Article

Impact of cover, ecotype and endomycorrhizae on morphology and quality of piquin chili

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Abstract

The research was carried out with piquin chili ecotypes from the states of Coahuila, Nuevo León and Zacatecas. Piquin chili plants were evaluated in its second year of production. Two environments were used: a) macro tunnels of white mesh, red, blue, black raschel type with 30% shade; and b) open field with 100% light transmission. 50 spores of a conglomerate of arbuscular mycorrhizal fungi (*Glomus mosseae*, *Rhizophagus intraradices*, *Sclerocystis coremioides* and *Gigaspora albida*) were inoculated directly to the radical system. A factorial arrangement 5 x 6 x 2 (cover, ecotypes and mycorrhizae) was used and its distribution corresponded to a random block design with four repetitions. The yield and some morphological characteristics of the plant were evaluated such as plant height (AP), stem diameter (DT), root length (LR), fresh plant weight (PFP), dry weight of the plant (PSP), fresh root weight (PFR), dry root weight (PSR), yield per plant (RPP). The results indicate that the white mesh favored the morphological characteristics of the plant with agronomics with 320% AP, 322.7% DT, 235.8% LR, 8 times PFP, 8.5 times PSP, 327.2% PFR, 5 times PSR, 6.8 times PTR, compared to plants developed in open field. In addition, the quality of piquin chili provides conditions for the development of endomycorrhizae (spores and percentage of colonization). Blue mesh with the lowest photosynthetically active radiation (RFA) positively influenced agronomic, quality variables and inoculation (number of spores and percentage of colonization). The ecotype that influenced SST, Vit C, phenols and NE was SNL and RTZ in agronomic variables and % colonization. The inoculation with the mycorrhizae consortium improved the agronomic characteristics and quality of the piquin chili fruit.

Keywords: *Capsicum annuum*, quality, shadow mesh, solar radiation.

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Introduction

Piquin chili *(Capsicum annuum* L.) is a phytogenetic resource widely distributed in Mexico, given its nature the wild form of this species is the predominant one, its fruit is usually obtained by harvesting in wild populations, but it is threatened by its genetic diversity (Pagán *et al*., 2010). Piquin chili has not been completely domesticated since it is observed low germination and morphological and genetic variability (García-Federico *et al*., 2010; Hernández-Verdugo *et al*., 2015).

Piquin chili yields are affected by environmental conditions, soil moisture and fertilization (Rodríguez *et al*., 2005). In relationto environmental factors it has been shown that direct sunlight causes more compact plants, whereas with 80% shade the plants grow quickly and become larger. *Capsicum* plants' response to different irradiance conditions may vary according to cultivar. The photoperiod, quality and quantity of sunlight coincide, directly, in the photosynthesis of plants and other phenotypic and functional characteristics (Peixoto *et al*., 2014).

The phenotypic responses of plants to varied light conditions have not been adequately used to modify the morpho-physiological characteristics of crops and obtain desired yield and quality (Kelly *et al*., 2015). The use of colored meshes is an alternative to avoid excessive radiation as with red mesh, which provides 42.6% total solar radiation (350 to 1 050 nm) and blue mesh 36% more blue light (400 to 500 nm) than black mesh (Ayala-Tafoya *et al*., 2015). These differences in radiation can cause differential response in photosynthesis and photomorphogenesis that produce effects on stem growth, foliar expansion, chloroplast development, chlorophyll synthesis and secondary metabolites. In cucumber the use of red and blue meshes increased yield 48.1 and 46.1% compared to the witness (Ayala-Tafoya *et al*., 2015).

On the other hand, the use of biofertilizers in the cultivation of piquin chili has shown positive effects on plant height, root length, increased dry biomass in saline and non-salty soils (Rueda *et al*., 2010). It has also been shown that mycorrhizal fungi have an affinity in different *Capsicum* species such as *annuum*, *bacatum*, *chinense*, *frutescens* and *pubescens* which confirms their positive effect (Cardona *et al*., 2008).

However, the application of commercial inoculums does not assure the positive effect on plant-mycorrhizal fungus synergism, due to the indiscriminate use of agrochemicals in traditional production systems (Koyama *et al*., 2017; Caruso *et al*., 2018). In addition, in other crops such as tomato, mycorrhizae *(Glomus mossae* and *Glomus cubense)* have been used in liquid form (10 and 20 spores) and solid form (20 and 40 spores) and have produced beneficial results for the plant (Mujica *et al.*, 2012).

The objective of this research work was to evaluate the effect of inoculation with native endomycorrhizae of three locations in the northeast of the country under photoselective cover and open field in the morphology and quality of piquin chili.

Materials and methods

The study was carried out, in the Horticulture Department of the Antonio Narro Agrarian Autonomous University, Saltillo, Coahuila, Mexico, located at 25° 22’ north latitude and 101° 22’ west longitude, at a height of 1 580 meters above sea level. Six ecotypes of piquin chili were collected, called: MZC= Múzquiz, Coahuila; SAC= San Alberto, Coahuila; LNL= Linares, Nuevo León; SNL= Santiago, Nuevo León; PTZ= Tepetatilla Bridge, Zacatecas and RTZ= Tuxpan River, Zacatecas.

Seed planting was carried out in germinating boxes to which it was applied GA3 (500 ppm) to speed up the germination process, after one month the seedlings were transplanted into polyethylene bags with a capacity of 10 L and placed at a distance of 40 cm between plants and 1 m between grooves, as a substratum was used sphagnum peat moss (Pro Mix®) and perlite (Hortiperl®) in a proportion 2:1 (v/v). In the experiment these chili ecotypes were used in their second year of production, different luminosity environments (cover color) were used: a) macro tunnels of white mesh (MA), red (MR), blue (MA), black (MN) raschel type with 30% shade, with a hole size of 6 x 8 mm; each tunnel 4 m wide, 6 m long and 2.30 m high; and b) open field with 100% light transmission.

The crop was fertilized with nutrient solution, 25% in seedling, 50% in vegetative development, 75% in flowering and 100% in fructification. At the beginning of this evaluation, phosphorus input was reduced to 25%, with the intention that endomycorrhizae presented synergy with plant roots. The water supply was made from 0.5 to 2.5 L plant^{-1} day^{-1}, with a fertigation system per stake.

The plants were inoculated after transplantation (50 spores) with a conglomerate of arbuscular mycorrhizal fungi (*Glomus mosseae*, *Rhizophagus intraradices*, *Sclerocystis coremioides* and *Gigaspora albida*), directly to the radical system, which were identified by comparative morphology (Sánchez-Sánchez *et al*., 2018).

These factors were evaluated by a factorial arrangement 5 x 6 x 2 (roof color:5, ecotypes:6 and mycorrhiza:2). The experimental design used corresponded to a random block with four repetitions. Microclimatic variables such as environmental temperature and relative humidity were recorded with a digital thermo hygrometer (Taylor® model 1452). Photosynthetically active radiation (RFA) was recorded with a Quantum portable sensor (Apogee® model SM-700). Measurements were made daily between 07:00 and 19:00 h, in the center of each deck, in clear sky conditions.

Morphological characteristics were evaluated in three plants by repetition and treatment, which included: plant height (AP), made with tape measure, stem diameter (DT), with a digital vernier (Digital Caliper®), 8 cuts were made, to obtain the average fruit yield per plant, were weighed with an electronic scale Rhino model Babol-100G with capacity of 100 g and resolution of 0.01 g. The dry weight of the plant (PSP) and the fresh weight of the plant (PFP) and of root (PFR) were determined on an OHAUS scale model CS-5000 with a capacity of 5 kg. To obtain the dry weight of the plant (PSP) and of root (PSR), the samples were placed on brown paper and subjected to 65°C for 48 hours on a Yamato drying stove model DX-602 and subsequently weighed on the aforementioned scale.

The number of leaves (NH), the number of fruits per plant (NFPP) was estimated by counting in each unit, evaluations were made in all experimental units. In quality variables three samples were evaluated per treatment and repetition for, total soluble solids (SST) with a digital refractometer HANNA 96-801, in which a drop of fruit pulp was placed in the cell of the apparatus, obtaining the content expressed in Brix. The content of ascorbic acid (Vitamin C), in fruits was determined by the AOAC methodology (2000).

The total phenol content (FT) was determined according to the methodology reported by Kim *et al.* (2006), with some modifications described below, 2 g of fresh piquin chili fruit was weighed and placed in 20 mL of 80% methanol, for 12 h at 4 °C, after the time was centrifuged at 12 000 rpm for 5 min, an aliquot of 200 µl was taken from the supernatant mixed with 150 µl of the agent Folin Ciocaltaeu 2 N, 2 ml of Na_2CO_3 to 2% were added, leaving it incubated for 25 min and finally the absorbance was read at 735 nm in spectrophotometer (Bio-145025 BIOMATE 5 Thermo elctron Corporation), the calibration curve was made with gallic acid.

The quantification of capsaicin (CAPs) was determined in fruits with physiological maturity, by the method described by Bennet and Kirby (1965), by a spectrophotometer (Bio-145025 Biomate-5 Thermo Electron Corporation) at a wavelength of 286 nm, in which capsaicin is in its organic phase. For the determination of the concentration a calibration curve was constructed of this antioxidant (Sigma, Co) in a range 0.5 to 1.5 mg ml^{-1}. In quality variables the evaluations were tripled for each treatment.

The number of spores was quantified by triplicate for each treatment and repetition in 100 g of soil with the method of wet sieving and decanting (Genderman and Nicolson, 1963), root cleaning and staining was performed with the Phillips and Hayman method (1970) and colonization (McGonigle, 1990) which consists of washing the roots with running water, cut them and place them in 25 ml falcon tubes, cover them with 10% KOH by 24 h at room temperature, then rinsed with plenty of running water, covered with H_2O_2, for 5 min, then rinsed with running water, covered roots with 10% HCl for 10 min, then HCl was decanted and without rinsing the roots, the 0.05% trypan blue solution was added by 24 h at room temperature, after time the dye was removed with the help of a sieve and they were placed in lactoglycerol, finally segments of 1 cm root were cut and deposited in a slide, were observed in an optical microscope (Axio Scope A1, Carl Zeiss, Microscopy GmbH, Gotting, Germany). The results obtained were analyzed using a variance analysis and the comparison of means by the Tukey test ($p\leq$ 0.05), using the SAS version 9.0 statistical program.

Results and discussion

In Figure 1 the RFA was measured noting that maximum radiation is between 13 and 15 h, and in CA there is 95% higher compared to the MR who captures radiation between 350 to 1 050 nm, MB and MN are similar and the lowest absorption was carried out with MA whose radiation is between 400 and 500 nm (Ayala-Tafoya *et al.*, 2015). In flower crops the black mesh reduces RFA between 55 and 60% depending on the season and red mesh from 41 to 51% (Arthurs *et al.*, 2013).

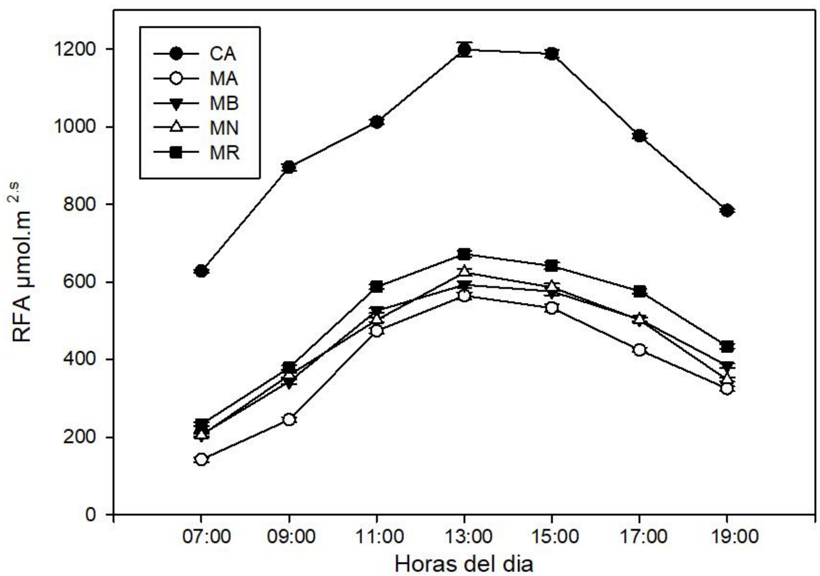

Figure 1. Photosynthetically active radiation in open field and colored meshes in piquin chili plants.

Table 1 shows the temperature values, the month with the highest record is may in open field decreasing between 1 and 2 degrees until September. It is also observed that MR and CA have similar temperature and MA decreases from 2 to 5 degrees in relation to CA. The Table 1 shows that HR increased each month until it reaches its maximum in September, also in CA the largest HR is observed only swing in September with MA and MB.

Table 1. Monthly average values of climate variables monitored in each environmental condition under open field and color meshes.

Cover	Variable	Months evaluated				
		May	June	July	August	September
CA	T (°C)	26.98	25.56	23.34	22.86	20.74
	HR (%)	34.54	43.1	57.32	62.38	69.36
MA	T (°C)	21.45	22.34	21.55	19.33	18.44
	HR (%)	34.22	42.11	55.32	63.44	70.44
MB	T (°C)	22.13	23.11	22.11	20.55	18.98
	HR (%)	34.58	44.55	55.67	61.78	65.89
MN	T (°C)	22.33	22.41	22.15	20.77	20.11
	HR (%)	33.77	42.33	55.65	60.43	66.51
MR	T (°C)	23.78	24.51	23.11	21.52	20.17
	HR (%)	33.11	41.22	56.73	60.22	68.55

T°= temperature; HR= relative humidity.

Table 2 shows that MB positively favors agronomic variables evaluated with 320% in AP, 322.7% in DT, 235.8% in LR, 8 times the PFP, 8.5 times the PSP, 327.2% the PFR, 5 times the PSR, 6.8 times the RPP and 4.8 times the NFPP compared to CA which was the least favorable environment, perhaps under normal growing conditions of piquin chili plants these are under the shade of a tree or shrub which prevent direct radiation. The MB reflects 70% radiation allowing favorable conditions for the growth of the plant, the pearl-colored mesh increased its transmission from 700 nm which favors cucumber photosynthesis (Ayala-Tafoya *et al*., 2015) and consequently the growth of the plant unlike what was reported by Shahak *et al*. (2008) indicating that production was increased in three crops of pepper chili with 16% pearl mesh and 32% with red mesh compared to black mesh.

The ecotype that favored the aforementioned variables was RTZ, only LNL increased the LR and MZC the PSR (Table 2). However, it has been reported that each morphotype presents different to the environmental conditions as is the case with morphotypes from Oaxaca whose phenotypic characteristics are very different (Castellón Martínez *et al*., 2014), to those from Nuevo León, Coahuila and Zacatecas used in this study. In Table 2, it is observed that inoculation with native mycorrhizae had a favorable response with the increase of 53% in AP, 32% in DT, 70.24% in PFP, 66.9% in PSP, 72.2% in R and 28.8% in NFPP. This matches Cardona *et al*. (2008) who found that arbuscular mycorrhizae colonize the roots of the genus *Capsicum*, for this the morphological characteristics of piquin chili were improved.

Table 2. Agronomic variables of the different factors evaluated in piquin chili plants.

Cover	AP (cm)	DT (mm)	LR (cm)	PFP (g)	PSP (g)	PFR (g)	PSR (g)	RPP (g)	NFPP
CA	22.94e	4.1f	15.1e	24.81e	11.34e	10.6e	4.36e	5.5f	36.52f
MN	44.62d	6.96e	28.44d	43.04d	19.56e	18.13d	7.21d	16.81d	80.1e
MR	53.69b	9.76b	29.79c	93.63b	36.59b	21.14c	11.74c	6.08c	113.08b
MA	47.25cd	9.39c	36.12a	64.13c	29.31c	24.31b	14.54b	22.8b	105.17c
MB	73.42a	13.23a	35.6ab	200.52a	96.71a	34.68a	21.32a	37.42a	177.08a
Ecotype									
SAC	33.75e	6.9e	28.22e	44.85d	20.89e	19.45f	10.75d	7.09f	59.50f
MZC	42.35d	8.46c	28.28d	76.44c	34.71cd	24.37b	14.53a	26.08b	133.75b
PTZ	44.6cd	8.73c	27.8f	75.63c	32.01d	20.81c	11.24c	10.57d	70.9e
RTZ	71.25a	9.8a	29.13c	115.54a	48.73a	20.7d	10.35f	50.48a	169.31a
LNL	52.46b	9.29b	30.68a	87.44b	43.87b	25.15a	13.43b	11.93c	94.19c
SNL	46.08c	7.68d	29.73b	74.94c	37.16c	20.15e	10.7e	9.27e	78.1d
Consortium									
SM	38.28b	7.31b	25.25b	58.57b	27.15b	18.03	9.05b	14.13b	88.24b
CM	58.56a	9.65a	32.69a	99.71a	45.3a	25.52a	14.62a	24.33a	113.68a
S*	**	**	**	**	**	**	**	**	**
CV (%)	9.53	5.82	8.54	13.78	14.59	9.91	14.86	5.89	5.81

AP= plant height; DT= stem diameter; LR= root length; PFP= fresh weight of plant; PSP= dry weight of plant; PFR= fresh weight of root; PSR= dry weight root; RPP= yield per plant; NFP= number of fruits per plant; RTZ= Tuxpan River, Zacatecas; PTZ= Tepetatilla Bridge, Zacatecas; LNL= Linares, Nuevo León; MZC= Múzquiz, Coahuila; SNL= Santiago, Nuevo León; SAC= San Alberto, Coahuila; MB= white mesh; MA= blue mesh; MR= red mesh; MN= black mesh; CA= open field; SM= without mycorrhiza; CM= with mycorrhiza. Means with the same letter within each column in each factor do not differ statistically (Tukey, $p \leq 0.05$). CV=coefficient of variation. S*= significance; **= highly significant ($p \leq 0.001$).

In Table 3, quality variables are presented, it is observed that for SST they are increased in fruits with 32% with MR and 37.6% in MB, vitamin C (42.9%) and total phenols also with (44.31%) MB and Capsaicin with MA 18.42%, in addition to the itching represented by Scoville units (SHU) in the MA, in the same table the ecotypes that present statistical differences for SST are the MZC and SNL ecotypes. For Vitamin C, SAC and SNL, total phenols SNL, for capsaicin and itching (US) PTZ.

Table 3. Quality variables of factors evaluated in piquin chili plants.

Cover	SST (°Brix)	Vit C (mg 100 g^{-1})	FT (µg EAG g-1)	CAPs (mg g^{-1})	U Scoville SHU
CA	6.41c	60.85f	34.77e	2.66d	42 560d
MN	6.69c	66.3e	37.09d	3.07b	49 120b
MR	8.46a	84.29b	42.42b	2.95c	47 200c
MA	7.52b	71.24c	43.05b	3.15a	50 400a
MB	8.82a	86.96a	50.18a	3.09ab	49 440ab
Ecotype					
SAC	6.71c	82.98a	34.91d	2.98bc	47 680bc
MZC	8.57a	68d	37.47c	2.93c	46 880c
PTZ	8.31a	79.95b	37.54c	3.09a	49 440a
RTZ	7.4b	78.38c	46.28b	3.02b	48 320b
LNL	7.41b	82.97a	35.82d	2.93c	46 880c
SNL	8.39a	45.21e	55.37a	3.02b	48 320b
Consortium					
SM	8.31a	72.27b	40.08b	2.92b	46,720b
CM	7.29b	73.56a	42.38a	3.07a	49 120a
S*	**	**	**	**	**
CV (%)	5.09	0.85	5.79	3.98	3.98

SST= total soluble solids; Vit C= vitamin C; FT= total phenols; CAPs= capsaicin; RTZ= Tuxpan River, Zacatecas; PTZ= Tepetatilla Bridge, Zacatecas; LNL= Linares, Nuevo León; MZC= Múzquiz, Coahuila; SNL= Santiago, Nuevo León; SAC= San Alberto, Coahuila; MB=white mesh; MA= blue mesh; MR= red mesh; MN= black mesh; CA= open field; SM= without mycorrhiza; CM= with mycorrhiza. Means with the same letter within each column in each factor do not differ statistically (Tukey, $p \leq 0.05$). CV= coefficient of variation. S*= significance; **= highly significant ($p \leq 0.001$).

The CM application produced better results in vitamin C (1%), total phenols (3.87%) and capsaicin (5.13%) increasing the antioxidants of the fruit and itching, only in SST was obtained the 27% increase SM. In relation to quality a study was carried out with different chili morphtypes such as piquin and solterito which produced higher phenol and flavonoid content in addition to capsaicin (Wei *et al*., 2013), although mycorrhizae were not added, the tendency of morphotypes is to produce higher antioxidant content.

In Table 4 shows that when analyzing the cover separately the MB is the one that increases by 96% the number of spores and 101.4% of colonization. It is also shown that the SNL ecotype increased NE and RTZ the % colonization, in relation to inoculation with native mycorrhizae favored NE and % colonization in piquin chili plants. This is consistent with a study about papaya where it is inoculated with *Glomus* sp. finding an increase in the percentage of colonization (Quiñones-Aguilar *et al*., 2014).

Table 4. Microbiological variables of factors evaluated in piquin chili plants.

Cover	NE	% Col
CA	23.33e	16.17c
MN	36.67b	23.67b
MR	31.15cd	25.67b
MA	35.83bc	25.92b
MB	45.73a	32.57a
	Ecotype	
SAC	29.27c	25.58ab
MZC	34.48ab	25.42ab
PTZ	34.79ab	23.42b
RTZ	35ab	27.58a
LNL	31.56bc	23.5b
SNL	37.19a	25.08b
	Consortium	
SM	5.38b	2.31b
CM	62.05a	47.89a
S*	*	*
CV (%)	25.75	23.62

NE= number of spores; % Col= colonization percentage; RTZ= Tuxpan River, Zacatecas; PTZ= Tepetatilla Bridge, Zacatecas; LNL= Linares, Nuevo León; MZC= Múzquiz, Coahuila; SNL= Santiago, Nuevo León; SAC= San Alberto, Coahuila; MB= white mesh; MA= blue mesh; MR= red mesh; MN= black mesh; CA= open field; SM= without mycorrhiza; CM= with mycorrhiza. Means with the same letter within each column in each factor do not differ statistically (Tukey, $p \leq 0.05$).

Principal component analysis (ACP), performed for the cover type and variables evaluated Figure 3, showed that MB positively influenced in the RPP, PFR, AP, PSR, DT and NFPP variables, contrary to what was obtained with MN and CA, while the MA influenced the content of Caps and LR, in turn the MR promoted an increase in the variables Vit C, SST, PFP and PSP, so the use of different cover promote diverse results in morphology and quality in piquin chili plants; consistent with what was reported in cucumber (Ayala-Tafoya *et al*., 2015) when colored meshes were used.

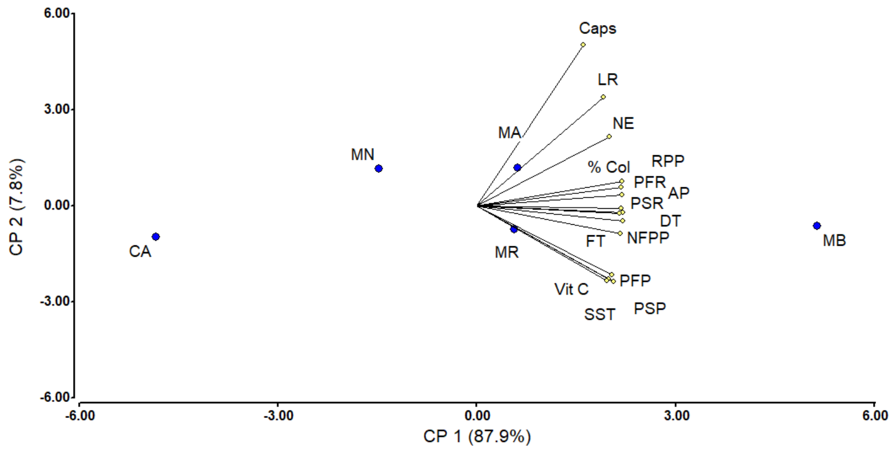

Figure 3. Principal components of analyzed variables and cover type. AP= plant height; DT= stem diameter; LR= root length; PFP= fresh weight of plant; PSP= dry weight of plant; PFR= fresh weight of root; PSR= dry weight of root; RPP= yield per plant; NFP= number of fruits per plant; SST= total soluble solids; Vit C= vitamin C; FT= total phenols; CAPs= capsaicin; NE= number of spores; % Col= colonization percentage; MB= white mesh; MA= blue mesh; IN= greenhouse; MR= red mesh; MN= black mesh; CA= open field.

The ACP for ecotypes and variables evaluated Figura 4, revealed a dispersed behavior of the ecotypes evaluated; however, it emphasizes that the RTZ ecotype favors the variables AP, PSP, RPP, NFPP, DT; while SAC and PTZ ecotypes are not favored in these variables, while LNL ecotype favored the increase in PFR and PSR and MZC and SNL ecotypes influenced the quality variables, Vit C, Caps and SST, demonstrating that the ecotype plays a primary role in the variables that were evaluated. This is consistent with Wei *et al*. (2013) about different chili morphotypes where it finds differences in the content of capsaicin and phenols.

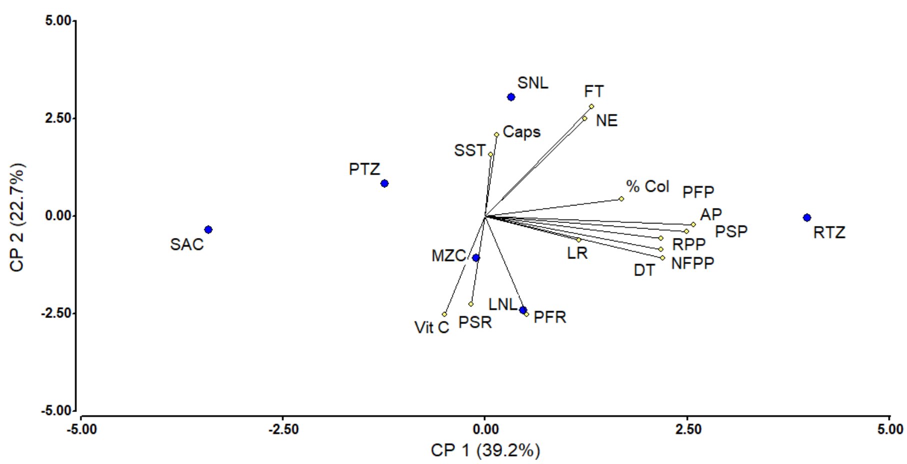

Figure 4. Principal components of the analyzed variables and the ecotype. AP= plant height; DT= stem diameter; LR= root length; PFP= fresh weight of plant; PSP= dry weight of plant; PFR= fresh weight of root; PSR= dry weight of root; RPP= yield per plant; NFPP= number of fruits per plant; SST= total soluble solids; Vit C= vitamin C; FT= total phenols; CAPs= capsaicin; NE= number of spores; % Col= colonization percentage; RTZ= Tuxpan River, Zacatecas; PTZ= Tepetatilla Bridge, Zacatecas; LNL= Linares, Nuevo León; MZC= Múzquiz, Coahuila; SNL= Santiago, Nuevo León; SAC= San Alberto, Coahuila.

The ACP with the application of mycorrhizae (CM) and the absence of these (SM) with the variables studied in Figure 5, demonstrates that there was a strong relationship in most variables evaluated when they were inoculated with *mycorrhizae (Glomus mosseae*, *Rhizophagus intraradices, Sclerocystis coremioides* and *Gigaspora albid*), with the exception of Vit C and the SST variable that was increased when the mycorrhizae were not applied. Similar results were obtained in tomato when was inoculated with *Glomus mossae* and *G. cubense* with 20 and 40 spores by increasing yield (Mújica, 2012).

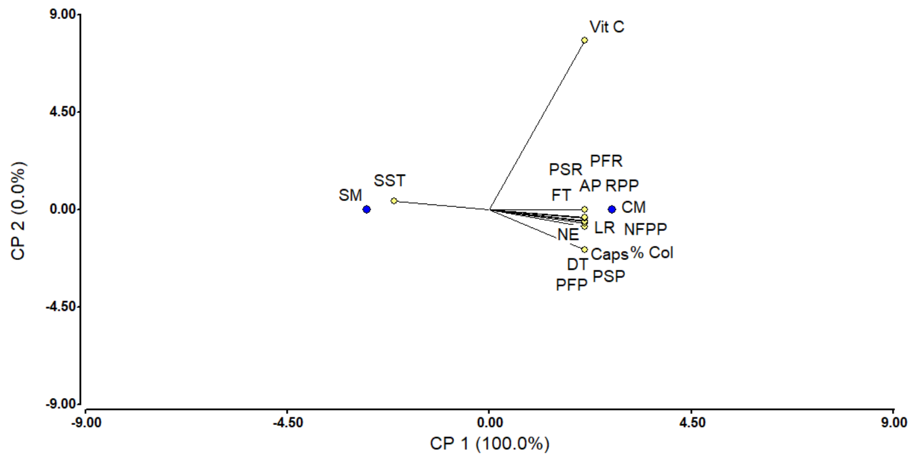

Figure 5. Principal components of the analyzed variables and Consortium. AP= plant height; D= stem diameter; LR= root length; PFP= fresh weight of plant; PSP= dry weight plant; PFR= fresh weight of root; PSR= dry weight of root; RPP= yield per plant; NFP= number of fruits per plant; SST= total soluble solids; Vit C= vitamin C; FT= total phenols; CAPs= capsaicin; NE= number of spores; % Col= colonization percentage; SM= without mycorrhizae; CM= with mycorrhizae.

Conclusions

Blue mesh with the lowest RFA had a positively impacted on the agronomic, quality and response variables to the number of spores and percentage of colonization. The SNL ecotype influenced quality variables such as SST, Vit C, phenols and NE, and RTZ on agronomic variables and % colonization. The inoculation with the mycorrhizae consortium produced favorable changes in agronomic characteristics and quality of the piquin chili fruit.

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