G.M. Abdel-Fattah et al. (2016) Int J Appl Sci Biotechnol, Vol 4(3): 372-379
DOI: 10.3126/ijasbt.v4i3.15775

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

THE IMPACT OF THE ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND PHYSIOLOGICAL PARAMETERS OF COWPEA PLANTS GROWN UNDER SALT STRESS CONDITIONS

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Abstract

A pot experiment was conducted to examine the effects of arbuscular mycorrhizal fungi on growth, nutrition and some physiological aspects of cowpea (*Vigna unguiculata* L.) plants grown at different salinity concentrations (0, 10, 15, 20, 25 and 30 mM NaCl). Under saline condition, arbuscular mycorrhizal fungal (AMF) inoculation significantly increased growth responses, photosynthetic pigments, nutrient contents, proline and total soluble protein of cowpea plants compared to non-AM ones. Those stimulations were related to the levels of mycorrhizal colonization in the associated plants. Interestingly, high proline, chlorophyll content and antioxidant enzymes in AM plants could be important for salt alleviation in plants growing in saline soils.

Keywords: Arbuscular mycorrhizal fungi; Soil salinity; Minerals content; Proline; Antioxidant enzymes; Cowpea.

Introduction

Salinity is one of the main abiotic factors negatively affecting plant growth and production all over the world (Evelin *et al*., 2012). Increasing salt concentrations in soil decreases the plant ability to absorb water, adversely affects metabolic processes, osmotic balance, nutrient absorbance, hydraulic conductivity, stomatal conductance and net photosynthetic rate. The development of salt- tolerant crops or desaliniation of soil by leaching excessive salts is not sufficient to overcome this problem. In recent years, the use of the biological applications; mycorrhizal symbiosis as a practical method to alleviate a soil stresses like salinity on plant growth and production has received a greater attention (Elhindi *et al*., 2016).

Most plant species form a symbiosis relationship with the arbuscular mycorrhizal (AMF) fungi, since they form symbiotic association with more than 90% of plant species (Harley and Smith, 1983). Many studies have reported the presence of the AMF association in salt stress conditions. In this concern, the introduction of AMF to sites with saline soil may improve plant tolerance and growth (Abo-Ghalia and Khalafallah, 2008). The different mechanisms AMF uses to enhance the salt resistance of host plants include enhancing water uptake, increasing the synthesis and effectiveness of some enzymes and increasing the plant capability to produce and accumulate proline in tissues of the symbiont plants. Moreover, AMF can enhance the physiological processes of the host plant and regulating the osmotic status as well as carbohydrate accumulation (Evelin *et al*., 2012).

Due to the detrimental effects of salinity and the function of AMF in improving the tolerance of plants and the urgent need to lessen the harmful effects of salinity and improve their growth. The present study was designed to study the efficiency of AMF in enhancing plant growth and physiology of cowpea plants grown in saline soil.

Materials and Methods

Preparation of AMF Inoculum

The inoculum of AMF species including *Glomus monosporium*, *G. nigra*, *G. clarum* and *Acaulospora leavis* were isolated from saline soils of South Hosseinieh, Sharkyia Governorate, Egypt using the wet sieving and decanting technique (Gerdemann and Nicolson, 1963), and identified by the author. The identified AMF spores were left to multiply for 5 months on maize (*Zea mays*) roots. A mixture of plant roots and sand soil which contained spores was used as mycorrhizal inoculum.

Plant and Growth Conditions

Cowpea seeds (*Vigna unguiculata* L.) **(**obtained from the Horticultural Research Center, Egypt) were surface sterilized in 7% sodium hypochlorite for 10 min, subsequently rinsed with sterilized water and sown in sterilized plastic pots (25 cm diam.) filled with 2kg sterilized soil. Pots were completely randomized and placed in the greenhouse of Botany Department, Faculty of Science, Zagazig University under natural conditions in two groups; the first was inoculated with AMF propagules at the rate of 50 g per pot at sowing date, the other was non-inoculated. AMF inoculums consisted of AMF spores, hyphae and colonized root fragments. Salt solutions were applied after germination at the levels (0, 10, 15, 20, 25 and 30 mM NaCl). Control treatments were irrigated with tap water. Each treatment was replicated three times.

Measurements

Growth parameters

After 60 days of salt application, cowpea roots were washed to remove soil particles and shoot height, root length, fresh and dry weights were determined.

Estimation of photosynthetic pigments

Chlorophyll a,b, carotenoids and total pigments in leaves were measured according to Metzner *et al*. (1965).

Detection the Levels of Mycorrhizal Colonization

It was described by Philips and Hayman (1970). The frequency of mycorrhizal colonization (F %), the intensity of colonization (M %) and the rate of arbuscular development (A%) of the stained roots were estimated by the method of Trouvelot *et al.* (1986).

Determination of the total soluble carbohydrates and protein contents

Carbohydrates were estimated by phenol sulphuric acid method according to Dubois *et al*. (1956). Total soluble protein content was measured according to Bradford (1976).

Determination of proline and lipid peroxidation

Proline was determined by the method adopted by Bates *et al.* (1973). Lipid peroxidation was determined by Ohkawa *et al*. (1979).

Estimation the activities of antioxidant enzymes (Catalase, peroxidase and superoxide dismutase)

A known fresh weight of shoot was homogenized in 0.05 M cold phosphate buffer (pH 7.0) containing 1 mM EDTA (Ethylene Diamine Tetra Acetic acid). The supernatant was used as enzyme source. Assay of catalase activity (CAT) was done according to Aebi (1983). Peroxidase activity was assayed according to Chance and Maehly (1955). Activity of superoxide dismutase (SOD) was measured according to Beyer and Fridovich (1987).

Statistical Analysis

The obtained data were analyzed statistically using one way analysis of variance (ANOVA). Treatment means were separated by LSD (Least significant difference) multiple range tests at (Level 0.01). All statistical analysis were carried out using the software SPSS, version 16 (Spiegel, 1975).

Table 1: Growth responses of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plants grown under different NaCl concentrations.

Treatments		**Fwt. (g/plant)**		**Dwt. (g/plant)**		**Shoot height**	**Root length**
NaCl conc.(mM)	**AMFStatus**	**Shoot**	**Root**	**Shoot**	**Root**	**(cm)**	**(cm)**
Control	**-AMF**	16.71**a**	2.53**b**	2.47**a**	0.34**b**	29.50**a**	21.0**b**
	+AMF	17.41**a**	3.04**a**	2.69**a**	0.47**a**	31.00**a**	25.0**a**
10	**-AMF**	9.30**bc**	1.96**cd**	1.27**b**	0.29**cd**	21.50**bc**	17.5**cde**
	+AMF	10.41**b**	2.03**c**	1.49**b**	0.32**bc**	24.00**b**	19.5**bc**
15	**-AMF**	6.00**d**	1.44**e**	1.01**cd**	0.17**e**	19.50**cd**	16.0**def**
	+AMF	10.33**b**	1.73**d**	1.41**b**	0.29**cd**	21.00**bcd**	18.0**cd**
20	**-AMF**	5.75**d**	1.20**ef**	0.87**cde**	0.15**ef**	18.00**d**	14.2**fgh**
	+AMF	8.68**c**	1.35**e**	1.09**c**	0.26**d**	20.50**cd**	15.0**efg**
25	**-AMF**	4.34**e**	1.00**fg**	0.56**f**	0.14**ef**	14.00**e**	13.0**gh**
	+AMF	5.46**d**	1.17**ef**	0.79**de**	0.17**e**	14.50**e**	14.0**fgh**
30	**-AMF**	4.18**e**	0.69**h**	0.54**f**	0.08**g**	10.00**f**	12.1**h**
	+AMF	4.97**d**	0.76**gh**	0.76**ef**	0.12**fg**	13.50**e**	13.8**fgh**
LSD		**1.49**	**0.27**	**0.22**	**0.04**	**3.19**	**2.62**

*Values with different letters within the same column are significantly different at $p < 0.01$; each value is the mean of three replicates.

Results and Discussion

Salinity stress as shown in Table 1 affected the growth of AM and non-AM plants in a negative way where shoot, root fresh and dry weights and shoot and root lengths decreased with increasing levels of NaCl over the control, those negative effects were more apparent in non-AM plants than AM one. These inhibitory effects are in agreement with Hashem *et al*. (2015). The reasons may be the non-availability of nutrients and the expenditure of energy to counteract the toxic effects of NaCl (Siddiqui *et al*., 2009). Also, salt stress is known to retard growth as a result of osmotic stress, ionic toxicity, nutritional imbalance and oxidative stress (Abdel Latef, 2011).

However, mycorrhization increased the growth of cowpea plants where AMF inoculation helps the plant to acquire nutrients through their hyphal network and improves the photosynthetic rate as well as water osmotic homeostasis. Also, AMF increase the uptake of P and other elements by extraradical mycorrhizal hyphae and transferring them to the root tissues (Abdel-Fattah and Asrar, 2012).

Chlorophyll Contents

Chlorophyll contents have been suggested as one of salt tolerance parameters in plants (Sirivastava *et al*., 1998); hence, their levels may determine the relative salt tolerance of the plants. The results in Table 2 showed that Chl. a, b, carotenoids and total pigments decreased with increasing salt concentration. But in most cases, the contents of the photosynthetic pigments of AM cowpea plant leaves were significantly greater than those of non-AM plants at all levels of salinity. This suggests that salt interferes with chlorophyll synthesis more in non-AM than in AM plants.

Mycorrhizal Colonization Levels and Spore Density

Salinity, not only affects the plant but also the development of AMF. Data recorded in Table 3 showed the frequency of root segments infection (F %), intensity of mycorrhizal colonization in root tissues (M %) and the rate of arbuscular formation in root segments (A%) in response to different NaCl concentrations. No mycorrhizal colonization was observed in non-AM treatments. Also our results showed an increase in colonization at 10, 15 and 20 mM NaCl and a decline in colonization rate at 25 and 30 mM NaCl.

Salinity can hamper colonization capacity and growth of fungal hyphae, probably due to the direct effect of NaCl on the fungi which can suppress the AMF formation. Thus, salinity affects directly the fungal development, reducing fungal mycelia formation and host root colonization (Sheng *et al*., 2008). Contrary to this, spore populations were gradually increased in the rhizosphere of AM cowpea plants grown in both non-salinized and salinized soil (Table 3), this was in harmony with Yamato *et al*. (2008). Contrary to our results, Juniper and Abbott (2006) found that germination of spores is delayed rather than prevented in the presence of NaCl. The discrepancies amongst studies may be because that various AM fungal species have varying tolerance to salinity (Porras-Soriano *et al*., 2009).

Table 2: Pigments content (mg/g Fwt.) of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plants grown under different NaCl concentrations.

Treatments		**Chl.a**	**Chl.b**	**Carotenoids**	**Total pigments**
NaCl conc.(mM)	**AMF Status**	**(mg/g Fwt.)**	**(mg/g Fwt.)**	**(mg/g Fwt.)**	**(mg/g Fwt.)**
Control	**-AMF**	0.83**c**	0.39**c**	0.68**b**	1.90**bc**
	+AMF	1.34**a**	0.57**a**	0.85**a**	2.77**a**
10	**-AMF**	0.88**c**	0.34**cd**	0.55**b**	1.77**bc**
	+AMF	0.80**c**	0.33**de**	0.57**b**	1.71**c**
15	**-AMF**	0.67**d**	0.29**de**	0.45**cd**	1.40**d**
	+AMF	1.05**b**	0.48**b**	0.53**bc**	2.06**b**
20	**-AMF**	0.49**e**	0.22**f**	0.40**de**	1.11**ef**
	+AMF	0.62**d**	0.28**e**	0.45**cd**	1.34**de**
25	**-AMF**	0.37**f**	0.18**fg**	0.34**e**	0.89**fg**
	+AMF	0.21**g**	0.14**g**	0.35**e**	0.71**g**
30	**-AMF**	0.36**f**	0.16**g**	0.21**f**	0.73**g**
	+AMF	0.40**ef**	0.19**fg**	0.33**e**	0.92**fg**
LSD		**0.11**	**0.05**	**0.08**	**0.24**

*Values with different letters within the same column are significantly different at $p < 0.01$; each value is the mean of three replicates.

Table 3: Spore density, frequency of mycorrhizal colonization (F %), intensity of mycorrhizal colonization (M %), arbuscular frequency (A %) of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plant roots grown under different NaCl concentrations.

Treatments		Mycorrhizal colonization levels (%)			Spore density (spore/g soil)
NaCl conc.(mM)	AMF Status	F	M	A	
Control	-AMF	0.0**c**	0.0**c**	0.0**d**	0.00 **d**
	+AMF	95.0**a**	85.0**a**	61.3**a**	36.00 **c**
10	**-AMF**	0.0**c**	0.0**c**	0.0**d**	0.00 **d**
	+AMF	95.0**a**	85.0**a**	56.5**a**	37.00 **c**
15	**-AMF**	0.0**c**	0.00**c**	0.0**d**	0.00 **d**
	+AMF	95.0**a**	83.0**a**	47.0**b**	39.00 **bc**
20	**-AMF**	0.0**c**	0.0**c**	0.0**d**	0.00 **d**
	+AMF	95.0**a**	79.0**a**	37.5**c**	45.00 **ab**
25	**-AMF**	0.0**c**	0.0**c**	0.0**e**	0.00 **d**
	+AMF	91.0**ab**	74.5**ab**	16.3**d**	47.00 **a**
30	**-AMF**	0.0**c**	0.0**c**	0.0**e**	0.00 **d**
	+AMF	90.0**b**	65.5**b**	11.4**d**	50.00 **a**
LSD		**4.25**	**12.88**	**6.97**	**6.89**

Osmolytes play a major role in the protection of plants from ultra-structure damage induced by salt stress, these are water soluble organic molecules that are non-toxic at high concentrations (Evelin *et al*., 2013). Their major functions are osmoprotection, osmotic adjustment, carbon storage, and radical scavenging under salinity stress.

Our results demonstrated that NaCl treatment gradually increased the total soluble protein in AM and non-AM shoots and roots as compared with control (Table 4), except at the highest NaCl concentration, where a decrease in its content has occurred. These results are in agreement with Abdul Qados (2011). Also AMF colonization increased its content in AM than non-AM ones which is a good conformity with the results of Ibrahim *et al*. (2011) which might be attributed to AM mediated activation of certain plant genes (Sheng *et al*., 2011) or due to the higher efficiency of the osmotic regulation mechanism in cowpea plants which in turn prevents protein reduction under salt stress (Kumar *et al*., 2010). This protein increment lead to membrane stabilization and helps plants to grow and develop under saline conditions (Abdel-Fattah and Asrar, 2012). Also the improvement of protein content by AMF inoculation might be due to accumulation of nutrients that are constituent of several metabolically active compounds (Marschner, 2002).

Lipid Peroxidation (Malondialdehyde Content)

Salinity stress can lead to membrane lipid peroxidation and enhancement the production and accumulation of ROS which cause peroxidation of unsaturated lipid component of membranes resulting in the loss of integrity, and hence leakage and desiccation (Evelin and Kapoor, 2013). The present study demonstrated that MDA content of cowpea plant leaves was significantly increased with increasing salinity stress in both AM and non-AM ones which in concurrence with Rasool *et al*. (2013). But it was clearly that with AMF inoculation, MDA content was significantly lower than those in non-AM plants. This may be due to the substantial increase in antioxidant activities in AM plants leading less lipid peroxidation where antioxidants scavenges the radical production before reacting with the membrane lipids and minimize the lipid peroxidation (Hashem *et al*., 2015).

Total Soluble Carbohydrates Content

Fig. 3 showed that NaCl treatment gradually lowered the carbohydrates content in shoots of AM and non-AM plants, also an increase in its content with AMF inoculation under both control and salt stress conditions. This may be due to (1) AMF enhanced photosynthesis and allowed higher allocation of sugars from shoots to roots, (2) the sink effect of AMF demanding sugars from shoot tissues and (3) hydrolysis of starch to soluble sugar in AM plants (Kapoor *et al*., 2013).

Table 4: Total soluble protein content (µg/g Fwt.) and MDA content (nM/g Fwt.) of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plants grown under different NaCl concentrations.

Treatments		Protein content (µg/g Fwt.)		MDA contnt (nM/g Fwt.)
NaCl conc.(mM)	**AMF Status**	**Shoot**	**Root**	
Control	-AMF	502.3**b**	305.6**d**	12.58**gh**
	+AMF	524.7**b**	342.5**cd**	11.42**h**
10	**-AMF**	494.0**a**	332.8**cd**	13.42**fgh**
	+AMF	532.4**b**	412.4**ab**	12.77**gh**
15	**-AMF**	473.2**a**	352.2**cd**	15.81**def**
	+AMF	551.8**b**	377.5**bc**	14.58**efg**
20	**-AMF**	452.8**a**	315.3**d**	18.00**cd**
	+AMF	530.5**b**	431.8**a**	16.65**cde**
25	**-AMF**	468.4**a**	303.7**d**	19.74**b**
	+AMF	519.8**b**	369.7**bc**	15.48**de**
30	**-AMF**	442.1**a**	220.2**e**	23.03**a**
	+AMF	465.4**a**	307.6**d**	18.97**bc**
LSD		**83.23**	**55.71**	**2.53**

*Values with different letters within the same column are significantly different at $p < 0.01$; each value is the mean of three replicates.

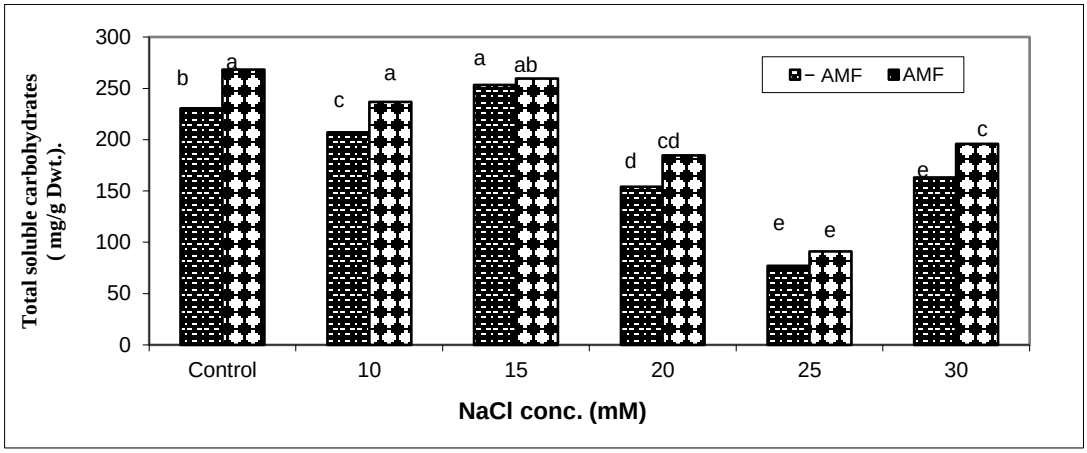

Fig. 3: Total soluble carbohydrates (mg/g Dwt.) of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plants grown under different NaCl concentrations. *Columns topped with different letters are significantly different at $p < 0.01$; each value is the mean of three replicates.

Proline Content

Salt concentrations stimulated the proline content in both AM and non-AM cowpea leaves compared with the control (Fig. 4). This may be due to the enhancement in the activity of proline synthesizing enzymes and reduction in catabolizing ones or its restricted incorporation during protein synthesis. Proline help in maintaining the water balance of plants so that the stress-induced ravage is allayed (Hashem *et al*., 2015). Proline influences protein turn over and directly regulates stress protective proteins. Further increment in proline with AMF colonization in salt stressed plants suggests the beneficial role of AMF in enhancing the stress tolerance by contributing to maintenance of cellular water content (Shekoofeh *et al*., 2012).

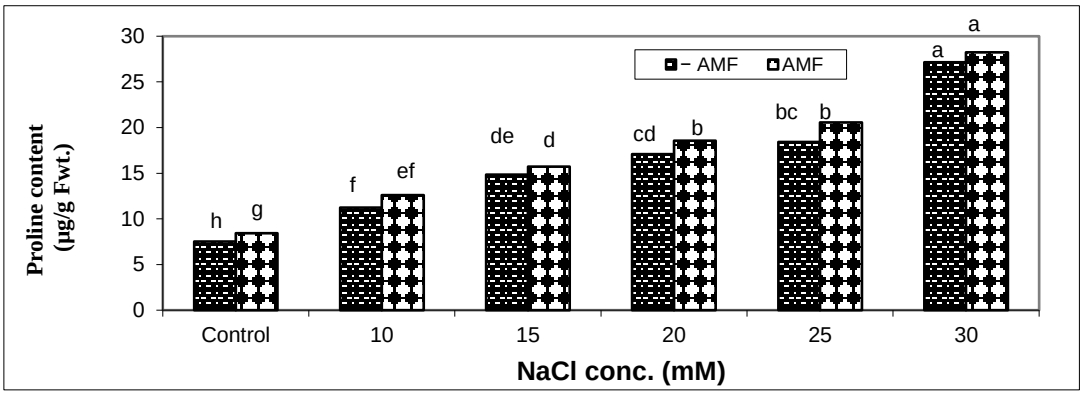

Fig.4: Proline content in leaves (µg/g Fwt.) of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plants grown under different concentrations of NaCl. * Columns topped with different letters are significantly different at $p < 0.01$; each value is the mean of three replicates.

Table 5: Catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activities in shoots of mycorrhizal (+AMF) and non-mycorrhizal (-AMF) cowpea plant leaves grown under different NaCl concentrations.

Treatments		**CAT**	**POX**	**SOD**
NaCl conc.(mM)	**AMF Status**	**(U µmol H_2O_2/min)**	**(U µmol H_2O_2/min)**	**(U/min)**
Control	-AMF	**143.58**h	**0.83**i	**0.75**e
	+AMF	182.11**g**	0.96**hi**	0.80**cde**
10	**-AMF**	192.20**fg**	1.01**ghi**	0.79**de**
	+AMF	227.06**ef**	1.10**fgh**	0.82**cde**
15	**-AMF**	271.10**de**	1.21**efg**	0.82**cde**
	+AMF	294.50**cd**	1.31**cde**	0.86**abcde**
20	**-AMF**	280.73**de**	1.30**de**	0.85**bcde**
	+AMF	308.72**bcd**	1.50**abcd**	0.89**abcd**
25	**-AMF**	324.31**abc**	1.34**bcde**	0.90**abcd**
	+AMF	351.83**ab**	1.57**a**	0.96**ab**
30	**-AMF**	341.28**a**	1.51**abc**	0.93**abc**
	+AMF	361.47**a**	1.63**a**	0.99**a**
LSD		**43.52**	**0.20**	**0.13**

*Values with different letters within the same column are significantly different at $p < 0.01$; each value is the mean of three replicates.

Antioxidant Enzymes

Antioxidant enzymes play an important role in scavenging of reactive oxygen species (ROS) and hence averting the oxidative stress induced damaging effects on several sensitive molecules like proteins, nucleic acids and lipids. Our results showed an increase in SOD, CAT and POX activities of cowpea plant shoots due saltinity. Also with AMF inoculation further increase in their activities occured (Table 5). Increased their activities help plants to maintain the ROS levels below to their deleterious levels and mediate quick removal of toxic ROS so that metabolism remains stable (Abd-Allah *et al*., 2015).

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