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Article

Monosporic Inoculation of Economically Important Horticultural Species with Native Endomycorrhizae under Greenhouse Conditions

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Abstract: Research into the symbiotic relationship between plants and arbuscular mycorrhizal fungi (AMF) is key for sustainable agricultural intensification. The objective of the present study is to evaluate native AMF at the monosporic level in greenhouse-grown, economically important crops. Agricultural soil samples from three locations (Saltillo, Zaragoza, and Parras) were obtained by combining portions resulting from a zigzag sampling pattern. From these samples, 15 morphotypes were extracted according to a modified Gerdemann's technique and monosporically inoculated on melon, cucumber, tomato, and onion, 30 days after their sowing. Under a completely random experimental design, 16 treatments with three repetitions were defined. Plant height, root length, stem diameter, total fresh weight, fresh root weight, dry root weight, bulb weight, fresh leaf weight, total dry weight, flower number, leaf number, fruit number, spore number, and percentage of colonization were all evaluated. The results were subjected to the analysis of variance (ANOVA) and the Tukey comparison test ($p \leq 0.05$), which showed that the monosporic inoculation favors significantly the AMF and the host, while the T6 (Saltillo spore + Steiner modified with 20% of the normal phosphorus concentration) showed a greater response uniformity on onion and melon, which indicates its great potential as an inoculum.

Keywords: endomycorrhizas; onion; tomato; melon; cucumber

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form mutualistic symbioses with 85% of terrestrial plants [1–3]. The AMF obtains sources of photosynthetic carbon from the host, which favors the absorption of nitrogen and phosphorus [4], increases productivity, promotes growth, reduces abiotic and biotic stresses, improves the soil quality for drought tolerance and saline stress, and above all, increases the chemical quality of horticulturally cultivated vegetables [5,6].

Plants from the Amaryllidaceae (onion and leek), Apiaceae (carrot), Asteraceae (lettuce), Cucurbitaceae (cucumber), Fabaceae (bean), and Solanaceae (tomato and bell pepper) families possess a high dependence on mycorrhizal colonization [5]. Inoculations of greenhouse onion plants grown in phosphorus deficient soil with 1–7 species of AMF in various combinations demonstrated increases in dry shoot weight, but a reduction in the concentrations of potassium and sulfur; *Glomeraceae* species had a greater impact on growth and nutrient absorption while *Acaulospopra* and *Racocetra* spp. did not have a significant impact [4]. The effects of mycorrhiza inoculation density (no inoculum, 1000 spore, and 2000 spore inocula) on cucumber plants subjected to saline stress (control, 50, 75, and 100 mM NaCl) has been studied. Inoculation with 2000 spores resulted in increases in dry and fresh fruit weight and root volume, in the absence of salt stress. In cucumber, dry and fresh weights increased with the inoculation of 1000 spores at all levels of salt stress [7]. Similarly, the interactions between four AMF (*Glomus intraradices, Glomus mosseae, Glomus claroideum,* and *Glomus constrictum*) and *Trichoderma harzianum* were evaluated in melon plants. The AMF reduced fresh plant weight compared to *T. harzianum*, while dual inoculation resulted in a decrease of fresh weight compared to AMF-inoculated plants, except for *G. intraradices* [8]. PKM-1, Gaurav, and Monarch tomato cultivars inoculated with *Glomus fasciculatum* were assessed. Root colonization of the AMF varied between 26.4% to 36.0% in 30-day-old plants and 40.2% to 47.0% in 60-day-old plants. Significant increases in plant height, and both shoot and root fresh and dry weights, as well as plant N, P, and K content were recorded [9].

The actual effects of commercial inoculations are not always positive [10,11], because the conventional agricultural systems coupled with the irrational use of pesticides reduces the positive effect of AMF [12,13]. Few studies objectively adjust their inoculum to the environment in order to avoid failure of the inoculation process. The objective of the present study is to evaluate the response to monosporic inoculation of native endomycorrhizae in melon, cucumber, onion, and tomato plants subjected to low doses of phosphorus fertilization under greenhouse conditions.

2. Materials and Methods

The experiment took place throughout 2015 and 2016 in a medium technology greenhouse belonging to the Antonio Narro Autonomous Agrarian University, located in Saltillo, Coahuila, Mexico (23° 37″ N, 100° 38″ W, at an altitude of 1581 m.a.s.l).

2.1. Sampling and Soil Analysis

Agricultural soils abandoned for 10 years in Parras, Saltillo, and Zaragoza, Coahuila, inhabited only by wild sunflowers of the family *Asteraceae*, were sampled in June 2015. Using a zigzag method, four 1-kg samples per point, with four points per location, were taken at a depth of 0–30 cm. Texture was determined by the Bouyoucus method [14], pH by a potentiometric technique (1:2), phosphorus by Olsen's technique [15], organic material (OM) by Walkey and Black's method [16], and electrical conductivity (EC) by electrometric methods (1:2).

2.2. AMF Spore Extraction by Modified Gerdemann's Method

Twenty soil samples were analyzed (five from Saltillo, seven from Zaragoza, and eight from Parras). The 200 g, unsifted soil samples were deposited in a container with 5 L of tap water, mixing until the lumps were gone, and leaving to stand for 2 h to separate the soil from the water. The soil was washed three times by pouring the contents through 40, 60, 200, 325, and 400 µm sieves. Subsequently, the residue from each sieve was collected in 50-mL Falcon tubes by rinsing the sieve with 20 mL of distilled water from a wash bottle. Each tube had 25 mL of 1.8 M sucrose solution added and the tubes were centrifuged at 3700 rpm for 4 min. The supernatant was poured through a 400-µm sieve and the residue washed until the sucrose was eliminated. The rinsed residue was deposited in 6-cm diameter, glass Petri dishes and visualized under a stereoscopic microscope (Olympus SZ51, Olympus America Inc, Center Valley, PA, USA.

2.3. Morphological Grouping and Washing of Spores

Using a stereoscopic microscope (Olympus SZ51), spores were grouped according to color, shape, size, and number of layers, according to the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) [17]. The morphotypes were washed with 1 mL of 1% sodium dodecyl sulfate (SDS) solution by inverting for 1 min before passing to an Eppendorf tube containing 1 mL of distilled water. Each sample was washed four times. The spores were stored at 4 °C for 3 days.

2.4. Plant Material Sowing and Inoculation

Peat moss for germination and sandy loam soil were sterilized for 30 min at 15 (lb in^{-2}) of pressure, three times. Red onion, cucumber var. Centauro, melon var. Magno F1, and tomato var. Híb MX7047 (Syngenta, Guanajuato, Mexico) seeds were germinated in 200-cell, Styrofoam trays and kept in the germination chamber for 15 days. The seedlings were moved to the greenhouse for transplanting into black, 3-L nursery pots filled with sandy loam soil that had been irrigated to capacity the day before. Inoculation was performed 30 days after sowing (d.a.s) by placing a single spore on the lateral root of each plant.

2.5. Crop Management

The plants were fertilized by drip irrigation with a Steiner solution modified to contain only 20% of the normal phosphorus concentration. Each pot received 900 mL of nutrient solution daily, divided amongst three irrigations (8:00 AM, 2:00 PM, and 8:00 PM). The average pH was 7.8 with an EC of 2.8 ds/m. The average temperature and relative humidity at 8 AM was 16.5 °C and 77%; at 2 PM, 36.3 °C and 30%; and at 8 PM, 20.2 °C and 56%. Pruning was performed, as well as chemical control of white fly and potato psyllid with imidacloprid. Powdery mildew and early blight were controlled with chlorothalonil and copper hydroxide.

At 66 days after transplant (d.a.t.), plant height (PH) and root length (RL) of each plant was measured with a tape measure, and stem diameter (SD) with digital Vernier calipers. Total fresh weight (TFW), root fresh weight (RFW), and leaf fresh weight (LFW) were measured with an analytical balance (Mettler AJ100 and Ohaus EP114C). Flower number (FLN), leaf number (LN), and fruit number (FRN) were also counted. The cucumber, melon, and tomato were in flowering, but the onion was in the vegetative phase start of bulb formation.

2.6. Determination of Root Colonization Percentage and Spore Number

The roots of the triple-repetition 16 treatments and the controls were washed under running water and then cut into 1-cm-long fragments, as per the root staining technique [18]. The root fragments were vertically aligned on slides (three slides per plant with 100 observations) with a drop of acidified glycerol added. The slides were observed under an Olympus CX21 Olympus America Inc, Center Valley, PA, USA. compound microscope. The percentage of colonization was estimated according to the McGonigle method [19]. The spores in 100 g of soil per pot and per crop were counted with an Olympus SZ51 stereoscopic microscope according to a modified Gerdemann's technique [20].

2.7. Statistical Analysis

The experiment consisted of 16 treatments with three repetitions: 15 treatments with 1 AMF spore from each location plus fertilization with Steiner solution containing 20% of the normal P concentration, and a control (no AMF) with the same fertilization scheme. The experimental design was completely random for each species studied. Analysis of variance (ANOVA) and Tukey's comparison of means test ($p \leq 0.05$) was performed with the Statistical Analysis Systems (SAS) software suite (2013).

3. Results

3.1. Soil Analysis

Table 1 presents the physicochemical characteristics of the studied soil. The pH of soil from Parras is moderately alkaline and alkaline in Zaragoza and Saltillo. Parras soil has high electrical conductivity (EC). Saltillo soil has low OM content, while Zaragoza soil has high OM, and Parras soil has moderately high OM. Zaragoza and Saltillo soil have moderately low total phosphorus (P), while Parras soil has medium levels of P.

Table 1. Analysis of soil from three locations in Coahuila.

Location	Texture	pH	EC (ds/m)	OM (%)	P (ppm)
Parras	Loamy	7.89	3.04	2.79	18.4
Zaragoza	Loamy	8.07	0.93	3.40	9.83
Saltillo	Sandy Loam	8.11	1.55	0.73	9.92

3.2. Spore Morphology

Table 2 shows that of the three locations studied, 15 treatments were obtained whose spores present a globose to sub-globose shape and a spore coat made up of one to three layers, with a wall thickness of 5.3–19.9 µm. Seven colors were identified, mostly the 0-10-60-0 orange–brown defined by the INVAM [17]. The spore equatorial diameter (ED) ranges from 93.1 to 217.8 µm and the meridian diameter (MD) from 96.5 to 208.05 µm. The number of strata (NS) varies from 1 to 3 and the thickness of the spore wall (TSW) from 3.09 to 13.90 µm.

Table 2. Characteristics of native AMF associated with wild sunflower from Coahuila, 2015.

Treatments	Color (INVAM 2015)		ED (µm)	MD (µm)	NS	TSW (µm)
1 Saltillo	0-10-60-0	Yellow–Brown	162.64	167.24	3	5.30
2 Saltillo	0-10-60-0	Yellow–Brown	143.10	146.55	3	11.38
3 Saltillo	0-10-40-0	Orange	125.86	129.89	2	6.92
4 Saltillo	0-10-40-0	Orange	149.43	142.53	3	8.75
5 Saltillo	0-10-60-0	Yellow–Brown	93.10	96.55	3	7.67
6 Saltillo	20-80-80-0	Orange–Brown	177.01	177.59	3	12.08
7 Zaragoza	20-80-80-0	Orange–Brown	133.33	135.06	3	13.84
8 Zaragoza	0-10-60-0	Yellow–Brown	110.92	122.41	1	3.09
9 Zaragoza	0-5-20-0	Cream with a pale pink tint	111.50	137.36	3	10.04
10 Zaragoza	0-10-40-0	Orange	132.76	137.36	2	12.08
11 Zaragoza	20-80-80-0	Orange–Brown	217.82	208.05	2	12.92
12 Parras	0-5-40-0	Hyaline/white	108.62	109.77	3	6.90
13 Parras	0-10-60-0	Yellow–Brown	125.86	137.93	2	13.90
14 Parras	0-20-80-0	Salmon	177.01	182.76	2	7.30
15 Parras	0-10-60-0	Yellow–Brown	106.34	111.56	3	8.12

3.3. Number of AMF Spores

Table 3 illustrates the capacity of the 15 inoculated treatments to produce spores under conditions totally different to their natural habitat. The T1 Saltillo ($p \leq 0.05$) in tomato presented the highest significant density of spores. The T5 Saltillo in onion, T9 Zaragoza in cucumber, T11 Zaragoza in melon, and T1 Saltillo in tomato also presented significant densities of spores. The letter in the table is the result of the Tukey comparison test.

Table 3. Average density of spores (Tukey's $p \leq 0.05$) in four horticultural species.

Treatment	Onion	Cucumber	Melon	Tomato
C	0.00 f	0.00 h	0.00 g	0.00 f
1 Saltillo	18.00 bdec	9.66 g	10.66 ef	30.33 a
2 Saltillo	17.00 bdec	14.66 fbecd	10.33 ef	25.33 bdac
3 Saltillo	14.66 de	18.00 ba	8.00 f	23.66 dec
4 Saltillo	15.33 bdec	14.33 fbecd	13.66 edc	23.66 dec
5 Saltillo	27.00 a	11.66 fge	14.33 bdc	18.33 e
6 Saltillo	13.33 e	14.33 fbecd	15.33 bac	26.33 bdac
7 Zaragoza	21.66 bac	15.66 becd	8.00 f	29.00 bac
8 Zaragoza	21.66 bac	13.33 fged	11.00 edf	26.33 bdac
9 Zaragoza	19.00 bdec	21.66a	15.00 bac	24.66 bdc
10 Zaragoza	21.00 bdac	16.33 bcd	15.66 bac	26.66 bdac

Table 3. *Cont.*

Treatment	Onion	Cucumber	Melon	Tomato
11 Zaragoza	17.33 bdec	17.33 bc	18.00 a	23.00 de
12 Parras	18.00 bdec	11.00 fg	17.33 ba	26.33 bdac
13 Parras	22.00 ba	13.00 fged	14.33 bdc	29.33 ba
14 Parras	17.33 bdec	12.66 fged	16.33 bac	27.00 bdac
15 Parras	15.00 dec	11.66 fge	14.66 bac	27.00 bdac
P	***	***	***	***
% C. V	12.93	10.21	9.49	7.54

C = control; *** = $p \leq 0.001$ ANVA; C. V = coefficient of variation.

3.4. Mycorrhizal Colonization

Table 4 demonstrates that the 15 monosporically-inoculated treatments did have the capacity to develop symbioses, even with low phosphorus fertilization. According to the statistical analyses, the T6 Saltillo established the greatest percentage of colonization in all four host species, though colonization in melon and cucumber was the highest among them.

Table 4. Average mycorrhizal colonization (Tukey, $p \leq 0.05$) in four horticultural species.

Treatment	Onion	Cucumber	Melon	Tomato
C	0.00 f	0.00g	0.00 g	0.00 g
1 Saltillo	14.00 ed	28.57 dfce	54.77 ba	28.00 edf
2 Saltillo	22.33 bdc	30.82 dce	53.07 bc	32.00 ebdfc
3 Saltillo	22.33 bdc	54.38 ba	52.66 bc	37.33 bac
4 Saltillo	24.33 bac	47.61 b	48.29 bc	24.00 f
5 Saltillo	22.00 bedc	35.50 c	38.69 fe	33.00 ebdac
6 Saltillo	33.00 a	58.36 a	60.33 a	41.00 a
7 Zaragoza	24.00 bc	32.89dc	52.70 bc	24.00 f
8 Zaragoza	13.33 e	34.67 c	32.10 f	38.33 bac
9 Zaragoza	21.00 bedc	22.73 fe	41.08 de	30.00 edfc
10 Zaragoza	25.66 bac	22.11 f	47.26 dc	36.00 bdac
11 Zaragoza	17.33 edc	36.37 c	51.33 bc	33.33 ebdac
12 Parras	18.66 bedc	24.58 fe	48.86 dc	34.00 bdac
13 Parras	26.66 ba	30.17 dfce	51.57 bc	25.33 ef
14 Parras	18.33 bedc	25.11 dfe	33.84 f	39.00 ba
15 Parras	18.66 bedc	29.37 dfce	47.76 dc	33.33 ebdac
P	***	***	***	***
% C.V	14.34	8.43	4.98	9.24

C = control; *** = $p \leq 0.001$ ANOVA; C.V = coefficient of variation.

The greatest difference in colonization percentages in onion was of 20% between the T6 Saltillo and T8 Zaragoza. In cucumber, the greatest difference was 36.25% between T6 Saltillo and T10 Zaragoza. In melon, it was 28.33% between the T6 Saltillo and T8 Zaragoza; while in tomato, the greatest difference was 17% between the T6 Saltillo and both the T4 Saltillo and T7 Zaragoza. The letter in the table is the result of the Tukey comparison test.

3.5. Agronomic Characteristics of Cucumber

Table 5 shows the results of Tukey's comparison test for the evaluated plant parameters in cucumber. A 23.67% increase in PH can be seen in T1 Saltillo over the control. T5 Saltillo had a 30.85% increase in SD, a 85.81% increase in TFW, and a 282.55% increase in RFW. T9 Zaragoza had an 23.21% increase in leaf number (LN) and a TDW increase of 286.89%. The FLN in T6 Saltillo increased 12.81% and RL in T13 Parras increased 5.4 times. The latter response is of great economic and environmental importance as it implies better absorption and utilization of nutrients and water as well as better plant anchoring. The letter in the table is the result of the Tukey comparison test.

Table 5. Average effects (Tukey, $p \leq 0.05$) of AMF on the agronomic parameters of greenhouse-grown cucumber.

T	PH (cm)	SD (mm)	LN	FLN	TFW (g)	TDW (g)	RFW (g)	RDW (g)	RL (cm)
C	103.50 bcde	7.26 e	28.0 abcde	18.50 ab	150.04 fg	42.34 b	43.00 gef	23.98 ab	13.66 g
1 Saltillo	128.00 a	8.833 abc	31.0 abc	15.0 bcd	199.81 cdef	58.77 b	44.00 gef	27.96 a	28.00 def
2 Saltillo	111.50 ab	9.23 ab	30.50 abcd	15.00 bcd	268.2 bcde	64.12 b	63.33 b	27.80 a	24.33 efg
3 Saltillo	81.00 fg	7.40 e	26.00 cde	10.66 efg	178.81 def	34.17 b	59.00 cb	24.32 ab	31.75 d
4 Saltillo	87.50 def	8.10 cde	28.0 abcde	11.66 defg	198.69 cdef	57.22 b	57.00 cbd	29.29 a	40.66 c
5 Saltillo	108.0 bc	9.50 a	27.33 bcde	17.50 abc	278.79 a	70.40 b	121.50 a	28.35 a	15.75 fg
6 Saltillo	105.5 bcd	8.35 abcd	30.0 abcd	20.87 a	258.10 ab	54.13 b	46.50 gefd	26.05 a	29.50 cde
7 Zaragoza	92.50 cdef	7.40 e	27.50 bcde	13.50 def	182.26 def	55.70 b	56.50 cbd	16.27 c	20.00 defg
8 Zaragoza	77.33 fg	8.26 bcde	31.0 abc	9.33 g	158.12 efg	60.47 b	60.50 b	26.84 a	23.00 defg
9 Zaragoza	102.50 bcde	8.65 abcd	34.50 a	9.50 g	243.72 abc	121.47 a	47.50 cefd	25.41 a	47.50 b
10 Zaragoza	66.66 g	7.55 de	24.0 de	13.66 cdef	175.19 defg	41.72 b	48.00 cefd	25.82 a	18.00 efg
11 Zaragoza	110.5 abc	7.60 de	33.50 ab	20.00 a	231.51 abcd	52.13 b	38.00 gf	24.72 ab	23.00 defg
12 Parras	93.66 bcdef	8.0 cde	27.0 bcde	10.0 fg	198.34 cdef	49.50 b	63.50 b	25.67 a	17.50 efg
13 Parras	106.0 bcd	8.20 bcde	29.33 abcd	11.66 defg	172.32 efg	59.05 b	51.66 cebd	17.27 bc	74.33 a
14 Parras	85.50 feg	7.76 cde	25.50 cde	14.00 cde	120.33 g	37.13 b	34.50 g	24.59 ab	13.75 g
15 Parras	76.33 fg	8.15 bcde	21.500 e	11.50 defg	151.92 efg	34.78 b	39.66 gef	24.86 ab	16.83 fg
P	***	***	***	***	***	***	***	***	***
CV	6.46	4.65	7.64	9.14	9.72	29.90	7.36	10.083	13.60

C = control; *** = $p \leq 0.001$ ANVA; C.V = coefficient of variation.

3.6. Agronomic Characteristics of Melon

The results of monosporic inoculation of melon with native AMF are summarized in Table 6. At 66 d.a.t., inoculation with a single spore results in significant differences in all the evaluated agronomic parameters. Of the 15 treatments, excelling T6 of Saltillo with six out of nine evaluated parameters: PH (101.6%), LN (80.6%), FLN (180.6%), TFW (157.61%), RFW (155%), and RL (164.86%), The T11 and T13 Parras resulted in an increase of biomass production statistically the same as T6 over the control. Those AMF are potentially significant candidates for melon seedling inoculation under greenhouse conditions.

Table 6. Average effects (Tukey, $p \leq 0.05$) of AMF on the agronomic parameters of greenhouse-grown melon. The letter in the table is the result of the Tukey comparison test.

T	PH (cm)	LN	FLN	FRN	TFW (g)	TDW (g)	RFW (g)	RDW (g)	RL (cm)
C	83.33 g	10.33 c	7.00 ef	0.33 ab	70.00 f	22.34 h	18.50 f	8.73 e	11.33 f
1 Saltillo	153.00 ab	12.00 bc	13.00 b	0.00 b	147.50 b	78.46 ab	21.50 ef	9.30 e	24.80 bc
2 Saltillo	116.33 defg	12.00 bc	13.00 b	1.00 ab	105.50 cde	42.95 bef	44.33 ab	19.97 ab	13.50 def
3 Saltillo	118.66 bcdef	11.66 bc	8.66 cdef	0.66 ab	116.00 cd	39.72 defg	24.00 def	10.99 de	16.50 def
4 Saltillo	142.00 abcde	13.66 b	9.33 cdef	0.33 ab	107.50 cde	64.75 bc	32.66 cd	15.13 bcd	14.66 def
5 Saltillo	124.66 bcdef	11.33 bc	14.00 b	1.00 ab	121.00 bcd	50.63 cd	29.33 cde	12.97 de	12.05 ef
6 Saltillo	168.00 a	18.66 a	19.66 a	2.00 a	180.33 a	79.23 ab	49.00 a	18.64 bc	34.45 a
7 Zaragoza	100.00 fg	12.33 bc	11.50 bcd	1.33 ab	122.00 bcd	48.67 de	37.50 bc	13.66 cde	19.83 cd
8 Zaragoza	133.00 bcdef	12.66 bc	6.50 f	0.66 ab	130.00 bc	35.28 efgh	30.00 cde	12.66 de	15.25 def
9 Zaragoza	151.00 abc	12.66 bc	7.50 ef	0.00 b	146.50 b	67.53 b	24.50 def	12.21 de	24.33 bc
10 Zaragoza	131.33 bcdef	12.00 bc	11.33 bcd	0.66 ab	84.00 ef	30.21 fgh	23.50 def	11.76 de	15.75 def
11 Zaragoza	118.00 cdef	12.00 bc	18.00 a	1.00 ab	197.33 a	83.53 a	37.33 bc	12.92 de	15.75 def
12 Parras	150.50 abcd	13.00 bc	8.00 def	0.66 ab	96.66 ef	27.37 gh	20.50 ef	8.81 e	18.60 cdef
13 Parras	115.66 efg	12.00 bc	9.00 cdef	1.00 ab	201.33 a	90.99 a	43.66 ab	24.15 a	25.80 bc
14 Parras	127.33 bcdef	18.00 a	11.66 bc	0.66 ab	115.00 cd	34.35 efgh	28.66 cdef	13.12 de	19.00 cde
15 Parras	132.33 bcdef	12.33 bc	11.00 bcde	0.66 ab	111.50 cde	33.48 fgh	33.66 bcd	14.94 bcd	28.75 ba
P	***	***	***	*	***	***	***	***	***
% CV	8.759	8.176	10.54	73.53	7.80	9.467	11.303	12.364	12.84

C = control; *** = $p \leq 0.001$ ANOVA; C.V = coefficient of variation.

3.7. Agronomic Characteristics of Tomato

The effects of monosporic inoculation of tomato plants with native AMF are presented in Table 7. Once again, the T5 Saltillo significantly improves PH (79.2%) and TFW (200%) when compared to the control. TDW and RDW were improved with T14 Parras inoculation by 2.6 and 5 times, respectively. T10 Zaragoza increased RFW by four times, while T15 Parras significantly increased RL by 104% over the control treatment. The LN parameter had no significant difference among treatments. The T2, T5, T6 Saltillo, and T7 Zaragoza had significant increases in FLN, when compared to the control.

Table 7. Average effects (Tukey, $p \leq 0.05$) of AMF on the agronomic parameters of greenhouse-grown tomato. The letter in the table is the result of the Tukey comparison test.

T	PH (cm)	SD (mm)	FLN	TFW (g)	TDW (g)	RFW (g)	RDW (g)	RL (cm)
C	57.00 h	7.00 abc	2.50 c	74.50 h	16.00 ij	11.00 fg	1.66 e	20.25 efg
1 Saltillo	59.00 gh	5.65 cd	4.00 bc	97.50 gh	11.00 j	9.0 g	2.66 de	16.33 fg
2 Saltillo	79.00 abcde	6.80 abc	8.00 a	131.0 defg	56.00 cdef	12.33 fg	2.66 de	25.00 cde
3 Saltillo	86.00 abc	7.40 ab	3.66 bc	168.0 bcde	68.00 abc	29.00 bc	5.66 ab	29.50 cd
4 Saltillo	77.00 bcde	7.33 ab	4.33 b	158.33 bcde	49.333 defg	27.66 bcd	3.33 cde	27.50 cde
5 Saltillo	91.00 a	7.50 ab	7.50 a	223.50 a	77.50 ab	23.00 bcde	5.00 abc	28.00 cd
6 Saltillo	89.50 ab	8.15 a	8.00 a	204.33 ab	62.00 bcde	29.50 b	3.66 bcde	27.50 cde
7 Zaragoza	85.00 abcd	7.30 abc	7.50 a	160.0 bcde	66.33 abcd	22.33 bcde	4.33 abcd	31.00 bc
8 Zaragoza	63.33 fgh	6.03 bcd	3.33 bc	169.00 bcde	31.66 hi	25.00 bcd	1.66 h	38.25 ba
9 Zaragoza	67.33 efgh	6.40 bcd	4.00 bc	128.00 efg	42.00 fgh	24.00 bcd	3.33 cde	22.50 defg
10 Zaragoza	87.66 abc	8.40 a	3.50 bc	187.00 abc	51.33 cdefg	44.00 a	3.33 cde	27.00 cde
11 Zaragoza	75.00 cdef	6.85 abc	4.00 bc	142.67 cdefg	51.66 cdefg	14.00 efg	3.00 cde	23.50 def
12 Parras	73.00 def	6.050 bcd	3.50 bc	177.0 abcd	47.50 efgh	19.00 def	3.50 bcde	22.25 defg
13 Parras	58.66 gh	4.93 d	3.50 bc	111.00 fgh	37.50 gh	12.00 fg	2.33 de	15.33 g
14 Parras	71.33 efg	4.93 abc	4.00 bc	135.00 defg	80.00 a	20.50 bcdef	6.00 a	29.66 cd
15 Parras	67.33efgh	4.93 bcd	4.00 bc	148.33 cdef	50.66 defg	19.66 cdef	3.00 cde	41.33 a
P	***	***	***	***	***	***	***	***
%CV	5.746	7.9771	12.6891	10.287	11.2045	15.04	20.97	9.1790

C = control; *** = $p \leq 0.001$ ANOVA; C.V = coefficient of variation.

3.8. Agronomic Characteristics of Onion

Table 8 summarizes the effects of AMF inoculation on greenhouse-grown onion plants. The T6 Saltillo significantly improved PH (89.03%), SD (four times), LN (100%) TFW and TDW (six times) RFW (three times) RL (132.12%), and LFW (ten times) compared to the control. The parameter did not have significant differences between treatments.

Table 8. Average effects (Tukey, $p \leq 0.05$) of AMF on the agronomic parameters of greenhouse-grown onion. The letter in the table is the result of the Tukey comparison test.

T	PH (cm)	SD (mm)	LN	TFW (g)	TDW (g)	RFW (g)	RL (cm)	LFW (g)
C	19.15 efd	1.30 h	3.00 b	2.66 e	1.29 d	1.23 ecd	11.61 gef	1.04 e
1 Saltillo	21.53 ecd	3.33 fbedc	4.66 ba	7.06 cbd	2.90 cb	1.51 becd	14.00 cefd	5.54 cb
2 Saltillo	17.86 ef	2.90 fgedc	3.66 b	4.29 ed	1.35 d	0.32 e	12.50 gefd	2.81 cebd
3 Saltillo	30.65 ba	4.79 ba	4.66 ba	7.91 cb	3.90 b	0.68 ecd	16.35 cebd	4.96 cbd
4 Saltillo	11.90 f	2.15 fgeh	3.33 b	3.16 e	1.59 d	0.453 ed	7.80 g	2.01 ced
5 Saltillo	22.80 becd	3.65 bdc	3.33 b	2.46 e	0.94 d	0.83 ecd	11.93 gef	1.14 e
6 Saltillo	36.20 a	5.42 a	6.0 a	16.09 a	7.88 a	3.65 a	26.95 a	10.90 a
7 Zaragoza	22.95 becd	3.40 bedc	3.33 b	3.49 e	1.37 d	0.683 ecd	21.0 b	3.72 cebd
8 Zaragoza	26.66 bcd	4.10 bac	3.33 b	5.10 ced	2.11 cd	0.98 ecd	15.75 ced	3.90 cebd
9 Zaragoza	23.56 becd	4.45 ba	4.0 b	7.48 cb	3.71 b	1.82 bcd	12.60 gefd	5.56 cb
10 Zaragoza	17.75 ef	4.65 ba	4.0 b	5.23 ced	2.18 cd	1.92 bc	17.00 cbd	1.65 ed
11 Zaragoza	11.40 f	1.93 fgh	3.0 b	3.08 e	0.94 d	0.55 ecd	10.25 gf	1.94 ced
12 Parras	27.87 bc	5.52 a	3.0 b	2.53 e	0.90 d	2.89 ba	9.75 gf	1.21 ed
13 Parras	18.25 ef	2.60 fgedh	3.3 b	2.82 e	1.05 d	0.44 ed	13.10 efd	0.90 e
14 Parras	27.36 bcd	4.76 ba	4.66 ba	8.54 b	4.10 b	1.08 ecd	18.20 cb	6.56 b
15 Parras	22.55 ecd	1.63 h	4.00 b	7.38 cb	3.86 b	1.80 bcd	13.04 efd	6.56 cebd
P	***	***	***	***	***	***	***	***
%CV	12.08	13.60	16.47	17.40	16.84	36.67	11.03	34.93

C = control; *** = $p \leq 0.001$ ANOVA; C.V = coefficient of variation.

4. Discussion

4.1. Effects of Host Species on AMF Colonization

AMF comprises more than one spore morphotype [21] and influences the growth and nutrient absorption of a certain plant species [22]. The physicochemical characteristics of soil that influence AMF diversity [23] are the pH, EC, OM, soil nutrients, and the host species [24,25]. There is evidence that each terrestrial plant species in symbiosis hosts more than one type of AMF [26]. However, the specificity of symbiosis can be at the species, family or ecological level [27,28]. Too little is known about the local and global distribution of AMF to understand their biogeography [29,30]. The results of this study demonstrate that the origin and physicochemical composition of the soil influences the diversity of AMF spores. Six treatments were collected from the Saltillo location, followed by five from Zaragoza and four from Parras. High genetic variation between species, and even between spores, has been shown at the molecular level, resulting in an increase in AMF diversity [27].

That being said, the morphological separation and monosporic inoculation carried out permits a deeper understanding of the true effects a single spore can have (a single species) on cucumber, melon, tomato, and onion plants. Nevertheless, the efficiency or inefficiency of the resulting symbiosis is regulated by the fertility of the soil at the time of inoculation, the level of disturbance at the site and probably, the co-adaptation with the partner species [28].

Based on the results obtained, T1 Saltillo in tomato has the necessary genetic conditions for greater sporulation and adaptation to greenhouse conditions compared to the other treatments and hosts. Studies show that the success of symbiosis and the development of spores is influenced by the environment, i.e., the ability to adapt to a new environment and period of inoculation [23]. Accordingly, the four host species are amenable to AMF spores. Previously, it was believed that the medium and host species had no effect on AMF [29]. However, the results obtained in this study demonstrate that the host species and environment play an important role in the successful establishment and sporulation of AMF. Chitin is known to stimulate the development and sporulation of AMF [30]. In this case, it may be that the tomato plant's chitinase defense mechanism presents a more localized or refined response to AMF colonization than the onion, cucumber, or melon plants [31].

In prior studies performed with greenhouse-grown wheat, 12 out to 21 native AMF morphotypes resulted in successful symbiosis following monosporic inoculation under conditions dissimilar to their natural habitat [25]. It can be said that the 15 evaluated AMF treatments possess the capacity to adapt to media completely different to their natural habitat with differing levels of affinity.

4.2. Effects of AMF Colonization on Host Species

The results are in line with the knowledge that AMF in mutualistic symbiosis stimulates the growth, photosynthesis, and absorption of nutrients in a wide range of host species. It should be emphasized that plants of the family *Cucurbitaceae* belong to those species in which the establishment of symbiosis is successful [32]. The T5 Saltillo increased cucumber plant vigor, as well as TFW and RFW, leading to a greater capacity for nutrient absorption as seen previously with spore inoculation under saline conditions [33]. In terms of FLN, PH, LN, and TDW parameters, the T8 Zaragoza, T10 Zaragoza, and T15 Parras were worse than the control, so they represent inocula without potential. Another study where three combinations of AMF, VT (*Claroideoglomus* sp., *Funneliformis* sp., *Diversispora* sp., *Glomus* sp., and *Rhizophagus* sp.), BF (*Glomus intraradices*, *G. microageregatum* BEG, and *G. Claroideum* BEG 210), and Fm (*Funneliformis mosseae*) were used to inoculate cucumber (*Cucumis sativus* L. cv. Zhongnong No. 106) plants found successful symbioses at 46 days after inoculation. The PH, SD, TDW, and RL increased significantly compared to the non-inoculated control [7]. A different study inoculated *Cucumis sativus* cv. Super N3 with *Glomus mosseae* under hydroponic conditions and found that inoculation with 1000 or 2000 spores resulted in increased shoot and root fresh weight, compared to their control. The 1000 spore inoculum was also able to increase antioxidant activity and phenol content [34].

AMF improve the absorption and translocation of mineral nutrients thanks to their ability to reach beyond the rhizosphere, which is reflected in the growth and development of the melon plants and other Cucurbitaceae [35]. Nevertheless, the TFW parameter did not show significant differences between treatments. A separate experiment involving melon inoculated with *Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum*, and *Glomus constrictum*, and challenged with Fusarium found a reduction in plant fresh weight and a failure to suppress the infection [8]. Regardless, the inoculation treatments in this experiment positively favored the melon plants.

A study of three tomato cultivars (PKM-1, Gaurav, and Monarch) inoculated with *Glomus fasciculatum* demonstrated improvements in plant height, fresh shoots, root length, and root dry weight compared to the non-inoculated control [9]. The Monarch cultivar benefited the most. After 30 days of cultivation, the dry shoot and root weights of inoculated Monarch plants were more than four times greater than the control. The Gaurav cultivar had the greatest biomass and dry weight production. In the present study, there was a significant, positive response (Tukey's, $p \leq 0.05$) in the PH, TFW, TDW, RFW, RDW, and RL of inoculated tomato plants.

Of the 15 inoculation treatments, four had outstanding effects on the previously mentioned parameters. These were the T5 Saltillo, T10 Zaragoza, T14 Parras and T15 Parras. A systematic quantitative analysis demonstrated that inoculation with AMF increases plant biomass by up to 34.9%, compared to non-inoculated plants. The same analysis found that inoculating with a single species yields an average improvement of 41.2% in plant growth, compared to inoculation with multiple species [30].

The genus *Allium* has been found to depend more on AMF than other plants due to their scarce radicular branching and lack of root hairs. There is also evidence that onion genetics influence the response to mycorrhizal symbiosis [36]. Based on the previous information, T6 Saltillo represents a promising inoculation candidate for the greenhouse production of onion.

5. Conclusions

The native endomycorrhizas associated with *Helianthus annus* L. wild and accustomed to different soil physicochemical conditions were able to establish symbiosis with a single spore in the four horticultural crops. The growth and development of cucumber plants was favored by treatment with the T1 Saltillo, T5 Saltillo, T6 Saltillo, T9 Zaragoza, and T13 Parras AMF. The melon and onion plants benefited the most with the T6 Saltillo. The T5 Saltillo, T10 Zaragoza, T14 Parras, and T15 Parras treatments favored the tomato plants. Overall, the single spore treatments of AMF from Saltillo elicited the best responses in the greenhouse-grown crops.

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