ARBUSCULAR MYCORRHIZAL FUNGI ALLEVIATE SALT STRESS ON SWEET *(Ocimum basilicum* L.) SEEDLINGS†

[HONGOS MICORRIZICOS ARBUSCULARES ALIVIAN EL ESTRÉS SALINO EN SEMILLAS DE ALBAHACA DULCES (*Ocimum basilicum* L.)]

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SUMMARY
Abiotic stress due to salinity is considered one of the main problems facing agriculture that has the detrimental effect of restricting the growth and development of plants. The objective of this study was to evaluate the effect of arbuscular mycorrhizal fungus species (*Rhizophagus fasciculatum*) as reliever of NaCl-stress in sweet basil seedlings in emergence stage. A completely randomized design with factorial arrangement with four replications was used; first factor was sweet basil varieties (Nufar, Genovese, and Napoletano), factor two were NaCl concentrations (0, 50, and 100 mM) and factor three was inoculation of seeds with *Rhizophagus fasciculatum* (+AMF) an arbuscular mycorrhizal fungi and non-inoculated seeds (−AMF). Chemical composition of the substrate, rate and emergence percentage, plant height, root length, fresh and dry biomass of aerial part and root, spore count, and colonization percentage were assessed. The results showed differences in all variables with Napoletano showing higher values of all variables at 0, 50 or 100 mM NaCl with AMF. Majority of variables decreased with NaCl concentrations. The substrate was suitable for growth of *Rhizophagus fasciculatum* and for sweet basil. No root colonization was found in any seedlings inoculated with AMF; however, the micrograph of the inoculated plants showed vegetative mycelium, a mycorrhizal structure that shows the initiation of the colonization process.
Keywords: *Ocimum basilicum*; emergence rate; emergence percentage; salt tolerance; NaCl; colonization.

RESUMEN
El estrés abiótico por salinidad es considerado como uno de los principales problemas que enfrenta la agricultura que tiene como efecto perjudicial restringir el crecimiento y el desarrollo de las plantas. El objetivo de este estudio fue evaluar el efecto de la especie de hongo micorrízico arbuscular (*Rhizophagus fasciculatum*) para mitigar el estrés por NaCl en plántulas de albahaca dulce en la etapa de emergencia. Se utilizó un diseño completamente al azar con arreglo factorial con cuatro replicas; factor 1 (Nufar, Genovese y Napoletano), el factor 2 fue las concentraciones de NaCl (0, 50 y 100 mM) y el factor 3 fue la inoculación de semillas con *Rhizophagus fasciculatum* (+ HMA), y semillas sin inocular (-HMA). Se evaluó la composición química del sustrato, el porcentaje y tasa de emergencia, la altura de las plántulas, la longitud de la raíz, la biomasa fresca y seca de la parte aérea y la raíz, conteo de esporas y el porcentaje de colonización. Los resultados mostraron diferencias en todas las variables siendo Napoletano la variedad que mostró mayores valores en 0, 50 ó 100 mM NaCl con HMA. La mayoría de las variables disminuyeron al incrementar las concentraciones de NaCl. El sustrato fue adecuado para el crecimiento de *Rhizophagus fasciculatum* y para la albahaca dulce. No se encontró colonización de la raíz en ninguna plántula inoculada con HMA, sin embargo, la micrografía de las plantas inoculadas mostró micelio vegetativo, estructura micorrízica que muestra que se inició el proceso de colonización.
Palabras clave: *Ocimum basilicum*; Tasa de emergencia; Porcentaje de emergencia; Tolerancia a la sal; NaCl; colonización

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INTRODUCTION

The intensive and disproportionate exploitation of resources has been the main tendency in productive agroecosystems for the past decades worldwide. Depleted and saturated soils have been caused by the excessive use of fertilizers, and salinity conditions are examples of the current situation (Aguado *et al*., 2012). Soil salinity is one of the main adverse factors crops have faced around the world (Das *et al*., 2015). This problem is a big concern in arid and semiarid areas where low rainfall, high evapotranspiration rates, and soil salinity are some of the limiting factors (Akram *et al*., 2010). Several environmental factors as salinity have an unfavorable impact on plant growth (Hashem *et al*., 2015a). Stress tolerance in plants is a complex phenomenon involving many changes at biochemical and physiological level. However, the mechanisms behind stress tolerance seem to be affected by the colonization of arbuscular mycorrhizal fungi (AMF) (Al-Karaki, 2000). Some studies have shown that inoculation with AMF greatly enhances growth, water uptake and essential nutrients for plants even in saline conditions (Zhu *et al*., 2012). The arbuscular mycorrhizal symbiosis is the evident result of the interaction between plant roots and a fungus, which is an example of the morphological alterations that roots have with the purpose of adapting themselves to the presence of a symbiont. In recent years, publications about the benefits of AMF in different crops have increased (Pérez *et al*., 2011).

There are mycorrhizal inoculants that are applied in small quantities, so they integrate to cultural practices (González *et al*., 2011; Harikumar, 2017) allowing them to become a promissory practice of biological basis for agricultural production. Sweet basil (*Ocimum basilicum* L.) is a crop with high demand in the international market. Among the crop limitations, its establishment is in areas where soil salinity is high (Batista-Sánchez *et al*., 2015). In the Peninsula of Baja California, Mexico, salinity in soil and water is one of the main problems agriculture confronts influencing in the decrease of basil yield, so studies with ecological alternatives should be performed to determine the tolerance of basil varieties able to establish in these conditions. It has been shown that arbuscular mycorrhizal fungi (AMF) are able to promote the growth of some plants and their tolerance to salinity The AMF use various mechanisms that favor the acquisition of nutrients, the production of plant growth hormones, the development of the rhizosphere, and even soil conditions (Qiang-Sheng *et al*., 2010; Harris-Valle *et al*., 2011; Mujica-Pérez and Fuentes-Martínez, 2012; Khalil, 2013; Sinclair *et al*., 2014; Hashem *et al*., 2015b; Elhindi *et al*., 2016; Khalid, *et al*., 2017; Wang *et al*., 2018). In studies carried out by Reyes-Pérez *et al*. (2014) and Batista-Sanchez *et al*. (2017) to determine the effect of NaCl on the growth and development of basil plants observed that Napoletano showed tolerance to NaCl-stress. The aim of this study was to evaluate the effect of the *Rhizophagus fasciculatum* strain, characterized as AMF as possible stress reliever by NaCl in the emergence rate, emergence percentage and morphometric variables of seedlings of different basil varieties.

MATERIALS AND METHODS

Ethics statement

The research conducted herein did not involve measurements with humans or animals. The study site is not considered a protected area. For location/activities, no specific permissions were required and the studies did not involve endangered or protected species. However, to carry out research activities on the lands administered by Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR®), permission was granted by Ing. Saul Edel Briseño-Ruiz, manager of the experimental open-field and shade-enclosure areas at CIBNOR®. The seeds of the basil commercial varieties used were obtained from a store of agricultural products at La Paz, Baja California Sur, México. The varieties of *Ocimum basilicum* L. used in the present study are not pondered an endangered species and their use therefore had insignificant effects on broader ecosystem functioning.

Study area and characteristics of the place

The experiment was developed in a shadow mesh structure (40% of shading, black color, model 20 mesh) in the experimental field of CIBNOR located northwest of La Paz, BCS, Mexico, 24° 08' 10.03 N and 110° 25' 35.31 W, 7 m.a.s.l. The average temperature, maximum and minimum inside the shadow mesh during the experimental period were 25.59 ± 4.14, 34.7 ± 4.12 and 16.9 ± 4.11 °C, respectively with 69.29 ± 13.31% of relative humidity. Data of the climatological variables recorded during the study period were obtained from a portable weather station (Vantage Pro2® Davis Instruments, USA), which was placed inside the shadow mesh structure. The experimental site has a weather of Bw (h') hw (e) considered as semiarid with xeric vegetation (García, 2004).

Genetic material

Basil varieties used were Napoletano, Genovese, and Nufar, which were reproduced by seeds (Vis Seed Company®, Arcadia, Cal., USA). Prior to the experiment, the quality of seed varieties was evaluated through a germination test according to the suggested methodology by ISTA (2010).

Experimental conditions

An arbuscular mycorrhizal fungi from a monosporic crop was used, containing the *Rhizophagus fasciculatum* species for inoculating the seed lot at seedtime using the method of coating seeds proposed by Fernández *et al*. (1999) and another seed lot was uninoculated as the control. The inoculum used in the experiment had a spore content count of 25-50 spores g^{-1} in the substrate. The seeds were sown in polystyrene trays with 200 cavities, which contained sterilized vermiculite as substrate. Saline treatments based on NaCl (0, 50, and 100 mM NaCl) were applied daily (08:30 h). The quantity applied to each treatment was 500 mL per tray in order to make sure the solution drained through the holes and avoided the accumulation of salts in the substrate, which could increase salt concentration. The emergence of each seedling was recorded daily, and the final emergence percentage was determined at 14 d. The emergence rate (M) is the speed that a seed germinated over a period of time (in days) was calculated using Maguire (1962) equation: $M = n_1/n_2 + t_1/t_2 + \ldots n_{30}/t_{14}$; where n_1, $n_{2\ldots}n_{30}$ is the number of germinated seeds in the times t_1, $t_2 \ldots t_{14}$ (in days).

Chemical analysis of the substrate

Samples of substrate were taken and sieved through a 10 mesh (2 mm). The pH and electrical conductivity (EC) were measured with a soil relationship solution of 1:5. The pH was measured using a potentiometer (Hanna®, model 211, USA). The EC (dS cm^{-1}) was measured using a conductimeter (Hach®, Model Sension5, Loveland, Colorado, USA). Extractable phosphorus (P, mg kg^{-1}) was determined from the aqueous extract with a soil relationship solution of 1:5 and using Multiskan Acent® (Model Labsystems, No. 354, Finland). Extractable potassium (K, mg kg^{-1}) was determined using a flame atomic absorption spectrophotometer (GBC®, model Avanta, Australia). Extractable calcium and magnesium were measured by complexometry by the volumetric titration method (EDTA titration with 0.01 N). Organic matter content (OM, %) was determined by the Walkley and Black method using a 35 mesh (0.5 mm). Total nitrogen (N) was determined by the Dumas method (Leco®, Model FP-528, USA) using a 100 mesh (0.150 mm).

Morphometric values evaluated

Emerged seedlings were maintained during 21 days and after this period, 10 seedlings per replication were randomly selected; then, plant height (cm), root length (cm), fresh and dry biomass of aerial part (g), fresh and dry biomass of root (g) were determined by the destructive method, dividing each seedling in root and aerial part, and weighing each one separately using an analytical balance (Mettler Toledo®, AG204, USA). Once the fresh weight of roots and aerial part were obtained, they were placed in paper bags and then in a drying oven (Shel-Lab®, FX-5, series-1000203, USA) at 70 °C until constant weight (about 72 h). Subsequently, they were weighed on analytical balance (Mettler Toledo®, AG204, USA) expressing the dry matter weight in grams.

Extraction and spores counting of the inoculum and percentage of colonization

The AMF spore extraction was developed by the wet sieving and decanting technique described by Daniels and Skipper (1982) and modified by Utobo *et al*. (2011) and the number of spores in the inoculum were quantified with a dissecting microscope. The percentage of mycorrhizal colonization was determined by the methodology described by Hashem *et al*. (2014). Colonization was assessed taking into account the presence of vesicles, arbuscules and/or coenocytic hyphaes (hyphaes) typical of the AMF.

Experimental design

The experiment was developed using a completely randomized design with factorial arrangement with four replications of 30 seeds each, using three factors in study, first factor was the varieties of sweet basil (Nufar, Genovese, and Napoletano), factor two was the NaCl concentrations (0, 50, and 100 mM) and factor three was mycorrhizal inoculation treatments [*Rhizophagus fasciculatum* (+ AMF) and non-inoculated (− AMF)].

Statistical analysis

Analysis of variance and differences among means of each factor and variable was performed (Tukey HSD $p<0.05$). To determine the linear or curvilinear relationship among NaCl concentrations and the response of all variables, a polynomial regression analysis was performed. The emergency percentage data were transformed by arcsine (Little and Hills, 1989; Statsoft, 2011). The statistical analyzes were carried out with Statistica® v. 10.0 for Windows® (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Napoletano showed greatest emergence rate (ER) followed by Nufar and Genovese; ER showed greatest values in 0 and 50 mM and lowest in 100 mM but inoculated seeds with AMF showed greatest ER (Table 1). Varieties × NaCl interaction showed greatest ER in Napoletano and Nufar with 0 and 50 mM while Genovese showed lowest ER in 50 mM

(Table 2). The interaction NaCl × AMF showed greatest ER in 0 mM with AMF while the lowest ER was with 100 mM without AMF (Table 2). Varieties × NaCl × AMF interaction showed that ER was higher in Napoletano with 0 and 50 mM with AMF, Nufar with 0 mM with AMF, while Genovese showed lowest ER in 100 mM without AMF (Table 3). One of the primary effects of salt stress is to delay seedling emergence (Martínez-Villavicencio *et al*., 2011). A significant decrease of ER in the varieties was showed as NaCl concentrations increased, that is attributed to the salinity detrimental effect on crops in early stages (Tavakkoli *et al*., 2011). In this study, ER was greatest in seedlings where seeds were inoculated with *Rhizophagus fasciculatum* (+ AMF) but no was due to inoculation, since in emergence, no arbuscules were found, which is a mycorrhizal structure that must be present for the plant to get the benefits of this endophyte (Colla *et al*., 2008). Studies carried out by Mata-Fernández *et al*. (2014) reported that the most common effect of salt stress is the reduction in the ability to absorb water. Napoletano showed greatest emergence percentage (EP) followed by Nufar and Genovese; EP was greatest in 0 and 50 mM and lowest in 100 mM but increased in those seeds treated with AMF (Table 1). Varieties × NaCl interaction showed greatest EP in Napoletano with 0 and 50 mM while Genovese showed lowest EP in 50 mM (Table 2). Varieties × AMF showed greatest EP in Napoletano with AMF but EP showed lowest values in Genovese without AMF (Table 2). The interaction NaCl × AMF showed greatest EP in 0 mM with AMF and lowest EP in 100 mM without AMF (Table 2).

Table 1. Average values by each factor (varieties, NaCl and AMF). The values correspond to the rate and emergence percentage and morphometric variables of sweet basil seedlings under NaCl stress from seeds inoculated with *Rhizophagus fasciculatum* (+ AMF) and non-inoculated (− AMF).

Varieties	ER	EP (%)	PH (cm)	RL (cm)	FBAP (g)	DBAP (g)	FRB (g)	DRB (g)
Genovese	2.59±0.73 c	61.66±12.93 c	1.18±0.15 c	2.02±0.51 c	0.148±0.02 c	0.018±0.007 b	0.041±0.02 c	0.010±0.007 b
Napoletano	4.01±0.84 a	81.94±11.87 a	2.12±1.24 a	4.32±0.97 a	0.334±0.13 a	0.121±0.014 a	0.163±0.11 a	0.079±0.049 a
Nufar	3.63±0.78 b	78.05±6.24 b	1.60±0.26 b	3.97±1.0 b	0.223±0.05 b	0.059±0.011 b	0.096±0.06 b	0.014±0.012 b
Significance	***	***	***	***	***	***	***	***
NaCl (mM)	ER	EP (%)	PH (cm)	RL (cm)	FBAP (g)	DBAP (g)	FRB (g)	DRB (g)
0	3.64±1.14 a	75.69±15.96 a	2.18±1.22 a	4.07±1.54 a	0.278±0.15 a	0.071±0.013 ab	0.166±0.116 a	0.043±0.079 a
50	3.48±0.99 a	75.13±14.24 a	1.40±0.29 b	3.28±1.12 b	0.219±0.07 b	0.028±0.011 b	0.081±0.051 b	0.017±0.009 b
100	3.10±0.73 b	70.83±10.82 b	1.32±0.20 b	2.96±1.06 b	0.208±0.05 b	0.099±0.006 a	0.054±0.024 c	0.012±0.008 b
Significance	***	***	***	***	***	***	***	***
AMF	ER	EP (%)	PH (cm)	RL (cm)	FBAP (g)	DBAP (g)	FRB (g)	DRB (g)
+ AMF	4.07±0.78 a	81.38±10.03 a	1.86±1.08 a	3.86±1.39 a	0.264±0.125 a	0.088±0.013 a	0.119±0.107 a	0.034±0.066 a
− AMF	2.75±0.68 b	66.38±13.08 b	1.40±0.31 b	3.02±1.13 b	0.206±0.045 b	0.050±0.011 b	0.082±0.051 b	0.014±0.007 b
Significance	***	***	***	***	***	*	***	***

ER = emergence rate; EP = emergence percentage; PH = plant height; RL= root length; FBAP = fresh biomass of aerial part; BSPA = dry biomass of aerial part; FRB = fresh root biomass; DRB = dry root biomass; NaCl = sodium chloride; AMF = Arbuscular mycorrhizal fungi; −AMF= without AMF (control), + AMF= with AMF (1 g of the AMF inoculum). Values in each column followed by the same letter(s) are not significantly different at $p \leq 0.05$ (Tukey HSD). Significance level: *= $p \leq 0.05$; ***= $p \leq 0.001$ (means ± SD).

Table 2. Average values of rate and percentage of emergence and morphometric variables regarding the interactions varieties × NaCl, varieties × AMF and NaCl × AMF in sweet basil seedlings under NaCl stress from seeds inoculated with *Rhizophagus fasciculatum* (+ AMF) and non-inoculated (− AMF).

Varieties	NaCl	ER	EP (%)	PH (cm)	RL (cm)	FBAP (g)	DBAP (g)	FRB (g)	DRB (g)
Genovese	0	2.62±0.76d	62.08±14.02c	1.34±0.05cd	2.26±0.46e	0.180±0.013c	0.023±0.005c	0.052±0.018d	0.010±0.007b
	50	2.51±0.65d	59.16±11.65c	1.08±0.08d	1.98±0.15e	0.167±0.14c	0.021±0.004c	0.036±0.018d	0.016±0.008b
	100	2.63±0.87d	63.75±14.30c	1.12±0.14cd	1.81±0.70e	0.098±0.024d	0.010±0.005c	0.033±0.010d	0.012±0.003b
Napoletano	0	4.30±0.95a	87.50±13.18a	3.40±1.46a	4.83±1.22ab	0.414±0.18a	0.049±0.01bc	0.292±0.10a	0.101±0.12a
	50	4.24±0.70a	86.66±7.35a	1.53±0.20c	4.22±0.80bc	0.325±0.06b	0.040±0.007bc	0.127±0.04bc	0.025±0.008b
	100	3.47±0.66bc	71.66±7.35b	1.42±0.09c	3.91±0.68c	0.263±0.02c	0.274±0.005a	0.072±0.02d	0.020±0.007b
Nufar	0	3.99±0.99ab	77.50±9.55b	1.80±0.23b	5.14±0.61a	0.242±0.08c	0.141±0.01b	0.153±0.06b	0.017±0.009b
	50	3.70±0.73ab	79.58±3.30b	1.59±0.22bc	3.63±0.57cd	0.236±0.02c	0.024±0.008c	0.081±0.02cd	0.009±0.005b
	100	3.21±0.35c	77.08±4.86b	1.42±0.17c	3.15±0.37d	0.192±0.01c	0.012±0.005c	0.056±0.01d	0.007±0.007b
Significance		***	***	***	***	***	***	***	**

Varieties	AMF	EP (%)	PH (cm)	FBAP (g)	DBAP (g)	FRB (g)	DRB (g)
Genovese	+ AMF	73.05±7.17c	1.22±0.146d	0.167±0.012c	0.020±0.006b	0.043±0.019c	0.015±0.008b
	− AMF	50.27±3.88d	1.13±0.145d	0.130±0.011d	0.016±0.007b	0.038±0.016c	0.010±0.002b
Napoletano	+ AMF	89.44±9.83a	2.64±1.581a	0.415±0.133a	0.124±0.014a	0.189±0.134a	0.077±0.005a
	− AMF	74.44±8.68c	1.60±0.342bc	0.253±0.028b	0.118±0.013a	0.138±0.057b	0.021±0.005b
Nufar	+ AMF	81.66±5.03b	1.73±0.241b	0.249±0.054b	0.101±0.012a	0.124±0.065b	0.013±0.001b
	− AMF	74.44±5.38c	1.47±0.223c	0.198±0.042b	0.017±0.007b	0.069±0.030c	0.010±0.008b
Significance		***	***	***	*	**	**

NaCl (mM)	AMF	ER	EP (%)	PH (cm)	FBAP (g)	DBAP (g)	FRB (g)
0	+ AMF	4.44±0.89a	86.38±11.59a	2.66±1.58a	0.360±0.173a	0.113±0.013a	0.212±0.138a
	− AMF	2.83±0.71de	65.00±12.19cd	1.70±0.32b	0.197±0.043cd	0.029±0.009bc	0.119±0.065b
50	+ AMF	4.10±0.79b	80.00±10.25bc	1.52±0.32bc	0.209±0.083bcd	0.031±0.01bc	0.083±0.062bc
	−AMF	2.87±0.78d	70.27±16.36c	1.27±0.18d	0.230±0.047b	0.025±0.011c	0.079±0.033bc
100	+ AMF	3.65±0.40c	77.77±6.09b	1.41±0.19cd	0.224±0.045bc	0.101±0.005ab	0.061±0.029c
	− AMF	2.55±0.52e	63.88±10.13d	1.23±0.16d	0.191±0.042d	0.097±0.005abc	0.047±0.017c
Significance		***	***	***	***	*	***

NaCl =sodium chloride (mM); + AMF = with AMF (1 g of the AMF inoculum); −AMF = without AMF (control); ER = emergence rate; EP = emergence percentage; PH = plant height; RL = root length; FBAP = fresh biomass of aerial part; DBAP = dry biomass of aerial part; FRB = fresh root biomass; DRB = dry root biomass. Values in each column followed by the same letter(s) are not significantly different at $p \leq 0,05$ (Tukey HSD). Significance level: *= $p \leq 0.05$. **= $p \leq 0.01$. ***= $p \leq 0.001$ (means ± SD).

The EP decrease in concentrations of 100 mM, attributed to the harmful effects caused by NaCl in early development stages such as emergence. Batista-Sánchez *et al*. (2015) suggested NaCl limits germination, emergence, and growth because most seedlings are sensitive to this condition. Seeds were inoculated (+ AMF) showed greatest EP; however, this result is mostly attributed to tolerance of the varieties to NaCl and not just to inoculation, as no root colonization was found in this stage. Emergence percentage decreased as NaCl concentrations increased, this result coincides with those reported by Parés *et al*. (2008). Napoletano showed greatest EP because it is a NaCl tolerant variety (Reyes-Pérez *et al*., 2013).

Napoletano had greatest plant height (PH) followed by Nufar and Genovese; pH was greatest in 0 mM and decreased as NaCl concentrations increased but was greatest in seedlings whose seeds were inoculated with AMF (Table 1). Varieties × NaCl interaction showed that PH was greatest in Napoletano in 0 mM, and in all varieties decreased as NaCl concentrations increased (Table 2). In the interaction varieties × AMF, Napoletano with AMF showed greatest PH while Genovese without AMF showed the lowest PH (Table 2). As far as the interaction NaCl × AMF, the PH was higher in 0 mM with AMF and decreased in 100 mM without AMF (Table 2). Concerning the interaction varieties × NaCl × AMF, Napoletano in 0 mM with AMF showed greatest PH whereas the PH decreased significantly in Genovese in 100 mM without AMF (Table 3). Lower plant height as NaCl increased interferes with mineral nutrition and cell metabolism (Amini *et al*., 2007), also NaCl stress modify the water uptake through the roots, phenomenon known as osmotic component (Fatma *et al*. 2014). Seedlings of inoculated (+ AMF) seeds showed greatest PH, but was not due to the inoculation.

Root length (RL) was longest in Napoletano followed by Nufar and Genovese and was longest in those plants treated with 0 mM and decreased as the concentrations of NaCl increased but the inoculation with AMF stimulated the root length (Table 1). Varieties × NaCl interaction showed that root was longest in 0 mM in both Nufar and Napoletano, decreasing as the NaCl increased (Table 2). The interaction varieties × NaCl × AMF showed that root was longest in Napoletano in 0 mM with AMF while lowest LR was showed by Genovese in 100 mM and without AMF (Table 3). NaCl stress causing reduced causes structural changes in meristematic cells and inducing structural changes in root and ion exhaust, and alterations of cell membranes (Abd-Allah *et al.*, 2015). The results of this research are consistent with the assertions of Fatma *et al*. (2014) that NaCl causes osmotic stress caused by salinity, plants reacts with a wide range of physiological responses at molecular and cellular level, including changes in plant development and morphology as an increase or decrease in root growth and changes in life cycle.

Fresh biomass of aerial part (FBAP) was greatest in Napoletano followed by Nufar and Genovese; was greatest in 0 mM and decreased as NaCl increased but was greatest in seedlings with seeds inoculated with AMF (Table 1). Varieties × NaCl interaction showed that FBAP was greatest in Napoletano at 0 mM but decreased in all varieties as NaCl increased (Table 2). In the interaction varieties × AMF, FBAP was greatest in Napoletano with AMF and lowest in Genovese without AMF (Table 2). The interaction NaCl × AMF showed greatest FBAP in 0 mM with AMF and lowest in 100 mM without AMF (Table 2). Varieties × NaCl × AMF indicated that FBAP increased their values in Napoletano with 0 mM with AMF and was lowest in Genovese with 50 mM with AMF (Table 3). Studies related to FBAP indicate NaCl causes biochemical changes such as increase of abscisic acid synthesis and osmoprotectants solute, physiological such as the change of membrane permeability, ions and water, reduction of transpiration and photosynthesis (Chávez and González, 2009), as well as water availability for plants (Elhindi *et al*., 2016). Other studies have attributed this effect to the impact of salt stress on growth and the effect of osmotic stress in the root zone of seedlings which entails a reduction in stem weight coinciding with a reduction in fresh biomass (Reyes-Pérez *et al*., 2013).

Dry biomass of aerial part (DBAP) was greatest in Napoletano while Nufar and Genovese showed lowest values; DBAP showed greatest values at 100 mM and lowest at 50 mM and increased in those seedlings whose seeds were inoculated with AMF (Table 1). Regarding the analysis of the interactions, varieties × NaCl revealed that DBAP was greatest in Napoletano in 100 mM and decreased in Nufar and Genovese decreasing slightly as NaCl increased (Table 2). Varieties × AMF showed that DBAP was greatest in Napoletano with and without AMF and Nufar with AMF (Table 2). The interaction NaCl × HMA showed that DBAP was greatest in 0 mM with AMF, but DBAP exhibited similar values in 100 mM with AMF (Table 2). In the interaction varieties × NaCl × AMF, the DBAP exhibited highest values in Napoletano with 100 mM with and without AMF and in Nufar with 0 mM with AMF (Table 3). The DBAP was greatest in Napoletano at 100 mM with AMF, which is attributed to the tolerance of this variety to salinity (Reyes-Pérez *et al*., 2013) while DBAP decreased in Genovese and Nufar as NaCl increased. This result agrees with those reported by Parés *et al*. (2008). Another result was obtained (Baracaldo *et al*., 2014) has registered a decrease in the growth of leaves, in terms of dry weight, when increasing the concentration of salinity. In the same way, these results coincide with those indicated for other species, such as coquia (García *et al*., 2011), wheat (Argentel *et al*., 2006) and guava (Casierra-Posada, 2006), in which there was a lower accumulation of dry biomass as the concentration of salts in the irrigation water increased.

Table 3. Average values of rate and emergence percentage and morphometric variables regarding the interaction varieties × NaCl × AMF of sweet basil seedlings under NaCl stress from seeds inoculated with *Rhizophagus fasciculatum* (+ AMF) and non-inoculated (− AMF).

Varieties	NaCl (mM)	AMF	ER	PH (cm)	RL (cm)	FBAP (g)	DBAP (g)	FRB (g)	DRB (g)
Genovese	0	+ AMF	3.29±0.56 f	1.36±0.04 defgh	2.52±0.57 fgh	0.185±0.01 fgh	0.026±0.006 b	0.059±0.020 def	0.009±0.001 b
	50	+ AMF	3.10±0.54 ef	1.10±0.09 gh	2.04±0.20 hi	0.019±0.01 i	0.022±0.001 b	0.034±0.018 ef	0.021±0.010 b
	100	+ AMF	3.41±0.73 ef	1.21±0.15 fgh	2.40±0.41 ghi	0.185±0.01 fgh	0.013±0.001 b	0.036±0.008 ef	0.016±0.001 b
	0	− AMF	1.94±0.56 g	1.32±0.05 fgh	2.00±0.01 hi	0.174±0.01 gh	0.021±0.005 b	0.045±0.015 ef	0.011±0.001 b
	50	− AMF	1.92±0.98 g	1.06±0.07 gh	1.92±0.06 hi	0.176±0.01 gh	0.019±0.004 b	0.039±0.021 ef	0.011±0.001 b
	100	− AMF	1.85±1.78 g	1.02±0.01 h	1.23±0.2 j	0.150±0.01 h	0.008±0.007 b	0.031±0.013 f	0.008±0.003 b
Napoletano	0	+ AMF	5.15±0.81 a	4.75±0.42 a	5.88±0.60 a	0.581±0.03 a	0.056±0.008 b	0.373±0.048 a	0.181±0.111 a
	50	+ AMF	4.84±0.67 b	1.72±0.03 bcde	4.70±0.56 abc	0.377±0.02 b	0.042±0.010 b	0.118±0.044 cde	0.027±0.005 b
	100	+ AMF	4.06±0.64 cd	1.45±0.13 defg	4.17±0.25 cd	0.286±0.01 cd	0.275±0.006 a	0.077±0.038 def	0.022±0.005 b
	0	− AMF	3.46±0.87 def	2.06±0.05 b	3.78±0.44 cde	0.247±0.02 de	0.042±0.006 b	0.211±0.022 b	0.022±0.005 b
	50	− AMF	3.65±0.31 de	1.34±0.02 efgh	3.75±0.76 cde	0.273±0.03 cd	0.038±0.001 b	0.135±0.022 bcd	0.022±0.006 b
	100	− AMF	2.88±0.11 f	1.40±0.02 defgh	3.65±0.92 cdef	0.241±0.02 def	0.273±0.005 a	0.067±0.055 def	0.018±0.005 b
Nufar	0	+ AMF	4.89±0.95 ab	1.87±0.32 bc	5.62±0.48 ab	0.313±0.03 c	0.259±0.006 a	0.204±0.049 bc	0.013±0.006 b
	50	+ AMF	4.36±0.81 bc	1.76±0.14 bcd	3.98±0.64 cde	0.232±0.01 defg	0.031±0.005 b	0.098±0.005 def	0.013±0.001 b
	100	+ AMF	3.49±0.27 def	1.56±0.14 cdef	3.42±0.11 defg	0.202±0.008 efgh	0.014±0.005 b	0.069±0.013 def	0.005±0.008 b
	0	− AMF	3.08±0.59 ef	1.73±0.11 bcd	4.66±0.19 bc	0.171±0.04 gh	0.022±0.002 b	0.102±0.005 def	0.021±0.001 b
	50	− AMF	3.03±0.33 ef	1.42±0.15 defgh	3.28±0.18 defg	0.240±0.02 def	0.017±0.006 b	0.064±0.023 def	0.006±0.003 b
	100	− AMF	2.92±0.39 f	1.28±0.04 fgh	2.88±0.34efgh	0.183±0.01 fgh	0.011±0.006 b	0.042±0.006 ef	0.003±0.001 b
Significance level			***	***	***	***	*	**	**

NaCl = sodium chloride (mM); + AMF = with AMF (1 g of the AMF inoculum); − AMF = without AMF (control); ER = emergence rate; EP = emergence percentage; PH = plant height; RL= root length; FBAP = fresh biomass of aerial part; DBAP = dry biomass of aerial part, FRB = fresh root biomass; DRB = dry root biomass. Values in each column followed by the same letter(s) are not significantly different at $p \leq 0.05$ (Tukey HSD). Significance level: *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$ (means ± SD).

Napoletano had greater fresh root biomass (FRB) and was greater in 0 mM decreasing as the concentrations of NaCl increased; FRB was greatest in seedlings whose seeds were inoculated with AMF (Table 1). In regard to the analysis of the interactions, varieties × NaCl showed that FRB was greatest in Napoletano in 0 mM and all varieties showed a trend to decrease as NaCl increased (Table 2). The interaction varieties × AMF revealed that all varieties increased FRB with AMF, with greatest values in Napoletano (Table 2). In the interaction NaCl × AMF, FRB was always greatest in seedlings whose seeds were inoculated with AMF even though the greatest value was in 0 mM (Table 2). Varieties × NaCl × AMF showed that FRB was greatest in Napoletano at 0 mM with AMF while lowest FRB was showed by Genovese at 100 mM without AMF (Table 3).

NaCl stress causes a decrease in germination and dry weight of stem and root and an increase in stem/root ratio (Ceccoli *et al*., 2011). High levels of Na^+ and Cl^- in the plant cause premature death of young tissues and produce marginal chlorosis on the leaves, which modifies the photosynthetically active area of seedlings, growth of stem and root and ion content in the plant (Batista-Sánchez *et al*., 2015).

Dry root biomass (DRB) was greatest in Napoletano regarding to Nufar and Genovese and showed greatest values in 0 mM, decreased as NaCl increased; nevertheless seedlings from seeds inoculated with AMF showed greatest DRB (Table 1). Varieties × NaCl interaction showed that Napoletano in 0 mM had greatest DRB (Table 2). In the interaction varieties × AMF, DRB exhibited greatest values in Napoletano with AMF while lowest DRB was showed in Nufar and Genovese without AMF (Table 2). Varieties × NaCl × AMF interaction showed that DRB was greatest in Napoletano in 0 mM with AMF while Genovese in 100 mM without AMF displayed lowest values (Table 3). The accumulation of dry biomass is widely used as a measure of plant growth because it expresses a balance between total productions of photoassimilates and breathing. Studies related to DRB of *Chloris gayana* under NaCl concentrations (0, 100 and 200 mM) dropped by 43% at concentrations of 200 mM compared to the control (Ceccoli *et al*., 2011), NaCl is known to reduce dry root biomass (Chávez and González, 2009).

The polynomial regression analysis shows that the relationship of NaCl concentrations and rate and percentage of emergence was nonlinear or quadratic. The relationship of NaCl concentrations with height of seedlings, length of root, fresh biomass of aerial part, fresh and dry biomass of root was linear while dry biomass of aerial part showed a quadratic relationship. These results are consistent with what was reported by Miranda *et al*. (2012) who observed a differentiated effect on growth variables under different saline solutions (Table 4).

Non-colonized roots were observed in the emergence stage of sweet basil seedlings. At 21 days a short period of AMF symbiosis was established; although at the beginning of the process was observed the site where the mycelium of *Rhizophagus fasciculatum* (+ AMF) makes contact with seedlings roots (Fig. 1). In seedlings roots from non-inoculated seeds (− AMF) no signs of colonization were detected (Fig. 2). The beneficial effects of this symbiosis happen because of a complex molecular interchange between the two symbionts (Camarena-Gutiérrez, 2012).

Table 4. Linear or curvilinear relationship among NaCl and all variables measured in sweet basil seedlings inoculated with AMF. Relationship determined by polynomial regression analysis.

Variables	Adjustment model	Correlation coefficient (R)	Coefficient of determination (R^2)	Number of data (N)	F-value (ANOVA)	P value	t value
PH (cm)	2.066-0.008×NaCl	-0.43	0.185	72	15.949	0.000) ***	-3.993
RL (cm)	3.999-0.0112×NaCl	-0.35	0.119	72	9.534	0.002 **	-3.087
FBAP (g)	$0.271\text{-}7E^{-3}\times$NaCl	-0.26	0.065	72	4.893	0.030 *	-2.212
DBAP (g)	0.2285-0.214×NaCl +0.057×NaCl ^2	0.075	0.274	72	4.754	0.032 *	2.180
FRB (g)	0.156-0.001×NaCl	-0.51	0.263	72	25.002	0.0004 ***	-5.000
DRB (g)	$0.039\text{-}0.3E^{-3}\times$NaCl	-0.25	0.064	72	4.806	0.0316 *	-2.192

PH = plant height; RL= root length; FBAP = fresh biomass of aerial part; DBAP = dry biomass of aerial part, FRB = fresh root biomass; DRB = dry root biomass.

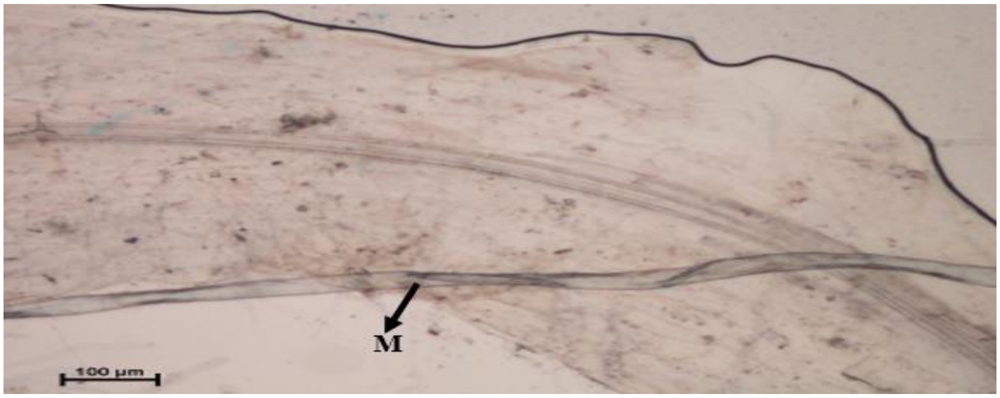

Figure 1. Micrograph of inoculated basil roots with *Rhizophagus fasciculatum* (+AMF). M= Vegetative mycelium.

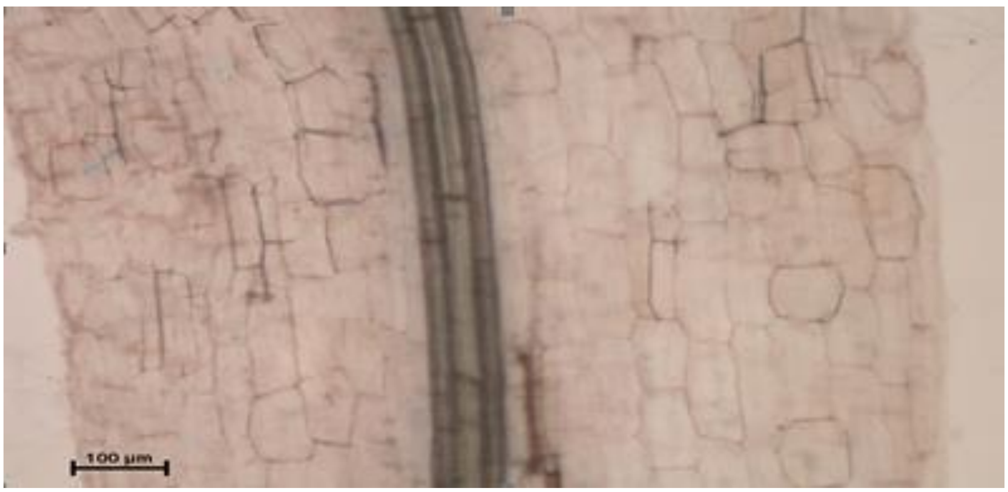

Figure 2. Micrograph of non-inoculated basil seedlings roots (−AMF).

CONCLUSIONS

No root colonization was found in any seedlings whose seeds were inoculated with AMF. Vegetative mycelium structure was observed in inoculated basil roots with *Rhizophagus fasciculatum* (+AMF). This mycelium is able to interconnect the roots of plants with the soil, which allows the flow of water and nutrients between the roots. The number of spores found in the inoculum is suitable to use in studies with AMF. The substrate used is suitable for the development of *Rhizophagus fasciculatum* and for sweet basil. Napoletano showed the highest values in most of the variables evaluated. Plant height, root length, fresh biomass of aerial part, fresh and dry root biomass showed linear relationship with NaCl concentrations while dry root biomass showed quadratic relationship. Rate and percentage of emergence do not shows linear or curvilinear relationship with NaCl concentrations.

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