7] Kloss, S. (2013) Biochar Application to Temperate Soils: Effects on Soil Fertility and Crop Growth under Greenhouse Conditions. *Journal of Plant Nutrition and Soil Science*, **177**, 3-15. <https://doi.org/10.1002/jpln.201200282>
- [8] Biederman, L.A. and Harpole, W.S. (2012) Biochar and Its Effects on Plant Productivity and Nutrient Cycling: A Meta-Analysis. *GCB Bioenergy*, **5**, 202-214. <https://doi.org/10.1111/gcbb.12037>
- [9] Gunarathne, V., Mayakaduwa, S. and Vithanage, M. (2017) Biochar's Influence as a Soil Amendment for Essential Plant Nutrient Uptake. In: Naeem, *et al.*, Eds., *Essential Plant Nutrients*, Springer International Publishing, New York, 47-67. https://doi.org/10.1007/978-3-319-58841-4_3
- [10] Rawat, J., Saxena, J. and Sanwal, P. (2019) Biochar: A Sustainable Approach for Improving Plant Growth and Soil Properties. <https://doi.org/10.5772/intechopen.82151>
- [11] Egamberdieva, D., Hua, M., Reckling, M., Wirth, S. and Bellingrath-Kimura, S.D. (2018) Potential Effects of Biochar-Based Microbial Inoculants in Agriculture. *Environmental Sustainability*, **1**, 19-24. <https://doi.org/10.1007/s42398-018-0010-6>
- [12] Sieverding, E. (1991) Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Eschborn, GTZ.
- [13] Hepper, C. and O'Shea, J. (1984) Vesicular-Arbuscular Mycorrhizal Infection in Lettuce (*Lactuca sativa*) in Relation to Calcium Supply. *Plant and Soil*, **82**, 61-68. <https://doi.org/10.1007/BF02220770>

- [14] Marques, G., Tampakaki, A. and Alsina I. (2014) Working with Microbial Symbioses of Legumes: Handbook of Protocols. Eurolegume. FP7 Research Project No. 613781.
- [15] Giovannetti, M. and Mosse, B. (1980) An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *New Phytologist*, **84**, 489-500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>
- [16] Phillips, J. and Hayman, D. (1970) Improved Procedures for Clearing Roots and Staining Parasitic and Vesicular-Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. *Transactions of the British Mycological Society*, **55**, 158-161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- [17] Singleton, V.L. and Rossi Jr., J. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, **16**, 144-158.
- [18] Siddiqui, A.R., Nazeer, S., Awais Piracha, M., Masood Saleem, M., Siddiqi, I., Muhammad Shahzad, S. and Sarwar, G. (2016) The Production of Biochar and Its Possible Effects on Soil Properties and Phosphate Solubilizing Bacteria. *Journal of Applied Agriculture and Biotechnology*, **1**, 27-40.
- [19] Blackwell, P., Krull, E., Butler, G., Herbert, A. and Solaiman, Z. (2010) Effect of Banded Biochar on Dryland Wheat Production and Fertiliser Use in South-Western Australia: An Agronomic and Economic Perspective. *Australian Journal of Soil Research*, **48**, 531-545. <https://doi.org/10.1071/SR10014>
- [20] Warnock, D.D., Lehmann, J., Kuyper, T.W. and Rillig, M.C. (2007) Mycorrhizal Responses to Biochar in Soil-Concepts and Mechanisms. *Plant & Soil*, **300**, 9-20. <https://doi.org/10.1007/s11104-007-9391-5>
- [21] Gosling, P., Mead, A., Proctor, M., Hammond, J. and Bending, G. (2013) Contrasting Arbuscular Mycorrhizal Communities Colonizing Different Host Plants Show a Similar Response to a Soil Phosphorus Concentration Gradient. *New Phytologist*, **198**, 546-556. <https://doi.org/10.1111/nph.12169>
- [22] Ibrahim, M. (2018) Response of Seeds Quality of Sunflower to Inoculation with Single and Mixed Species of Indigenous Arbuscular Mycorrhizal Fungi. *The Open Agriculture Journal*, **12**, 123-129. <https://doi.org/10.2174/1874331501812010123>
- [23] Jansa, J., Smith, A. and Smith, S. (2007) Are There Benefits of Simultaneous Root Colonization by Different Arbuscular Mycorrhizal Fungi? *New Phytologist*, **177**, 779-789. <https://doi.org/10.1111/j.1469-8137.2007.02294.x>
- [24] Lone, R., Shuab, R., Sharma, V., Kumar, V., Mir, R. and Koul, K. (2015) Effect of Arbuscular Mycorrhizal Fungi on Growth and Development of Potato (*Solanum tuberosum*). *Plant Journal of Crop Science*, **7**, 233-243. <https://doi.org/10.3923/ajcs.2015.233.243>
- [25] Zare-Maivan, H., Khanpour-Ardestani, N. and Ghanati, F. (2017) Influence of Mycorrhizal Fungi on Growth, Chlorophyll Content, and Potassium and Magnesium Uptake in Maize. *Journal of Plant Nutrition*, **40**, 2026-2032. <https://doi.org/10.1080/01904167.2017.1346119>
- [26] Zhang, H., Xu, N., Li, X., Long, J., Sui, X., Wu, Y., Li, J., Wang, J., Zhong, H. and Sun, G.Y. (2018) Arbuscular Mycorrhizal Fungi (*Glomus mosseae*) Improves Growth, Photosynthesis and Protects Photosystem II in Leaves of *Lolium perenne* L. in Cadmium Contaminated Soil. *Frontiers Plant Science*, **9**, 1156. <https://doi.org/10.3389/fpls.2018.01156>
- [27] Mena-Violante, H., Ocampo-Jiménez, O., Dendooven, L., Martínez-Soto, G., González-Castañeda, J., Davies Jr., F. and Olalde-Portugal, V. (2006) Arbuscular

- Mycorrhizal Fungi Enhance Fruit Growth and Quality of Chile Ancho (*Capsicum annuum* L. cv San Luis) Plants Exposed to Drought. *Mycorrhiza*, **16**, 261-267. <https://doi.org/10.1007/s00572-006-0043-z>
- [28] Rajasekaran, S., Nagarajan, S.M., Arumugam, K., Sravanamuthu, R. and Balamurugan, S. (2006) Effect of Dual Inoculation (AM Fungi and Rhizobium) on Chlorophyll Content of *Arachis hypogaea* L. CV. TMV-2. *Plant Archives*, **6**, 671-672.
- [29] Cecatto, A.P., Martínez Ruiz, F., Oliveira Calvete, E., Martínez, J. and Palencia, P. (2016) Mycorrhizal Inoculation Affects the Phytochemical Content in Strawberry Fruits. *Acta Scientiarum Agronomy*, **38**, 227-237. <https://doi.org/10.4025/actasciagron.v38i2.27932>
- [30] Candidoia, V., Campanelli, G., D'Addaboc, T., Castronuova, D., Perniola, M. and Camelea, I. (2015) Growth and Yield Promoting Effect of Artificial Mycorrhization on Field Tomato at Different Irrigation Regimes. *Scientia Horticulturae*, **187**, 35-43. <https://doi.org/10.1016/j.scienta.2015.02.033>
- [31] Sellitto, V., Golubkina, N., Pietrantonio, L., Cozzolino, E., Cuciniello, A., Cenvinzo, V., Florin, I. and Caruso, G. (2019) Tomato Yield, Quality, Mineral Composition and Antioxidants as Affected by Beneficial Microorganisms under Soil Salinity Induced by Balanced Nutrient Solutions. *Agriculture*, **9**, 110. <https://doi.org/10.3390/agriculture9050110>
- [32] Jugran, A.K., Bahukhandi, A., Dhyani, P., Bhatt, I.D., Rawal, R.S., Nandi, S.K. and Palni, L.M.S. (2015) The Effect of Inoculation with Mycorrhiza: AM on Growth, Phenolics, Tannins, Phenolic Composition and Antioxidant Activity in *Valeriana jatamansi* Jones. *Journal of Soil Science and Plant Nutrition*, **15**, 1036-1049. <https://doi.org/10.4067/S0718-95162015005000072>
- [33] Lima dos Santos, E., Alves da Silva, F. and Barbosa da Silva, F.S. (2017) Arbuscular Mycorrhizal Fungi Increase the Phenolic Compounds Concentration in the Bark of the Stem of *Libidibia ferrea* in Field Conditions. *The Open Microbiology Journal*, **11**, 283-291. <https://doi.org/10.2174/1874285801711010283>
- [34] Sarker, U. and Oba, S. (2018) Drought Stress Enhances Nutritional and Bioactive Compounds, Phenolic Acids and Antioxidant Capacity of *Amaranthus* Leafy Vegetable. *BMC Plant Biology*, **18**, 258. <https://doi.org/10.1186/s12870-018-1484-1>
- [35] Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. and Zheng, B. (2019) Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. *Molecules*, **24**, 2452. <https://doi.org/10.3390/molecules24132452>
- [36] Caretto, S., Linsalata, V., Colella, G., Mita, G. and Lattanzio, V. (2015) Carbon Fluxes between Primary Metabolism and Phenolic Pathway in Plant Tissues under Stress. *International Journal of Molecular Sciences*, **16**, 26378-26394. <https://doi.org/10.3390/ijms161125967>
- [37] Vallverdu-Queralt, A., Medina-Rejon, A., Martínez-Huelamo, M., Jauregui, O., Andres-Lacueva, C. and Lamuela-Raventos, R.M. (2011) Phenolic Profile and Hydrophilic Antioxidant Capacity as Chemotaxonomic Markers of Tomato Varieties. *Journal of Agricultural Food Chemistry*, **59**, 3994-4001. <https://doi.org/10.1021/jf104400g>
- [38] Martí, R., Roselló, S. and Cebolla-Cornejo, J. (2016) Tomato as a Source of Carotenoids and Polyphenols Targeted to Cancer Prevention. *Cancers*, **8**, 58. <https://doi.org/10.3390/cancers8060058>