

EFFECT OF ENDOMYCORRHIZAL FUNGI ON MAIZE PLANTS UNDER DIFFERENT LEVELS OF SALINITY

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ABSTRACT

A greenhouse experiment was conducted to study the effect of two isolates of arbuscular mycorrhizal fungus (G3 and G4, *Glomus intraradices*) on the maize (*Zea mays* L.) growth, Na and some nutrients uptake in shoots, and roots content of salicylic acid, proline, arginine, phenylalanine and soluble sugar under Na Cl stress. Plants were subjected to four levels of salt stress (0.4, 2.0, 4.0 and 6.0 dSm⁻¹), one week after germination.

Mycorrhizal inoculation significantly reduced Na uptake in maize shoots. AMF treatments significantly increased salicylic acid content in roots (G3: 66.7 and 95.6%) and (G4: 17.4 and 50.4%) under 4.0 and 6.0 dSm⁻¹, respectively, more than the non-inoculated ones at the end of the experiment. Proline and soluble sugar contents in roots showed pronounced increases. N and P uptake in AM shoots significantly increased under salt stress, as compared to the non-inoculated plants. Results conclusively indicate that mycorrhizal inoculation increased the salt stress tolerance of maize plants.

Keywords: Arbuscular mycorrhizal fungi; salicylic acid; salt stress; maize

INTRODUCTION

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semi-arid areas (Al-Karaki 2006). Saline soils occupy 7% of the earth's land surface (Ruiz-lozano *et al.* 2001) and increased Salinization of arable land will result in to 50% land loss by the middle of the 21st century (Wang *et al.* 2003). High levels of salinity (> 4 dSm⁻¹ or > 0.1% soil content; Juniper and Abbott 1993) in soils is mainly due to the soluble salts in irrigation water and fertilizers used in agriculture (Copeman *et al.* 1996; Al-Karaki 2000), low precipitation and high temperature in these regions and over- exploitation of available water resources (e.g. ground water) (Mouk and Ishii 2006). The direct effects of salt on plant growth may involve : (a) reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought (Jahromi *et al.* 2008); (b) toxicity of excessive Na⁺ and Cl⁻ ions towards the cell – the toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of photosynthesis, respiration and protein synthesis (Feng *et al.* 2002); and (c) nutrient imbalance in the plant caused by nutrient uptake and/or transport to the shoot leading to ion deficiencies (Adiku *et al.* 2001).

Some microorganisms, particularly beneficial bacteria and fungi can improve plant performance under stress environments and, consequently, enhance yield (Creus *et al.* 1998). Arbuscular mycorrhizal fungi (AMF) are associated with the roots of over 80% terrestrial plant species (Smith and Read 1997). AMF have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition (Al-Karaki and Al-Raddad 1997), producing plant growth hormones, improving rhizospheric and soil conditions (Lindermann 1994), altering the physiological and biochemical properties of the host (Smith and Read 1995) and defending roots against soil-borne pathogens (Dehne 1982).

Salicylic acid (SA) is widely distributed in monocot and dicot plants. Recent studies show that SA can enhance plant resistance to environmental stresses, such as heat, chilling and drought (Janda *et al.* 1999; Tissa *et al.* 2000). Salicylic acid is a plant phenol, and today it is in use as internal regular hormone (Shahba *et al.* 2010).

This research studies the effect of AMF (*Glomus intrardices*) on the growth, salicylic acid and chemical contents of maize plants under salinity stress (NaCl).

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the faculty of Agriculture, Saba – Basha, Alexandria University, Egypt, during the autumn of 2010 with natural light at 30/20 degrees centigrade (day / night). Two mycorrhizal strains *Glomus intrardices* were used in this experiment. The first (G3) was isolated from the Experimental Station of Alexandria University at Abies (Aboul – Nasr 1993a), and the second (G4) was produced by " Amykor Company, Germany. The inoculum consists of expanded clay aggregates (2-4 mm in diameter, leca), containing chlamydospores and fungus mycelium, which had been produced on *Tagetes erecta* L. (Aboul – Nasr 2004). The inoculum was applied prior to sowing by mixing the carrier material with the substrate (10%, v.), the controls received the same amount of heat sterilized expanded clay.

A mixture of sterilized sand and peat moss (45%:45%,v.) at 110 °C for 24 hr were used as a substrate (mixture pH = 7.4). Three grains of maize (*Zea mays*. L.) var. Giza 321, were sown in plastic pots, (11 cm in diameter). Seedlings were irrigated with tap water as needed prior to stress applications. Salinity treatments were started, one week after germination. Four levels of NaCl solution were applied; irrigated with tap water (0.4 dSm⁻¹ control), 2 ,4 and 6 dSm⁻¹ of Na Cl solution. Plants were irrigated as needed with field capacity, Ca 70%. Liquid soil fertilizer "Super grow (Agrico., Egypt, 100g /200L), N:P:K = 20:20:20) was used and the plants received 30 ml / pot weekly. After 4 and 8 weeks from the beginning of salt treatments, i.e. the 5th and 9th week of plant age, three root samples from three randomized pots (one from each pot) were taken and the amount of salicylic acid in fresh roots was measured according to Iqbal and Vaid (2009). Shoot and root dry

weights g/ plant were also recorded. Three pots per each treatment from the shoot were analyzed for the determination of N by Nessler's method (Chapman and Pratt 1978), and P (Jackson 1973). The concentrations of K and Na were determined by flame photometry as described by Worth (1985). Carbohydrates and three amino acids; proline, arginine and phenylalanine were measured spectrophotometrically in root (Umbreit *et al.* 1972). At the end of the experiment soil's electrical conductivity (EC) from the different treatments was measured.

The experimental design consisted of 6(3×4) treatments crossing three mycorrhizal inoculation levels (non-AMF, *G.intraradices* (G3) and *G.intraradices* (G4) with four salt levels (Na Cl). Pots were arranged as factorial in a completely randomized block design. Six replicates of each treatment were applied totaling 24 pots for each AMF strain. The statistical analysis was done according to SAS (1989).

RESULTS AND DISCUSSION

1- Measurement of soil electrical conductivity (EC)

At the end of the experiment, soil samples from each treatment were randomized collected and the EC (dSm^{-1}) was measured. Figure (1) shows that the soil EC at the beginning of the experiment was 0.8 dSm^{-1} . In case of non-mycorrhizal plants the soil salinity was increased according to the irrigation of NaCl level. It reached 0.33, 1.54, 2.41 and 3.54 dSm^{-1} in treated plants with 0.4, 2.0, 4.0 and 6 dSm^{-1} NaCl solution, respectively. *Glomus intraradices* (G3) reduced soil salinity within all salt concentrations by – 41.6, – 24.5 and –24.3% than the un-inoculated and irrigated plants with 2.0, 4.0 and 6.0 dSm^{-1} respectively. G.4 had also a positive effect on soil EC and inoculated soil pots with G.4 reached - 31.8, – 17.8 and – 13% reduction than the un-inoculated ones in case of irrigated plants with 2.0, 4.0 and 6.0 dSm^{-1} , respectively.

2- Na uptake in shoots (ppm)

Figure (2) shows the significant effect of both AM strains on the reduction of Na uptake in shoots. Na uptake in shoots of non-AM maize seedlings was 10.6, 17.0, 22.6 and 32.3 ppm in case of irrigated pots with tap water, 2.0, 4.0 and 6.0 dSm^{-1} of NaCl solution. The percentage decrease of Na uptake in case of *G. intraradices* (G3) reached 50% when plants irrigated with moderate and high concentration of Na Cl (4 and 6 dSm^{-1}) as compared to non –inoculated plants. G4 showed the same trend in Na uptake, but Na uptake was more reduced in G3 than G4. Similar results were obtained by He *et al.* (2010). One of the harmful effects of salinity on plant growth involves the excessive accumulation of Na^+ and Cl^- (Ashraf and Hrris 2004 ; Munns *et al.* 2002). More Na^+ accumulation resulted in ionic imbalance, specific ion effects and nutrient deficiency symptoms in plants. They found that the Na^+ concentration in shoots and roots of non – AM tomato seedlings was 1.11 and 1.22 times of AM plants respectively, under saltless condition, 1.57 and 1.26 times under 0.5% salt stress, 1.07 and 1.06 times under 1% salt stress respectively. They also reported that AM colonization seemed to decrease

Na⁺ under 0.5% salt stress, remarkably. Our results showed that AM helped to sustain ion balance and lower Na⁺ accumulation which played key role in improving salt tolerance of AM maize.

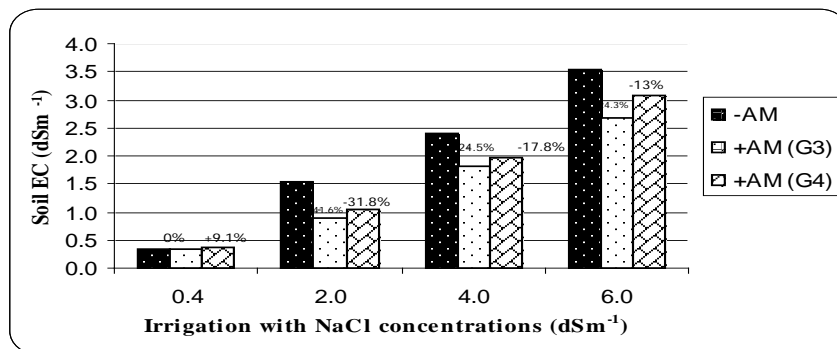


Figure (1). Soil EC (dSm⁻¹) at the end of maize experiment

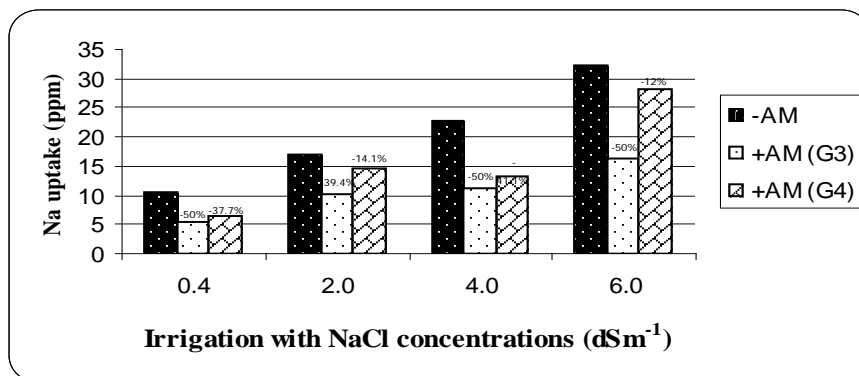


Figure (2). Effect of two strains of *Glomus intraradices* on Na uptake in maize shoots (ppm) under different NaCl stress

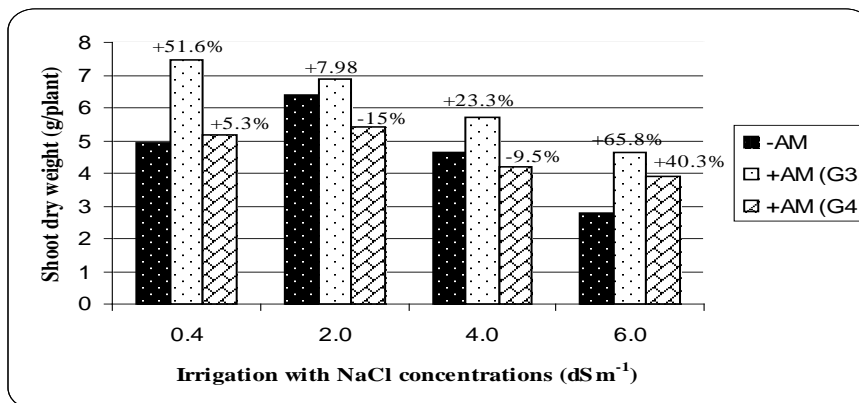


Figure (3). Effect of two strains of *Glomus intraradices* on shoot dry weight (g/plant) under different NaCl stress

3- Shoot and root dry weights (g / plant)

Na Cl stress significantly reduced plant growth (Figures 3 and 4). Shoot and root dry weights increasement were significantly larger than corresponding non-AM seedlings. The percentage increase in shoot dry weight in case of inoculated plants with G3 was 8, 23.3, 65.8% more than un-inoculated ones under different salt level stress (2, 4 and 6 dSm⁻¹), respectively. Whereas the percentage increase was 39.3, 66.2 and 24.5%, respectively, in root dry weight compared to non-AM. Inoculated plants with G4 increased the shoot dry weight in case of the highest concentration of Na Cl (6 dSm⁻¹). The same trend was also found in root dry weight. Similar results were reported by Al-Karaki *et al.* (2001) and He *et al.* (2010).

4- Salicylic acid (SA) (mg / 100g root fresh wt)

Salicylic acid was measured spectrophotometrically in maize fresh roots (mg /100g fresh root). Data in Figures (5a and 5b) show the significant increase in salicylic acid's amount under the different levels of Na Cl, especially in inoculated plants either with G3 or G4 when plants were 5 and 9 weeks old. The highest percentage increase appeared in G3 at the end of the experiment. It reached 54, 67 and 95.6% in the presence of 2, 4 and 6 dSm⁻¹, respectively, as compared to the un-inoculated plants. The same trend was shown in case of G4. Salicylic acid is a plant phenol, and it is in use as internal regulator hormone, because its role in the defensive mechanism against biotic and abiotic stresses (Shahba *et al.* 2010).

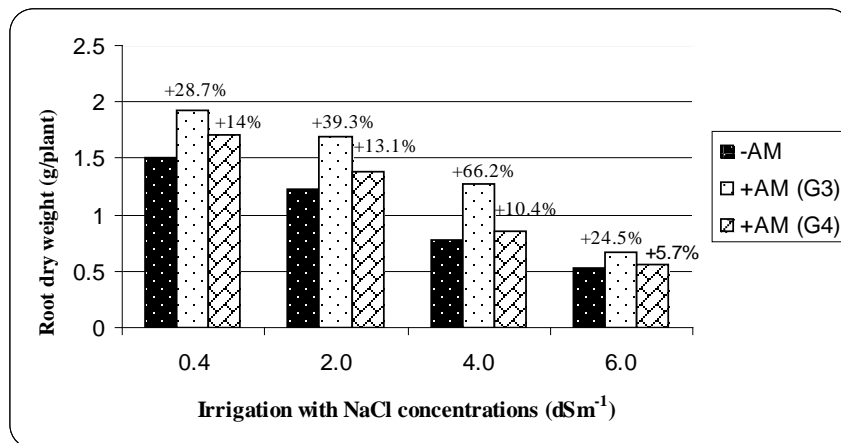


Figure (4). Effect of two strains of *Glomus intraradices* on root dry weight (g/plant) under different NaCl stress

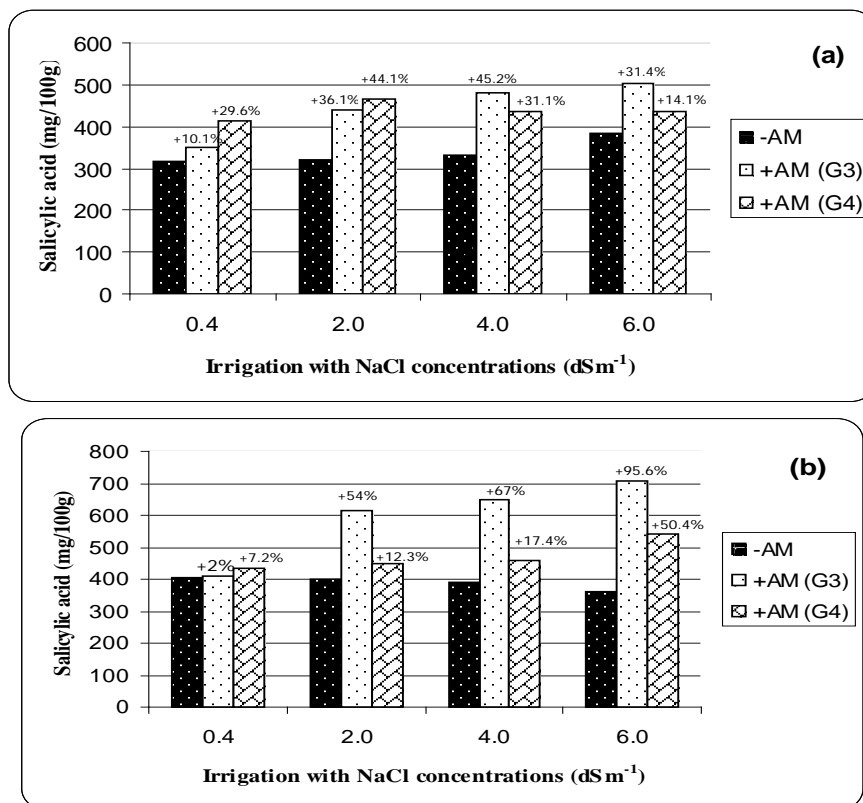


Figure (5). Effect of two strains of *Glomus intraradices* on salicylic acid content in root fresh weight (mg/100g) under different NaCl stress a) after 5 weeks from planting b) after 9 weeks from planting

Klessig and Malamy (1994) and Clarke *et al.* (2000) reported that, salicylic acid (SA) has been firmly established as a natural signal molecule in the induction of systemic acquired resistance (SAR) in tobacco, cucumber and some other plants. Recent studies show that SA can enhance plant resistance to environmental stresses, such as heat, chilling and drought (Janda *et al.* 1999 and Tissa *et al.* 2000).

5- Free amino acids

Proline, arginine and phenylalanine were determined in maize roots. Figure (6) shows that with increasing salinity, the root proline increases. Significant increases were obtained in AM roots as compared to the un-inoculated ones. The high amount of accumulated proline in roots was in case of G3 and G4 under 4 and 6 d Sm⁻¹.

Figures (7) and (8) show the amount of arginine and phenylalanine as compared to the un-inoculated plants. The plants under salinity condition change their metabolism to overcome the changed environmental condition. One mechanisms used by the plants for overcoming the salt stress effects

might be via accumulation of compatible osmolytes, such as proline and soluble sugar. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm, (Shahba *et al.* 2010 ; Ayala – Astorga and Alcaraz – Meléndez 2010).

6- Soluble sugar (mg /100g root dry weight)

Figure (9) shows carbohydrate content in maize roots (mg /100g dry weight). Soluble sugar was increased in AM roots than corresponding non-AM under the different levels of Na Cl stress. Under continuous salt stress, our results showed that the enhanced salt tolerance was closely related to the increase of soluble sugar accumulation in roots of AM plants. Results obtained by Feng *et al.* (2002) indicated that improved tolerance of maize plants to salt stress by arbuscular mycorrhiza was related to the higher accumulation of soluble sugars in roots. He *et al.* (2010) explained that the greatest sugar accumulation in AM plant under salt stress may be due to (1) the ability of plants to cope with salt stress, (2) the sink effect of the mycorrhizal fungus demanding sugars from shoot tissues, (3) the fact that AM colonization enhanced photosynthesis and therefore allowed higher allocation of sugars from leaves to roots Moreover, the salt stressed seedlings accumulated more total soluble carbohydrates in the roots than in the leaves, thus showing a change in behavior of shoot / root carbon partitioning. This probably reflects the maintenance or even induction of root elongation at low water potentials, which can be considered as an adaptive response to drought and salinity (Balibrea *et al.* 2000).

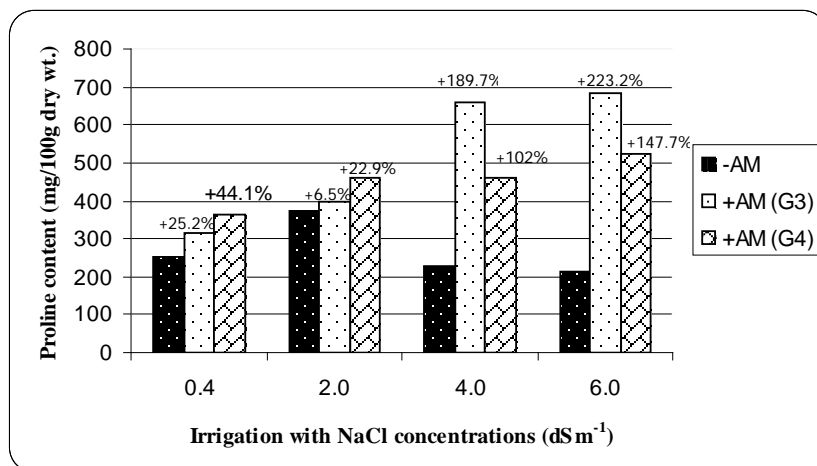


Figure (6). Effect of two strains of *Glomus intraradices* on proline content in root dry weight (mg/100g) under different NaCl stress

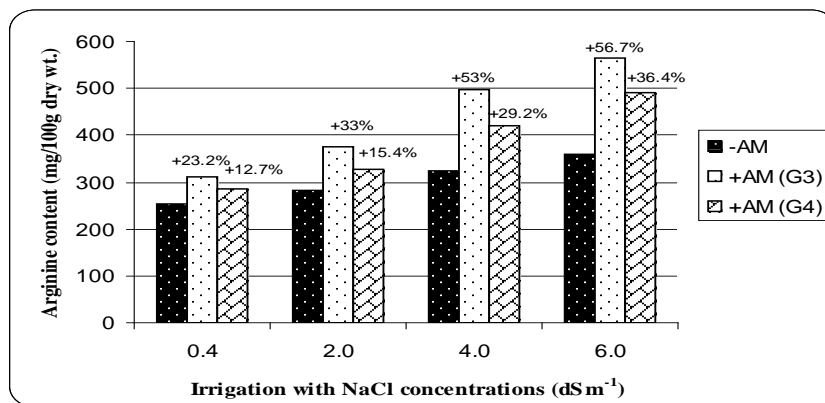


Figure (7). Effect of two strains of *Glomus intraradices* on arginine content in root dry weight (mg/100g) under different NaCl stress

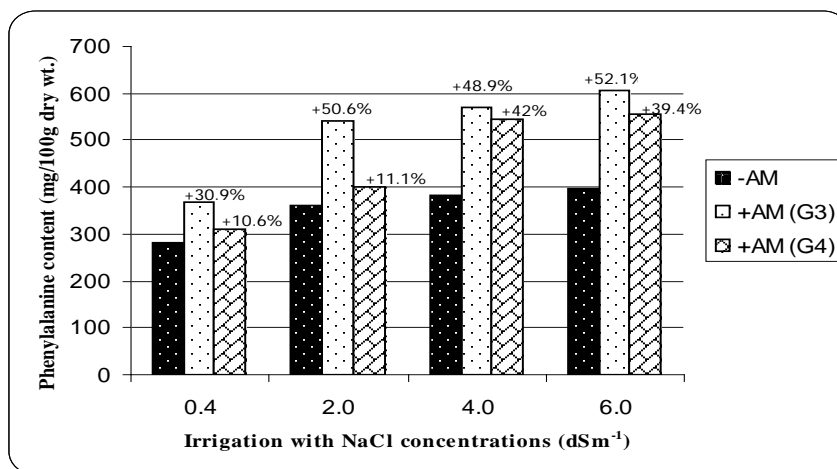


Figure (8). Effect of two strains of *Glomus intraradices* on phenylalanine content in root dry weight (mg/100g) under different NaCl stress

7- N P K uptake (mg /100g dry weight)

N P K uptakes in maize shoots were determined at the end of the experiment under salt stress.

a- N uptake:

Figure (10) shows that AM inoculation significantly increased N uptake under the different levels of NaCl as compared to the non-inoculated plants. Studies have reported that improved N nutrition may help to reduce the toxic effects of Na ions by reducing its uptake and this may indirectly help in maintaining the chlorophyll content of the plant. The form of available N (NO₃⁻ or NH₄⁺) strongly influences Na⁺ accumulation (Giri and Mukerji

2004). However, the exact mechanisms used by AMF to N uptake under salt – stress conditions are not clearly understood.

b- P uptake (mg /100 dry weigh)

AMF have been shown to have a positive influence on the composition of mineral nutrients (especially poor mobility nutrients such as phosphorus) of plants grown in salt – stress conditions (Al- Karaki and Clark 1998).

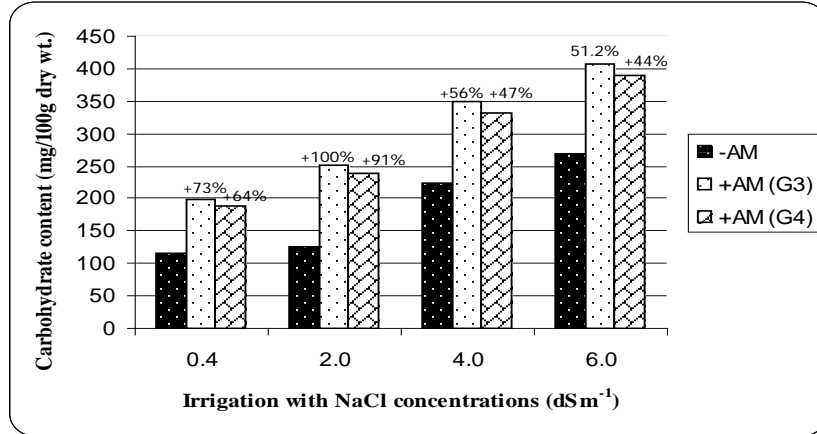


Figure (9). Effect of two strains of *Glomus intraradices* on carbohydrates content in root dry weight (mg/100g dry wt.) under different NaCl stress

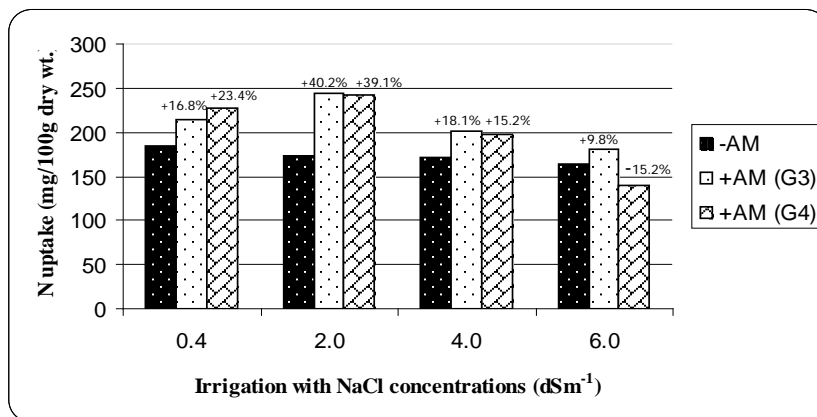


Figure (10). Effect of two strains of *Glomus intraradices* on N uptake (mg/100g dry wt.) in shoot dry weight under different NaCl stress

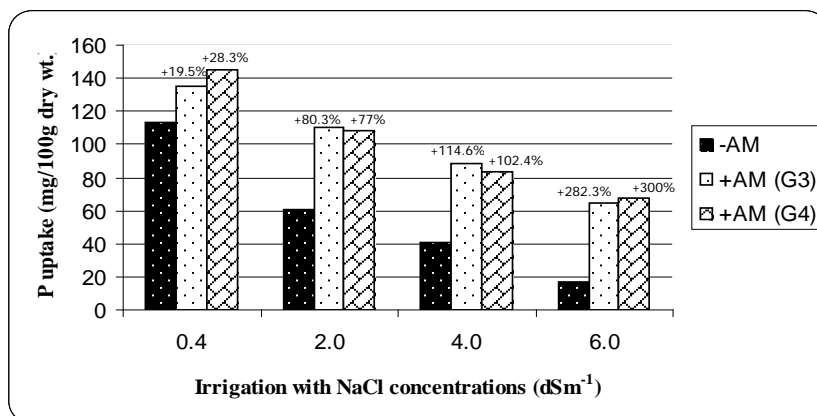


Figure (11). Effect of two strains of *Glomus intraradices* on P uptake (mg/100g dry wt.) in shoot dry weight under different NaCl stress

Figure (11) shows that AM inoculation resulted in highly significant increase of P uptake as compared to the non-AM plants under all salt stress levels. The percentage was increased in case of G3 and G4 by increasing NaCl level compared to non-inoculated maize plants. At salt level 4 dSm⁻¹ P uptake was increased to 114.6 and 102.4 % in case of G3 and G4, respectively, more than non-inoculated ones. Whereas the percentage increase of P uptake reached 282.3 and 300 % more than the non-inoculated plants for the same AM strains, respectively. Mycorrhizal inoculation can increase P concentration in plants by enhancing its uptake facilitated by the extensive hyphae of the fungus which allows them to explore more soil volume than the non-mycorrhizal plants (Ruiz – Lozano and Azcon 2000). It is estimated that external hyphae deliver up to 80% of a plant's P requirements (Matamores *et al.* 1999). Studies have shown higher P content in mycorrhizal than non-mycorrhizal *Acacia nilotica* plants in saline soils at varied levels of soil salinity (Giri *et al.* 2007).

c- K uptake (mg / 100g dry wt)

Data in Table (1) shows the effect of *G. intraradices* on K uptake in maize shoots under NaCl stress. No significant differences were found due to AM inoculation. When Na⁺ or salt concentration in the soil is high, plants tend to take up more Na⁺ resulting in decreased K⁺ uptake. Na⁺ ions compete with K⁺ for binding sites essential for various cellular functions. Potassium plays a key role in plant metabolism. It activates a range of enzymes, and plays an important role in stomatal movements and protein synthesis (Blaha *et al.* 2000).

Zuccarini and Okurowska (2008) reported that mycorrhizal colonization can enhance K⁺ absorption under saline conditions, while preventing Na⁺ translocation to shoot tissues. There are contrasting reports that the uptake of K⁺ increased in shoot tissues of mycorrhizal plants even at a high salinity level (9.5 dSm⁻¹). This increases the K⁺ : Na⁺ ratio in roots and shoots of AM plants (Giri *et al.* 2007).

Table (1). Effect of two strains of *Glomus intraradices* on K uptake (mg/100g dry wt.) in shoot dry weight under different NaCl stress

Parameter	K uptake (mg/100g shoot dry weight)							
	0.4		2.0		4.0		6.0	
NaCl (dSm ⁻¹)	mg/100g	±%	mg/100g	±%	Mg/100g	±%	mg/100g	±%
- AM	1361 a	--	1828 a	--	1618 a	--	1152 a	--
+ AM (G3)	1592 a	+17	1642 a	-10	1603 a	-1	1383 a	+20
+ AM (G4)	1488 a	+9.3	1772 a	-3	1521 a	-6	1207 a	+4.8
Inoculation L.S.D. _{0.01}	315.312 (ns)							
Salinity L.S.D. _{0.01}	364.091**							
Interaction L.S.D. _{0.01}	630.64 (ns)							

Conclusion:

The role of arbuscular mycorrhizal fungi (AMF) in alleviation salt stress is well documented. Our study explained some mechanisms of AMF employ to enhance the salt tolerance of maize plants such as enhanced nutrient acquisition (P, N), reduced Na⁺, biochemical changes (accumulation of proline and carbohydrates) and enhanced plants resistance to salt stress by increasing the amount of salicylic acid which has been proposed as an internal regular plant hormone.

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تأثير الميكوريزا الداخلية علي نباتات الذرة تحت تأثير مستويات مختلفة من الملوحة

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أجريت التجربة في صوبه زجاجية لدراسة تأثير عزلتين من فطر الميكوريزا الداخلية علي نمو نباتات الذرة ، امتصاص الصوديوم وبعض العناصر الغذائية في المجموع الخضري وتقدير حمض الساليسليك ، البرولين ، الأرجينين ، الفينيل ألانين والسكريات الذائبة في جذور الذرة تحت تأثير أربعة مستويات من الملوحة. وكانت بداية استخدام المحلول الملحي بعد اسبوع من الانبات. أظهرت النباتات الملقحة بالميكوريزا انخفاضا معنويا في امتصاص الصوديوم في المجموع الخضري. كما اظهرت زيادة في محتوى الجذر من حمض الساليسليك فقد كانت العزلة ج 3 تزيد 66.7 و 95.6 % عن النباتات الغير ملقحة أما العزلة ج 4 فزاد حمض الساليسليك الي 17.4 و 50.4 % عند تركيز 4 و 6 عن النباتات الغير ملقحة وذلك في نهاية التجربة. كان هناك زيادة معنوية في كلا من الوزن الجاف للمجموع الخضري و المجموع الجذري في حالة النباتات الملقحة مقارنة بالغير ملقحة. زاد ايضا امتصاص النباتات الملقحة لكل من النيتروجين ، الفوسفور و البوتاسيوم تحت ظروف الملوحة مقارنة بالنباتات الغير ملقحة. كما اظهرت النتائج زيادة معنوية في محتوى النباتات من الأحماض الأمينية (البرولين ، الأرجينين و الفينيل الانين) مقارنة بالنباتات الغير ملقحة وكذلك ايضا زيادة معنوية في محتوى النباتات من السكريات الذائبة. أوضحت التجربة مدي تحمل نباتات الذرة الملقحة بفطر الميكوريزا الداخلية للنمو تحت الظروف الملحية.

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