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ORIGINAL ARTICLE

The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.)

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KEYWORDS

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Abstract Salinity is one of the serious abiotic stresses adversely affecting the majority of arable lands worldwide, limiting the crop productivity of most of the economically important crops. Sweet basil (*Osmium basilicum*) plants were grown in a non-saline soil (EC = 0.64 dS m^{-1}), in low saline soil (EC = 5 dS m^{-1}), and in a high saline soil (EC = 10 dS m^{-1}). There were differences between arbuscular mycorrhizal (*Glomus deserticola*) colonized plants (+AMF) and non-colonized plants (−AMF). Mycorrhiza mitigated the reduction of K, P and Ca uptake due to salinity. The balance between K/Na and between Ca/Na was improved in +AMF plants. Growth enhancement by mycorrhiza was independent from plant phosphorus content under high salinity levels. Different growth parameters, salt stress tolerance and accumulation of proline content were investigated, these results showed that the use of mycorrhizal inoculum (AMF) was able to enhance the productivity of sweet basil plants under salinity conditions. Mycorrhizal inoculation significantly increased chlorophyll content and water use efficiency under salinity stress. The sweet basil plants appeared to have high dependency on AMF which improved plant growth, photosynthetic efficiency, gas exchange and water use efficiency under salinity stress. In this study, there was evidence that colonization with

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AMF can alleviate the detrimental salinity stress influence on the growth and productivity of sweet basil plants.

1. Introduction

Salinity is one of the main abiotic factors negatively affecting plant production all over the world (Koca et al., 2007). Increasing salt concentrations in soil decreases the plant ability to absorb water, adversely affects metabolic processes and affects osmotic balance, nutrient absorbance, hydraulic conductivity, stomatal conductance, net photosynthetic rate, and intercellular CO_2 concentrations, all of this results in negatively affecting the plant ability to grow and develop (Al-Karaki et al., 2001). Furthermore, salinity stress affects the plant ability to uptake water in the root zone through decreasing the water potential of the soil (Sabir et al., 2009). This deficiency in available water under saline condition raises the potential of cells to be dehydrated which is a result of the osmotic stress caused by salinity. The higher ratios of toxic

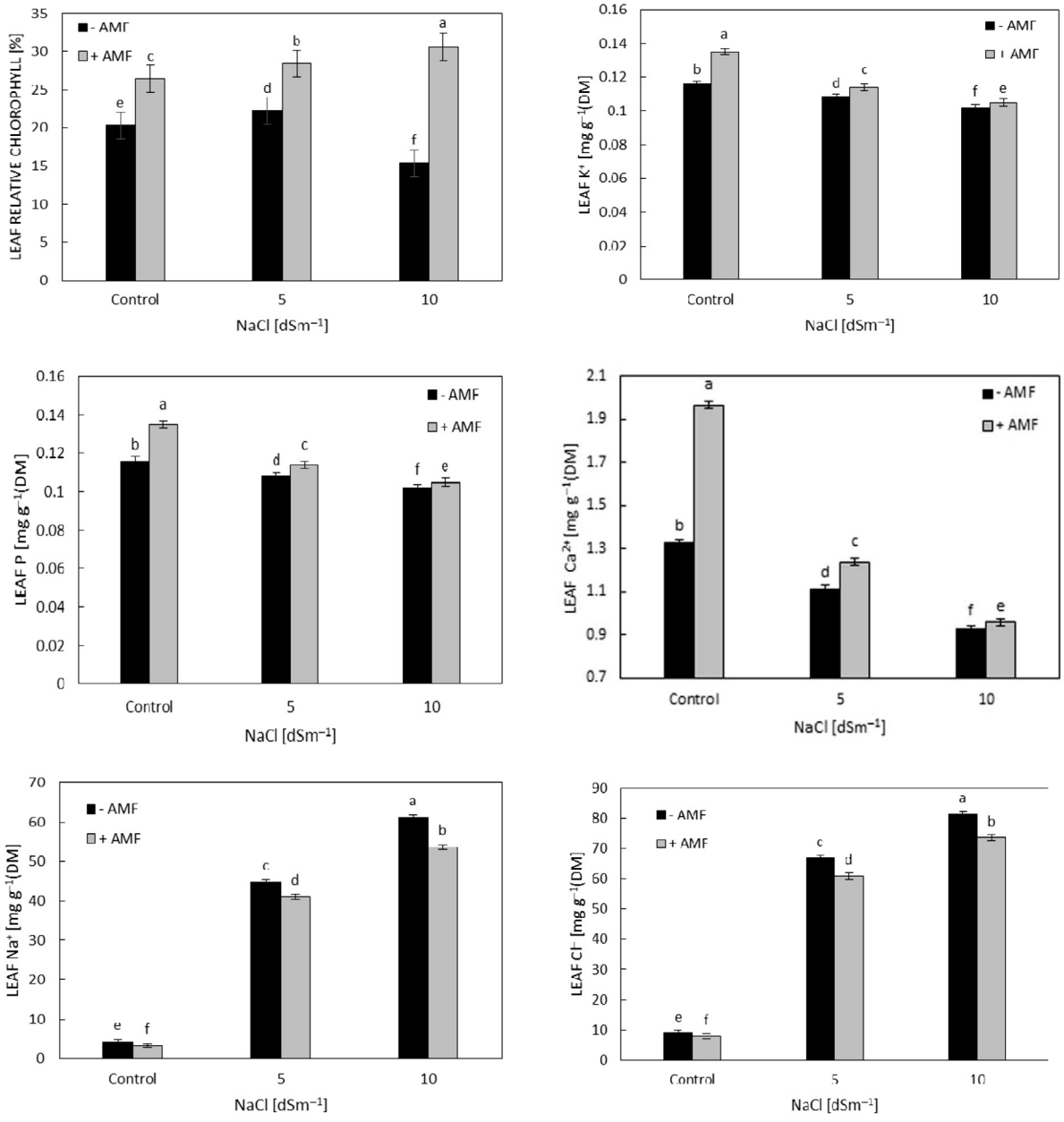

Figure 1 Influence of different salinity levels on leaf relative chlorophyll content and uptake of selected mineral nutrients by sweet basil plants colonized (+AMF) or not (−AMF) with AMF. Values in each bar followed by *the same letter* are not significantly different at $P \leqslant 0.05$. (*Duncan*'s multiple range tests). Vertical bars indicate mean ± SE.

ions like Na^+ and Cl^- damage the balance between ions through reducing the plant ability to absorb other ions like K^+, Ca^{2+}, and Mn^{2+} (Hasegawa et al., 2000). In this respect, the use of a non-hazardous biological method, such as mycorrhizal fungi applications to mitigate the negative effects of salinity stress and use of moderately salt-tolerant plants are an excellent environmental choice to make (Mayak et al., 2004). In this regard, the application of AM fungi in saline soil sites could improve plant growth and tolerance through helping the plants to face the adverse effects of salinity (Al-Karaki, 2006; Daei et al., 2009; Kumar et al., 2010; Abdel-Fattah and Asrar, 2012; Asrar et al., 2014).

Most plant species form a symbiosis relationship with the arbuscular mycorrhizal (AM) fungi (Al-Karaki and Al-Raddad, 1997). The plant benefits from these relations as they enhance the plant to grow and uptake water and nutrients under various abiotic stresses e.g. salinity, low fertility and drought (Zuccarini and Okurowska, 2008). The different mechanisms AMF uses to enhance the salt resistance of host plants include improving nutrient uptake, especially P (Evelin et al., 2009), reducing the uptake of sodium and chloride ions and impairing their movement to plant aerial parts (Al-Karaki, 2006; Daei et al., 2009), enhancing water uptake (Ruiz-Lozano and Azcön, 2000), keeping ionic balance by improving uptake of nutrients and stimulating selective uptake (Evelin et al., 2012), increasing the synthesis and effectiveness of some enzymes (Wu et al., 2010) and increasing the plant capability to produce and accumulate proline in tissues of the symbiont plants (Ibrahim et al., 2011). Other AMF mechanisms help plants in adjusting their osmotic status (osmotic adjustment), which help in keeping the turgor pressure of leaves in a good state, and enhancing the balance between photosynthesis and transpiration, water use efficiency and stomatal conductance in the symbiont plants (Augé, 2001; Augé et al., 2008; Choe et al., 2006). Moreover, AMF can enhance the physiological processes of the host plant such as water absorption capacity via increasing hydraulic conductivity of roots and regulating the osmotic status as well as carbohydrate accumulation (Rosendahl and Rosendahl, 1991; Ruiz-Lozano and Azcón, 2000). This may increase plant ability to grow and alleviate the toxic effect of salinity related ions (Juniper and Abbott, 2006). These stated aid of AM fungi have made it an appropriate candidate for bio-solving the problem of salinity stress. The increased capability of mycorrhizal plants to grow in salinity stressed environments also has been primarily referred to AMF mediated enhanced K, Ca, Mg uptake and improved K:Na and Ca:Na ratios in plants (Giri et al., 2007; Daei et al., 2009) under salinity stress. Sweet basil as well as many other plants from the Laminaceae family has the capability to form a symbiosis relationship with mycorrhizae (Wang and Qiu, 2006).

Sweet basil (*Ocimum basilicum* L.) is an annual plant which grows in many areas around the world. The plant is one of the most important applied in meal (Machale et al., 1997). The essential oil of sweet basil is applied as perfumes (Bauer et al., 1997). Although many investigations (Al-Karaki, 2000; Elhindi, 2013) have mentioned retardation of growth at great salinity, Species of plant vary in their sensitivity or hardiness to salt stress (Mer et al., 2000). Arbuscular mycorrhizal (AM) plants, generally, shows a higher osmotic potential than non-AMF plants (Subramanian et al., 2006; Wu et al., 2008, 2010). While, the utilization of AM colonization to alleviate the negative impact of salt stress on biomass and product of aromatic and medicinal herbs is still uninvestigated, the strategies for controlling salt stress through enhancing nutrient uptake and water use efficiency can be accomplished by injecting soils with appropriate AMF species. In addition, AMF is a dualistic relationship between fungi and plants which plays a fundamental part in nutrient cycling and stress resistance, while, few information is clear around the mycorrhiza-mediated improvement of growth rate and salinity resistance of the sweet basil (*O. basilicum* L.) plants growing under arid and semi-arid conditions.

Therefore, this experiment was carried out to examine the impact of the AM fungi, *Glomus deserticola* on growth rate, photosynthetic efficiency and water relations of sweet basil plants grown either under normal or salinity stress. The differences in gas exchange including transpiration rate, stomatal conductance and photosynthetic rate in sweet basil leaves due to ability of AMF were also studied.

2. Materials and methods

2.1. Treatments and experimental design

This experiment was determined using a 2×3 randomized block design with three salinity levels [control, (0.64 dS m^{-1}), low salinity (5 dS m^{-1}), and high salinity (10 dS m^{-1})] combined two mycorrhizal inoculations [*Glomus deserticola* (+AMF) and non-inoculated (−AMF)] treatments. The two salinity levels in the soil were achieved through dissolving NaCl at rates of 0.2, 0.4, 0.6, 0.8 and 1.0 g in 150 ml deionized water and adding each solution to pots including 450 g dry soil in a preliminary experiment to reach the water saturation level. Then, EC was computed in the soil extracts after a full day. The NaCl levels added to the soil was plotted against the values of EC.

2.2. Preparation of arbuscular mycorrhizal fungal inoculum

The mycorrhizal fungus inocula in this study consisting of soil including spores, hyphae, and infected root fragments of sudangrass (*Sorghum halepense* L.) plants from a stock culture of *G. deserticola*, were provided by the stock mycorrhizal cultures collected from saline soils of Al-Kassab region with electrical conductivity (EC) of ~10 dS m^{-1}. The AM inoculums including 5 g of rhizosphere soil and 0.5 g of infected root fragments of sudangrass through a level of infection of 88.7% were colonized to each mycorrhizal pot. The mycorrhizal inoculums were placed at 3–5 cm depth in pots directly prior to the growing of the sweet basil plants to promote fungal inoculation of plant roots. Non-AMF treatments accepted the equal mass of autoclaved growth.

2.3. Plant and growth conditions

Sweet basil seeds (*O. basilicum* cv. Nano Compatt) were surface-sterilized by 1% sodium hypochlorite solution for 15 min and growing in 10 cm pots including sterile perlite. Uniform germinated rooted cuttings were grown into pots (30 cm) which involve 4 kg of autoclaved soil (60% sand, 25% silt, and 15% clay). The soil was obtained from the top surface layer

(0–30 cm) of the soil in the Experimental Agricultural Research Station at Dirab, Riyadh, Saudi Arabia. Characteristics of soil in this study were as follows: pH 7.6; available nitrogen (26.9 mg kg^{-1}); organic matter (0.63%); EC (0.20 dS m^{-1}); P (8.11 mg kg^{-1}) and K (83 mg kg^{-1}). Pots were arranged in a greenhouse with a temperature range of 24°/17 °C day/night, 13/11 h light/dark period, a relative humidity of 75–82% and with a photon flux density of about 400–500 µmol m^{-2} s^{-1}. Plants were maintained in the greenhouse during the experiment, watered every three day with distilled water daily as well as received every ten days with standard Hoagland's solution without P for AMF plants added for non-mycorrhizal plants (Graham and Fardelmann, 1986). Water application to the pots was calculated depending on the soil field capacity.

2.4. Leaf chlorophyll measurement

A portable chlorophyll meter CCM 200 (Opti-Sciences, Tyngsboro, MA, USA) was used to measure leaf greenness of the plants. Chlorophyll concentration was calculated using the formulae from Strain and Svec (1966).

2.5. Proline

It was conducted following the method characterize by Sadasivam and Manickam (1996) using ninhydrin.

2.6. Gas exchange measurements

Gas exchange measurements included net photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (g_s) were measured as according to Long and Bernacchi (2003).

2.7. Growth analysis

After sowing, four plants at 70 days were randomly sampled from each pot. Plants were harvested and separated into leaves and stems. Leaf area was measured using a portable leaf area meter (*Li-Cor*, Lincoln, NE, USA). After then, the samples were dried at 65 °C for 72 h and their dry weights were determined. Water use efficiency (WUE) was determined by Sinclair et al. (1984), defined as the ratio of net photosynthesis rate (A) to transpiration rate (E). These indices were calculated using the following relationships:

$$\mathrm{WUE} = A/E.$$

2.8. Determination of ion concentrations

K^+, Ca^{2+}, and Na^+ concentrations were measured with a flamephotometer (*Corning 400*, UK). Phosphorus (P) concentration was determined using spectrophotometrically following Jackson (1973). For Cl^-, the ground leaf samples (100 mg) were extracted in100 mL of distilled water and was shaken for one hour and then filtered. Chloride was measured using an ion chromatography analyser (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

2.9. Estimating the levels of mycorrhizal colonization

It was evaluated by Phillips and Hayman (1970). AM inoculation levels [the frequency of mycorrhizal inoculation (F), intensity of mycorrhizal inoculation (M) and rate of arbuscular development (A)] of the stained roots were evaluated following the method of Trouvelot et al. (1986). The Mycorrhizal dependency (MD) was described by Menge et al. (1978) as:

$$(M - NM)/NM \times 100,$$

where M is the parameter value of plants inoculated with AMF and NM is the parameter value of non-inoculated plants under the same level of salinity stress. The salt tolerance index (STI) was assessed in AM and non-AM plants by the following formula (Shetty et al., 1995) as:

$$(\text{Parameter value at } S_0 - \text{Parameter value at } S_x)/\text{parameter value at } S_0 \times 100,$$

where S_0 is a control treatment and S_x is a NaCl treatment level.

2.10. Statistical analysis

Data were statistically analyzed with a two-factor analysis of variance (ANOVA). Means were separated by least significant difference (LSD, $P \leqslant 0.05$) by *Costat* software (*Cohort*, Berkeley, CA, USA). All of the measurements were performed four times for each treatment, and the calculated means and standard errors (SE) were mentioned.

3. Results

3.1. Growth measurements

Both shoot and root of the dry matter, height of the shoot, number of leaves and branches, inflorescence length and leaf area in both AM and non-AM plants under salinity stress were negatively affected and lower with a significant difference when compared to the control plants (Table 1). On the other hand, the manner of reductions in most growth parameters was more apparent in non-AM than in AM plants. The shoot and root dry matter, the shoot height, the leaf area and leaves number and branches characters were significantly higher in AM plants than in non-AM plants regardless the NaCl stress level. Interactions between salinity and AM inoculation was significant for the dry matter of both shoot and the whole plant, and was not significant for shoot height and branch number parameters ($P < 0.05$).

3.2. Leaf nutrients content

In the non-stress treatments, the content of K^+, P, and Ca^{2+} was recorded higher in AM plants than the respective non-AM plants, except Na^+ and Cl^- content in sweet basil (Fig. 1). The content of K^+, P, and Ca^{2+} was significantly higher in the leaves of AM plants than those of non-AM plants, regardless of NaCl treatments. The nutrient content decreased with increasing levels of NaCl treatments, except for Na^+ and

Table 1 Growth parameters of mycorrhizal (+AMF) and nonmycorrhizal (−AMF) sweet basil grown under salt stressed and nonstressed conditions.

Treatments		DM [g plant^{-1}]		SH [cm plant^{-1}]	LN [plant^{-1}]	BN [plant^{-1}]	LA [cm^2 plant^{-1}]	IL [cm plant^{-1}]
NaCl [dS m^{-1}]	AMF status	Shoot	Root					
0 (Control)	−AMF	17.21 ± 0.25^{b}	1.47 ± 0.01^{c}	68.31 ± 1.01^{b}	353.68 ± 1.12^{b}	10.51 ± 0.24^{b}	198.37 ± 0.89^{c}	7.11 ± 0.41^{b}
	+AMF	21.28 ± 0.65^{a}	2.54 ± 0.10^{a}	74.44 ± 0.91^{a}	585.36 ± 1.01^{a}	12.09 ± 0.50^{a}	235.44 ± 1.12^{a}	8.97 ± 0.68^{a}
5	−AMF	12.31 ± 0.35^{d}	1.33 ± 0.02^{d}	58.46 ± 0.81^{d}	336.67 ± 1.12^{d}	8.34 ± 0.30^{d}	192.30 ± 0.89^{d}	6.33 ± 0.11^{c}
	+AMF	14.47 ± 1.36^{c}	1.83 ± 0.06^{b}	62.01 ± 1.23^{c}	346.29 ± 0.89^{c}	9.58 ± 0.36^{c}	227.46 ± 0.97^{b}	6.90 ± 0.11^{bc}
10	−AMF	8.95 ± 0.29^{f}	1.00 ± 0.04^{e}	45.35 ± 0.80^{f}	196.51 ± 1.31^{f}	6.50 ± 0.23^{f}	146.36 ± 1.16^{f}	4.81 ± 0.17^{e}
	+AMF	10.41 ± 0.17^{e}	1.07 ± 0.07^{e}	49.36 ± 0.81^{e}	216.54 ± 1.36^{e}	7.43 ± 0.20^{e}	166.39 ± 0.88^{e}	5.61 ± 0.36^{d}
Significance level								
NaCl		***	***	***	***	***	***	***
AMF		***	***	***	***	***	***	***
NaCl × AMF		*	***	NS	***	NS	***	*

DM – dry matter, SH – shoot height, LN – leaf number, BN – branch number, LA – leaf area, IL – inflorescence length. Values in each column followed by *the same letter(s)* are not significantly different at $P \leqslant 0.05$ (*Duncan*'s multiple range tests), where NS − not significant, $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$; $^{***}P \leqslant 0.001$ ($n = 4$; means ± SE).

Table 2 Concentrations of K^+/Na^+ and Ca^{2+}/Na^+ ratios in the leaves and proline of mycorrhizal (+AMF) and nonmycorrhizal (−AMF) sweet basil grown under salt stressed and nonstressed conditions.

Treatments		K/Na		Ca/Na		Proline	
NaCl [dS m^{-1}]	AMF status	Shoot	Root	Shoot	Root	Shoot	Root
0 (Control)	−AMF	14.70 ± 0.20^{b}	3.78 ± 0.01^{b}	3.94 ± 0.02^{b}	2.36 ± 0.01^{b}	207.00 ± 1.00^{f}	42.00 ± 1.00^{e}
	+AMF	19.57 ± 0.25^{a}	5.56 ± 0.02^{a}	5.06 ± 0.02^{a}	3.88 ± 0.01^{a}	215.00 ± 1.00^{d}	46.00 ± 1.00^{d}
5	−AMF	1.76 ± 0.01^{d}	0.78 ± 0.01^{d}	0.38 ± 0.02^{d}	0.54 ± 0.01^{d}	213.00 ± 1.00^{e}	43.00 ± 1.00^{e}
	+AMF	3.54 ± 0.02^{c}	1.56 ± 0.01^{c}	0.78 ± 0.02^{d}	1.07 ± 0.02^{c}	221.33 ± 1.53^{c}	54.00 ± 1.00^{c}
10	−AMF	0.75 ± 0.01^{e}	0.28 ± 0.01^{f}	0.17 ± 0.02^{e}	0.25 ± 0.01^{e}	262.00 ± 1.00^{b}	98.00 ± 1.00^{b}
	+AMF	1.55 ± 0.04^{d}	0.63 ± 0.02^{e}	0.37 ± 0.01^{d}	0.54 ± 0.01^{d}	296.00 ± 1.00^{a}	117.00 ± 1.00^{a}
Significance level							
NaCl		***	***	***	***	***	***
AMF		***	***	***	***	***	***
NaCl × AMF		***	***	***	***	***	***

Values in each column followed by *the same letter(s)* are not significantly different at $P \leqslant 0.05$ (*Duncan*'s multiple range tests), where $^{***}P \leqslant 0.001$ ($n = 4$; means ± SE).

Cl^- content. Under stress conditions, inoculation was positively effective in enhancing leaf-K^+, P, and Ca^{2+} content in plant. On the other hand, under stress at all salinity, low content levels of Na^+ and Cl^- were recorded when plants were inoculated with mycorrhiza. The salinity had significant reducing effect on the ratio between K^+ and Na^+ and between Ca^{2+} and Na^+. Accordingly, mycorrhizal plants were higher with a significant difference in the ratio between K^+ and Na^+ and between Ca^{2+} and Na^+ ratios in leaves compared to non-mycorrhizal (Table 2). It was observed that under salinity treatments the K^+/Na^+ ratio was the highest in AMF plants at all treatments of salinity regardless of its level. The significant NaCl × AMF interactions on both Na^+ and Ca^{2+} uptake and K^+/Na^+, Ca^{2+}/Na^+ ratios were significant for the alleviation of salt-induced ion imbalance by the help of AM fungi.

Chlorophyll content (Chl): the content of Chl was higher in plant under non-stress conditions. However, the synthesis of plant Chl in sweet basil plant was found to be decreased with increasing levels of NaCl, but reductions in Chl content due to salt stress was more apparent in non-AM than in AM plants. The Chl content was higher in AM plants than in non-AM plants regardless of the salinity treatment level, Except for the 5 dS m^{-1} treatment the difference in Chl content between AM and non-AM plants was not significant (Fig. 1).

Table 3 Gas exchange parameters [Net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s)] and water use efficiency (WUE) of mycorrhizal (+AMF) and nonmycorrhizal (−AMF) sweet basil grown under salt stressed and nonstressed conditions.

Treatments		A [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	WUE [μmol m^{-2} s^{-1}]
NaCl [dS m^{-1}]	AMF status				
0 (Control)	−AMF	6.52 ± 0.02[a]	3.22 ± 0.01[b]	54.00 ± 1.00[b]	2.03 ± 0.03[b]
	+AMF	6.34 ± 0.01[c]	4.16 ± 0.01[a]	59.00 ± 1.00[a]	1.52 ± 0.01[d]
5	−AMF	4.58 ± 0.01[d]	2.38 ± 0.01[d]	33.00 ± 1.00[d]	1.92 ± 0.01[c]
	+AMF	6.38 ± 0.01[b]	3.11 ± 0.01[c]	48.00 ± 1.00[c]	2.05 ± 0.01[b]
10	−AMF	2.05 ± 0.01[f]	0.58 ± 0.01[f]	8.00 ± 1.00[f]	3.54 ± 0.08[a]
	+AMF	3.58 ± 0.01[e]	1.73 ± 0.02[e]	19.00 ± 1.00[e]	2.07 ± 0.03[b]
Significance level					
NaCl		***	***	***	***
AMF		***	***	***	***
NaCl × AMF		***	***	***	***

Values in each column followed by *the same letter*(*s*) are not significantly different at $P \leqslant 0.05$ (*Duncan*'s multiple range tests), where $^{***}P \leqslant 0.001$ ($n = 4$; mean ± SE).

3.3. Proline content

There was an apparent increase in the content of Proline in both mycorrhizal and non-mycorrhizal plants (in shoots and roots) with the increase of NaCl level (Table 2). Yet, mycorrhiza inoculated plants had lower proline content in both shoots and roots when compared to non-inoculated plants with all treatments, and the difference was more apparent with salinity treatments. Statistically significant NaCl × AMF interactions for proline in both shoots and roots give a powerful clue for the role for antioxidant defenses in sweet basil as one of the mechanics promoted by mycorrhizal inoculation for the sake of alleviating salinity stress effect.

3.4. Gas-exchange parameters

In general, photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), Transpiration rates (E, mmol H_2O m^{-2} s^{-1}), and stomatal conductance (g_s, mmol m^{-2} s^{-1}) in leaves of the mycorrhizal (+AMF) plants were significantly greater than those in the nonmycorrhizal (−AMF) plants grown either in control or NaCl stress conditions (Table 3), and the effects were more remarked in control treatment. Such stimulations in gas-exchange parameters were directly related to the degree of the mycorrhizal inoculation for each treatment. The change in A, E, and g_s values between mycorrhizal Salinity stressed plants and mycorrhizal unstressed plants was not significant.

Water use efficiency (WUE): Table 3 shows results that evidently illustrate the efficient water usage made by mycorrhizal plants as the amount of absorbed water to get one unit of dry matter was less in AM plants than it was in non-AM plants, which means that the mycorrhizal plants was higher in WUE. The WUE for control (untreated with salinity) plants was greater than it in treated plants for both AM and non-AM plants. In our experiment, mycorrhizal sweet basil significantly WUE throughout the improvement plant water relations under salt stress corresponding of mycorrhiza's improvement in P uptake to AM fungi–plants.

Table 4 Frequency of mycorrhizal colonization (F [%]), intensity of mycorrhizal colonization (M [%]), and arbuscular development (A [%]) in the root tissues of mycorrhizal (+AMF) and nonmycorrhizal (−AMF) sweet basil grown under salt stressed and nonstressed conditions.

Treatments		F [%]	M [%]	A [%]
NaCl [dS m^{-1}]	AMF status			
0 (Control)	−AMF	0.0	0.0	0.0
	+AMF	92.98 ± 2.13[a]	71.13 ± 1.99[a]	60.16 ± 2.28[a]
5	−AMF	0.0	0.0	0.0
	+AMF	90.12 ± 1.69[b]	68.22 ± 2.56[b]	58.15 ± 1.13[a]
10	−AMF	0.0	0.0	0.0
	+AMF	79.35 ± 2.13[c]	60.63 ± 3.01[c]	50.02 ± 2.12[b]
Significance level				
NaCl		***	***	***
AMF		***	***	***
NaCl × AMF		***	***	***

Values in each column followed by *the same letter*(*s*) are not significantly different at $P \leqslant 0.05$ (*Duncan*'s multiple range tests), where $^{***}P \leqslant 0.001$ ($n = 4$; means ± SE).

3.5. Mycorrhizal colonization levels

There was an inverse relationship between the salinity level and some mycorrhizal parameters namely intensity of mycorrhizal colonization (M) and the arbuscular frequency (A) in the root tissues of plants (Table 4). There was no observed colonization in roots of plants that have not been colonized with *G. deserticola*. Interactions for all levels between NaCl × AMF were significant of AM inoculation in root tissues of plants.

Table 5 Mycorrhizal dependency (MD) of sweet basil grown under salt stressed and nonstressed conditions.

Parameters	−NaCl	+5 NaCl	+10 NaCl
DM	23.65 ± 2.00[a]	17.90 ± 8.95[b]	16.40 ± 2.77[b]
LA	18.69 ± 0.92[a]	18.28 ± 0.05[a]	13.39 ± 1.50[b]
BN	15.18 ± 7.45[a]	14.99 ± 7.66[a]	14.39 ± 2.10[a]
IL	26.67 ± 5.41[a]	16.59 ± 3.55[b]	9.04 ± 5.08[c]
RC	30.27 ± 9.40[b]	65.13 ± 8.76[a]	28.04 ± 5.27[b]

DM – dry matter, LA – leaf area, BN – branch number, IL – inflorescence length, RC – relative chlorophyll. Values in each column followed by *the same letter*(*s*) are not significantly different at $P \leqslant 0.05$ (*Duncan*'s multiple range tests, $n = 4$; means ± SE).

3.6. Mycorrhizal dependency

Data in Table 5 show the mycorrhizal dependency (MD) for enhancing plant growth and relative chlorophyll content of plants planted with or without NaCl treatments. There was a positive relationship between response of plants to mycorrhizal inoculation and the NaCl stress, but, the varied relative chlorophyll content between AM plants grown with or without NaCl treatment was not significant ($P < 0.05$). Under salt stress, improved growth of sweet basil plants depends highly on arbuscular mycorrhizal fungi, this is a clear proof that the arbuscular mycorrhizal colonization has the ability to mitigate the negative effects of salinity stress (NaCl) on the growth of plants.

3.7. Salt tolerance index (STI)

The STI of sweet basil plants in terms of shoot dry matter, leaf area, branch number, inflorescence length, and relative chlorophyll of AM plant was significantly higher compared to the same parameters in non-AM plants at both NaCl treatments (Table 6). Such improvements in these characters due to mycorrhizal inoculation were remarked at high levels of NaCl stress. Our results have suggested that AM inoculation increased STI of sweet basil plants at high levels of salt stress, besides having the ability to protect the plants from the negative effects of salinity stress.

4. Discussion

Salinity whether in soil or in irrigation water is a serious challenging obstacle facing the agricultural production as, salinity has many growth reducing negative effects on most of the economically important crops. Salinity stress as shown in the result has affected the growth of AM and non-AM plants in a negative way, those negative effects were more apparent in non-mycorrhizal plants, the fresh and dry weight parameter clearly proves that the inoculation with mycorrhiza *G. desert-icola* has a significant positive growth improving effects on sweet basil plants under control and salinity. The inoculation of AMF has the capability to enhance the plant productivity under salinity stress conditions as reported by Tain et al. (2004). The positive salinity tolerance related influence of mycorrhizal plants colonization on sweet basil plants may be a result of the enhancement of nutrients balance and absorbance (Zandavalli et al., 2004). As the salinity tolerance is partially enhanced due to the better status of P absorbance caused by mycorrhiza (Evelin et al., 2009; Kaya et al., 2009; Ibrahim et al., 2011). Many researchers have mentioned that AM fungi-colonized plants grow better than non-colonized plants under salt stress (Al-Karaki, 2000; Cantrell and Linderman, 2001; Giri et al., 2003; Zuccarini, 2007; Zuccarini and Okurowska, 2008).

The present study shows that the NaCl treatment has caused the leaf content of Na^+ and Cl^- to increase significantly, and the K^+, P and Ca^{2+} to decrease significantly in non-mycorrhizal sweet basil plants (Fig. 1). There was an observed enhancement in the uptake of K^+, P and Ca^{2+} in the mycorrhizal (*G. deserticola*) inoculated plants, as the inoculation has reduced the uptake of Na^+ and Cl^-, which has reduced their negative toxic effect and, hence enhancing the growth of mycorrhiza treated plants. The results of this study come in compliance with those of other researchers (Giri and Mukerji, 2004; Tain et al., 2004; Cengiz et al., 2009). From the reasons or mechanisms that enabled mycorrhizal plant to show better salinity tolerance was the enhancement of leaves stomatal conductance (Augé et al., 2008), the enhanced transpiration rate (Augé, 2001) also, K^+, P, and Ca^{2+} uptake rate. K^+ which is vital for a plant to be able to face or tolerate water stress, its cationic solute has a key role in controlling the stomatal conductance (Ruiz-Lozano et al., 1995). This study shows a positive relationship between the raised salinity tolerance degree of mycorrhizal plants and the K^+ level in those plants. This study also shows a positive relationship between the Na^+ and Cl^- contents in the leaves and the degree of salinity (NaCl) treatment. Under stress conditions, the Na^+ content in AM plants was significantly lower when compared with that of non-AM plants, this may be explained by the management made by mycorrhiza to the absorbance of Na^+

Table 6 Salt tolerance index (%) of mycorrhizal (+AMF) and nonmycorrhizal (−AMF) sweet basil plants grown under salt stress conditions (5 and 10 dS m^{-1}).

Parameters	Salinity [dS m^{-1}]			
	5		10	
	−AMF	+AMF	−AMF	+AMF
SH	28.50 ± 1.00[a]	31.81 ± 8.46[a]	27.21 ± 3.87[a]	27.64 ± 7.05[a]
LA	3.05 ± 0.45[c]	3.39 ± 0.87[c]	23.89 ± 0.95[b]	26.85 ± 0.08[a]
BN	20.54 ± 4.70[a]	20.61 ± 6.14[a]	21.96 ± 5.47[a]	22.38 ± 0.92[a]
IL	10.74 ± 5.01[b]	22.71 ± 6.57[a]	24.07 ± 1.54[a]	18.71 ± 5.63[ab]

SH – shoot height, LA – leaf area, BN – branch number, IL – inflorescence length. Values in each column followed by *the same letter*(*s*) are not significantly different at $P \leqslant 0.05$ (*Duncan*'s multiple range tests, $n = 4$; means ± SE).

when its concentration is high and toxic to the plants (Allen and Cunningham, 1983; Evelin et al., 2012). So, it can be clearly concluded that the tolerance of mycorrhizal plants towards salinity is partially a result of the prevention of Na^+ uptake and transfer by the roots to the shoots. Furthermore the enhancement made by AM fungi to P uptake may also be a reason to the improved salinity tolerance of sweet basil plants as the results show in the present study. This may be elucidated by the role of P levels in helping the vacuolar membranes to hold its state, and make it easy to compartment Na^+ ions in the vacuoles (Bothe, 2012). This keeps the metabolic pathways safe and un-interrupted by Na^+ ions, which as a final result reduces the detrimental effects of salinity (Khalid and Teixeira da Silva, 2010). In accordance with what has been stated in this discussion, higher root colonization by *G. deserticola* has raised the nutrient absorbance (Fig. 1) while maintaining the uptake of Cl^- at lower ratios, which helps in raising the tolerance of colonized plants, and hence increasing its growth and productivity under salinity stress. The Cl^- ions may also be compartmentalized vacuoles (Cantrell and Linderman, 2001). Which as stated before means that the role of mycorrhiza in enhancing the P levels which, helps the vacuolar membrane to maintain it state may help to prevent the Cl^- ions from interrupting the metabolic pathways. Some authors relate the Cl^- accumulation role mediated by mycorrhizal inoculation to the draining of Carbon maid by the mycorrhizal hyphae, this improves the transfer of mobile anions such as Cl^- (Graham and Syversten, 1984).

The data in Table 2 show a significant enhance in the Ca^{2+}/Na^+ and K^+/Na^+ ratios in AMF sweet basil plants when compared to non-AMF plants, this gives a clear clue to the function of AMF in enhancing the uptake of K^+ and Ca^{2+} under salinity stress conditions. Under salinity conditions plants keep high levels of K^+ and low levels of Na^+ in the cytosol, this could be attributed to the regulation of K^+, Na^+ transporters and H^+ Pumps expression, which create the necessary power to want to transport these elements (Parida and Das, 2005). Salinity stress increases the cytosol Ca^{2+} content as it is transported from the apoplast and intra cellular spaces this transit of Ca^{2+} promote the transduction of stress signals which result in raising the plant adaptation to salinity. In accordance with the K^+ higher content in AMF plants compared to it of non-AMF plants, Rabie and Almadini (2005) also noticed that higher contents of K^+ on AM plants under salinity have decreased the negative effects of Na^+ on plant growth.

The AMF plant leaves (Fig. 1) have higher contents of chlorophyll compared to non-AMF plants, this greater chlorophyll content means that the photosynthesis is in a higher rate and the carbon fixation is in higher rates, which enable the plant to afford the relation with mycorrhiza, as the cost for this relation is Carbon (Wright et al., 1998). One of the main negative impacts of salinity was the reduction of chlorophyll content, this is in accordance with the Singh et al. (2000) report about chlorophyll degradation under salinity.

Proline plays many roles in the plant reply to salinity as one of the factors controlling osmotic adjustment, the accumulation of proline is considered a sign of stress in salinity treated plants (Yoshiba et al., 1997). In the present study the proline content under salinity conditions was significantly lower in AM plants compared to non-AM plants (Table 2). Plants of sweet basil under stress conditions exhibited increased proline accumulation that further increased by the application of mycorrhiza and thus could improve tolerance of sweet basil plants to salinity by maintaining the osmotic balance and reducing the free radicals' damage induced by osmotic stress (Garg and Manchanda, 2009). The lower amounts of proline in AMF sweet basil plants give a clear indicator that these plants were less stressed, as they have produced lower amounts of the stress related molecule, which is proline. Proline as reported by Chen and Murata (2002) works as a sink for C, N and free radicals, helps in maintaining the membranes and proteins status.

The data shown in Table 3 clearly inform a negative relationship between the salinity level and the photosynthetic parameters of sweet basil plants. Those negative effects of salinity on the photosynthetic activities may be due to the variation happening in osmotic potential and water potential within the tissues because of salinity. While the relationship between the salinity and the osmotic potential is positive, it is a negative relation with the water potential (Ashraf and Foolad, 2005). So, the water potential is decreased under salinity treatments, this decreased water potential adversely affects the stomatal conductance (Chartzoulakis et al., 2002; Sudhir and Murthy, 2004), causing imbalanced gas exchange, and finally disturbing the photosynthetic apparatus. Those adverse effects of salinity on the stomatal conductance and transpiration rate have been well reported by many authors (Parida and Das, 2005). The present study data show a clear significant increase in the A, E, and g_s parameters in AM plants compared to non-AMF plants wither under control or salinity stress, the increase level was positively related to the degree of mycorrhizal inoculation, those results agree to those of Augé et al. (2008). A greater E content in leaves of the AM plants is related to the high content of g_s, which is considered necessary to guarantee the carbon supply to the mycorrhizal fungus (Augé, 2001; Maggio et al., 2004; Choe et al., 2006).

The salinity stress negatively affected the water use efficiency (WUE) in both AM and non-AM plants. The WUE percentage was high in AMF salinity stressed plant than it was for non-AMF salinity stressed plants with a significant difference. The inoculation with *G. deserticola* has raised the water content of salinity stressed AMF sweet basil plants. This is supported by what Abdella and Abdel-Fattah (2000) reported about the positive relationship between AMF colonization and the water uptake of the hos plants. The improvement of the water uptake mediated by AMF may be explained by the ability of AMF to manage the osmotic balance of cells (Rosendahl and Rosendahl, 1991).

The negative effect of Na^+ and Cl^- ions on water uptake exposed the salinity (NaCl) stressed plants to a physiological drought (Fuzy et al., 2008). The colonization with AMF enables the plants to have greater water content than that of non-AMF plants (Sheng et al., 2008). This could be explained by the increased hydraulic conductivity mediated by AMF on the plant roots even under stress conditions (Kapoor et al., 2008). Mycorrhizal fungi could alter root system morphology, enhance the length of plant roots, and then increase root conductance (Kothari et al., 1990). Moreover, Sheng et al. (2008) reported that the greater stomatal conductance of AM plants improves the demand for transpiration. The ability of mycorrhizal plants to accumulate solutes enables the inoculated plants to adjust their osmotic potential (Al-Garni, 2006). All previously stated parameters enable the mycorrhizal plants to improve their efficiency in using the water (WUE)

(Graham and Syversten, 1984) allowing them to retain a less intercellular carbon dioxide content. So, the gas exchange capacity improves in the AM plants.

The present experiment conducted that the levels of mycorrhizal inoculation in sweet basil root reduced with enhancing NaCl concentrations in the soil. Our results are in accordance that supply of salt to soil decreases growth of hyphae in soil and hyphal spreading after initial infection had occurred (Bourgou et al., 2012) and decreases the arbuscules number (Kaya et al., 2009; Al-Amri et al., 2013). In spite of the lower germination percentage of AMF spores, the mycorrhizal inoculation still has the ability to positively affect the plant growth as the data of our present study shows.

5. Conclusion

The present study data and measured parameters clearly determine the function of AMF in improving the salinity tolerance of sweet basil plants, as it enhanced the uptake of (K^+, P, and Ca^{2+}), and through affecting the biochemical status of colonized plants (proline, stimulating photosynthetic pigments content particularly chlorophyll), and physiological mechanisms (raising photosynthetic rate, gas exchange and WUE). From the results of our study the potential role of mycorrhizal inoculation as a bio-solution for the salinity stress is strongly supported.

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