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RESEARCH ARTICLE

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Arbuscular mycorrhizal colonization improves growth and biochemical profile in *Acacia arabica* under salt stress

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ABSTRACT

This study elucidated the individual and mixed mycorrhizal effects of two arbuscular mycorrhizal (AM) isolates on growth and biochemical status of *Acacia arabica* under salinity stress gradients. Salt treatment provided in soil hampered legume growth and its biochemical status. But, mycorrhizal colonizations in plant root system reduced the extent of deleterious salt effect and also helped in plant growth enhancement. Additionally, mixed mycorrhizal association (*Glomus mosseae* + *Glomus fasciculatum*) responded better towards osmolyte accumulation and in salt stress alleviation. Due to individual and mixed mycorrhizal colonizations in *A. arabica*; protein, carbohydrate and reducing sugar acquisitions were found maximum at soil salinity of 5.94 dS/m over corresponding non-mycorrhizal plant. However, mixed AM inoculation accumulated proline content and improved dry biomass to a higher magnitude at the highest soil salinity level. Mixed AM (*G. mosseae* + *G. fasciculatum*) colonization improved maximum amount of total chlorophyll (20.94%), protein (19.72%), carbohydrate (23.83%), reducing sugar (17.60%) at soil salinity of 5.94 dS/m and dry biomass (20.35%), proline content (10.99%) at salinity level of 8.26 dS/m when compared with non-mycorrhizal counterpart. Greater magnitude of AM root colonization was found in mixed AM treated plant and may be responsible for more improvement in growth and biochemical status and consequently mitigated adverse salt effect better.

Key words: *Glomus mosseae*, *Glomus fasciculatum*, *Acacia arabica*, growth, biochemical status, salt stress

Introduction

In the present scenario apart from urbanization and industrialization, abiotic stress mainly salinity is one of the major limiting factors for agricultural crop production worldwide. Indiscriminate use of various chemical fertilizers for prolonged periods, irrigation mismanagement etc. are responsible to convert agricultural fertile land into saline. In various parts of India saline soil is widely distributed and the high salt content in this soil results in nutrient imbalance, generates reactive oxygen species in plant cells, impairs various physiological processes including photosynthesis, carbohydrate metabolism, decreases enzyme activity etc. (Gao et al., 1998; Al-Karaki, 2000; Melloni et al., 2003). As a consequence, it causes deterioration of plant growth and yield. In essence, soil salinity generates adverse effect on morphological, physiological and biochemical properties of plant. Hence to remediate saline soil, various strategies have been evolved so far which includes use of organic amendments from biological sources, chemical amendments, plantations with medicinally important tree species, development of stress tolerant crops etc. (Jain & Singh, 1998; Ahmad et al., 2006; Tejada et al, 2006; Rai et al., 2011). However, these approaches became successful but their application is limited because of cost factor and some of them take extensive time (Cantrell & Linderman, 2001). Hence, to overcome the negative effect of saline soil, to improve soil quality and to maintain agricultural sustainability; an

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alternative approach by dealing with arbuscular mycorrhizal (AM) fungi and its association with host plant would be a better option (Harrier & Watson, 2003).

AM fungi form mutualistic symbiotic association in vascular plant roots and help in better water absorption, nutrient mobilization including phosphate uptake, protect host plant root from pathogenic attack, improve plant growth hormonal profile, increase abiotic stress tolerance to plants etc. (Allen, 1991; Selvaraj et al., 2004; Selvaraj et al., 2005). AM fungi ameliorate adverse salt effect on plant health mainly by stimulating host plant physiological process, enhancing antioxidant enzyme potential, improving mineral status of host (Juniper & Abbott, 1993; Lindermann, 1994; Zhong Qun et al., 2007). However, their beneficial effect varies among different plant species as well as among type of AM fungi (Burke et al., 2003; Tian et al., 2004). Hence, selection of specific AM fungus in association with particular host plant is required to investigate to achieve better benefit from this plant-fungus symbiosis as well as to obtain its efficient use in saline soil amelioration. The knowledge regarding mycorrhizal responses using two different AM isolates in combination towards tree legume host remains scarce. On this background, the present study was conducted to check the effectiveness of *Glomus mosseae* and *Glomus fasciculatum* as an individual and in mixed inoculation in improvement of growth and biochemical profiling of legume (*Acacia arabica*) under salt stress.

A. arabica (family: Fabaceae) is a tree legume and its gum is valued at industrial level. Additionally, fresh bark and leaf of this legume have medical application (Agrawal et al., 2010; Shittu, 2010). The main purpose of this study was to compare the individual and mixed (combined) effects of two AM isolates on growth and biochemical status of *A. arabica* for the selection of most suitable AM treatment in salt stress amelioration.

Materials and Methods

Seeds of *A. arabica* were purchased from Naik Seeds Pvt. Ltd., Pune, Maharashtra (India) and were surface sterilized by washing with dilute sulfuric acid followed by soaking in sterile distilled water. For germination, soaked seeds were kept in dark on sterilized fine sand moistened with sterile distilled water. To carry out green house study, soil (pH 6.0) was first mixed with river sand (particle size of <0.3 mm) [1:1 (v/v)] and then the mixture was autoclaved to remove indigenous AM propagules.

AM treatment

In this study, soil based inocula of two indigenous AM isolates namely *Glomus mosseae* (Nicol. & Gerd.) and *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe. were used. In our laboratory, these two AM isolates were previously isolated from rhizospheric soils of *A. arabica* (soil EC: 5.77 dS/m) and *Arachis hypogaea* (soil EC: 1.68 dS/m) respectively by wet sieving and decanting method (Gerdemann & Nicolson, 1963; Schenck & Perez, 1990). Soil based inocula of *G. mosseae* (~160 AM spores/ 10 g dry soil) and *G. fasciculatum* (~110 AM spores/10 g dry soil) were obtained by propagating spores of respective AM isolates in autoclaved sand: soil (1:1, v/v) for three months using *Zea mays* L. as host.

Green house experiment

The green house experiment was conducted in a complete randomized manner, comprised of four mycorrhizal treatments [NM: non-mycorrhizal (uninoculated), *Gm*: *Glomus mosseae* inoculated, *Gf*: *Glomus fasciculatum* inoculated, *Gm+Gf*: both *Glomus mosseae* and *Glomus fasciculatum* inoculated], five different soil salinity levels [soil electrical conductivity (EC) of 1.04 dS/m (control), 2.10, 3.78, 5.94 and 8.26 dS/m], with four replicates (three plants per replicate) using *A. arabica* as host plant. Variable amount of soil based inocula [50 g *G. mosseae* (for *Gm* treatment), 75 g *G. fasciculatum* (for *Gf* treatment), and 25 g *G. mosseae* + 40 g *G. fasciculatum* (for *Gm+Gf* treatment)] were used to maintain almost similar number of AM spores per pot. Soil based inoculum was placed below the seedling and seedlings of almost equal length were chosen for the study. NM labeled pot did not receive any mycorrhizal inoculation and in all the mycorrhizal and non-mycorrhizal treatments, constant sand: soil volume was maintained. Tap water (passed through 105 μ sieve) was used to irrigate plants on every alternate day and with P-free Hoagland solution (X/10) twice in a month (Hoagland & Arnon, 1940). Salinity stress was provided by applying gradient of NaCl solutions (50 to 200 mM) in soil after one month of seedling transplantation until the target salinity level (in terms of EC value) was achieved. NaCl solution was not supplied to the control pot which had soil EC of 1.04 dS/m.

Growth Parameters

Plant height and biomass

After the growth period of two months from the date of seedling transplantation, plant from each treatment was

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harvested and height (cm) was measured. To measure dry biomass, fresh plant from each treatment was washed in distilled water and kept in oven at 60°C for drying and finally dry weight was recorded.

Biochemical Parameters

To analyze the amount of total protein, carbohydrate, reducing sugar and proline, whole plant material was taken including both shoot and root tissues.

Total chlorophyll content

Leaf chlorophyll content was estimated by extracting fresh leaves (1 g) in 80% (v/v) acetone followed by centrifugation. Absorption of supernatant was measured and concentrations of chlorophyll *a* and chlorophyll *b* were calculated using the following formulae (Strain & Svec, 1966):

Chlorophyll *a* (mg/g) = [11.64 X (absorption at 663 nm) – 2.16 X (absorption at 645 nm)]

Chlorophyll *b* (mg/g) = [20.97 X (absorption at 645 nm) – 3.94 X (absorption at 663 nm)]

Total chlorophyll content was calculated by adding the concentrations of chlorophyll *a* and chlorophyll *b* and the value was expressed in terms of mg/g of fresh leaf.

Protein content

Soluble protein content was determined by dye binding assay using fresh plant material (Bradford, 1976).

Carbohydrate content

Soluble carbohydrate content of plant material was measured according to the method of Hedge & Hofreiter (1962**)**. After acid hydrolysis, sample solution was treated with anthrone reagent and the absorbance was measured at 630 nm.

Reducing sugar content

Plant material was taken to estimate reducing sugar content using 3,5-dinitrosalicylic acid (Miller, 1972).

Proline content

Free proline content in plant material was determined using ninhydrin acid reagent following aqueous sulphosalicylic acid extraction (Bates et al., 1973).

Electrolyte leakage (EL)

Fresh leaf was taken in test tube containing distilled water and was allowed to heat at 32°C in water bath. Initial electrical conductivity (EC) of the medium was measured at 25°C and then the sample in tube was autoclaved. After cooling, final EC was measured at 25°C. EL of leaf sample was calculated (Dionisio-Sese & Tobita, 1998).

Root colonization (%)

To measure percent root colonization, root sample was washed thoroughly and boiled in hot KOH solution (10%, w/v at 90°C) until root becomes colorless. Then, cleared roots were treated with HCl (10%, v/v) for 3min and stained with hot trypan blue solution (0.05%, w/v) for 15min at 90°C (Phillips & Hayman, 1970). Gridline intersect method was used to estimate percent root colonization (Giovannetti & Mosse, 1980).

Statistical analysis

Data were analyzed by ANOVA. Duncan's Multiple Range Test was used to determine significant ($P<0.05$) difference between means. Data was analyzed statistically using SPSS v. 9.0 software.

Results

In the present study it was observed that salt treatment provided in soil affected *A. arabica* growth and amount of its biochemical constituents. But AM colonizations in this plant root system reduced the negative impact of salt stress and helped in plant growth enhancement. This tree legume responded differently towards two different AM isolates and also variable effects were noticed due to their individual and mixed inoculations with respect to increment in plant biomass and biochemical status.

Plant height

Irrespective of mycorrhizal inoculations, control plant found relatively taller than plants from salt stressed treatments (2.10 to 8.26 dS/m) (Figure 1).

At various levels of soil salinity (2.10 to 8.26 dS/m) and at control treatment (1.04 dS/m), mycorrhizal plants had significantly better height than corresponding NM plants. Under the gradient of salt stress treatment (1.04 to 8.26 dS/m) NM, *Gm*, *Gf* and *Gm+Gf* plants had heights in the ranges of 39.7 to 15.6, 42.1 to 17.2, 41 to 16.3 and 47 to 18.7 cm respectively (Figure 1).

Plant biomass

NM plants grown under control treatment (1.04 dS/m) had dry biomass of 0.804 g and with increase in soil salinity; a steady, significant decrease was noticed. Although, plant dry biomass was reduced due to saline stress but mycorrhizae colonized plants had comparatively greater dry biomass than corresponding NM plants at control (1.04 dS/m) and soil salinity range of 2.10 to 8.26 dS/m. It was observed that, *A.*

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arabica inoculated with *Gm* and *Gf* isolates individually had dry biomass contents in the ranges of 0.881 to 0.131 g and 0.836 to 0.127 g respectively under provided salt stress treatments (1.04 to 8.26 dS/m) (Figure 2).

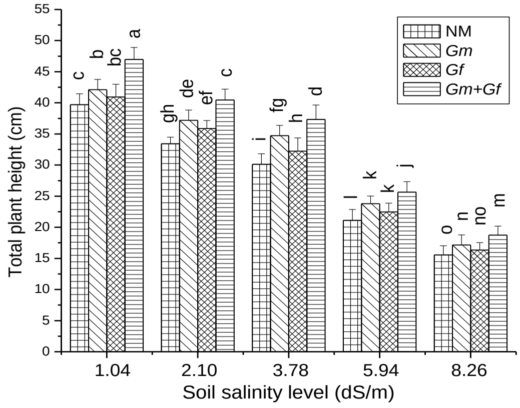

Figure 1. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on height of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

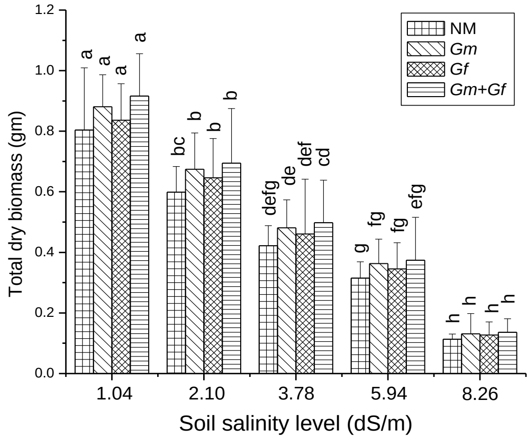

Figure 2. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on dry biomass of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

In comparison with individual mycorrhizal effects, mixed mycorrhizal inoculation (*Gm* + *Gf*) resulted in relatively greater amount of dry biomass and was in the range of 0.916 to 0.136 g at control (1.04 dS/m) and soil salinity treatment (2.10 to 8.26 dS/m) (Figure 2).

Chlorophyll content

By raising soil salinity stress levels from 1.04 (control) to 8.26 dS/m, a significant (*P*<0.05) reduction in total chlorophyll concentration was observed in NM and all the mycorrhizal plants (Figure 3).

At control treatment (1.04 dS/m), NM plant had chlorophyll content of 1.821 mg/g and the value was declined to 0.577 mg/g at the highest level of soil salinity (8.26 dS/m). Salinity stress in soil reduced chlorophyll content in plant but, mycorrhizal associations significantly enhanced the concentration of photosynthetic pigment. However in this regard, mixed inoculation with two different AM isolates was found superior. *Gm* and *Gf* plants had total chlorophyll contents in the ranges of 2.009 to 0.653 and 1.938 to 0.612 mg/g respectively while growing at control (1.04 dS/m) and soil salinity of 2.10 to 8.26 dS/m. Whereas, mixed mycorrhizae inoculated test plant from control treatment had chlorophyll content of 2.114 mg/g and the concentration was significantly decreased to 0.667 mg/g at the highest level of soil salinity (8.26 dS/m) (Figure 3).

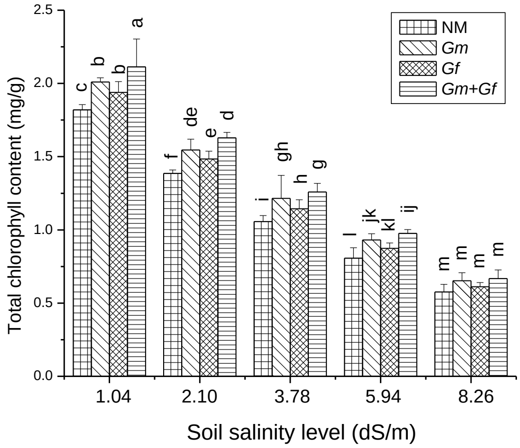

Figure 3. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on total chlorophyll content of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

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Protein content

Data from Figure 4 indicate that, with an increase in soil salinity from 1.04 (control) to 2.10 dS/m, protein content in test plant was increased significantly irrespective of mycorrhizal treatments. But, with further increment in soil salinity stress levels from 2.10 to 8.26 dS/m, almost significant reduction in protein content was noticed in *A. arabica* (Figure 4). At control (1.04 dS/m) and soil salinity treatments (2.10 to 8.26 dS/m), NM plant had soluble protein content in the range of 6.96 to 11.047 mg/g (Figure 4).

However, *Gm* and *Gf* plants had relatively higher amount of soluble protein than NM counterpart and were found in the ranges of 7.872 to 12.229 mg/g and 7.363 to 11.564 mg/g respectively at control (1.04 dS/m) and soil salinity of 2.10 to 8.26 dS/m. Additionally, amount of soluble protein was found highest in mixed mycorrhizae inoculated plant and was recorded in the range of 8.275 to 12.505 mg/g (Figure 4).

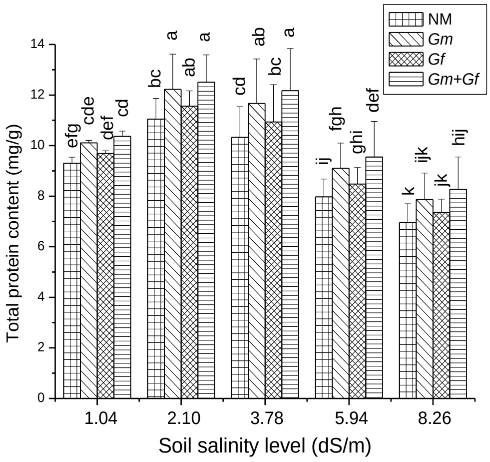

Figure 4. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on protein content of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

Carbohydrate content

Soluble carbohydrate content of *A. arabica* was significantly increased by increasing soil salinity level up to 3.78 dS/m and then the concentration was found to become less with further increment in soil salinity irrespective of mycorrhizal treatments. At control (1.04 dS/m) and soil salinity treatments (2.10 to 8.26 dS/m), NM, *Gm* and *Gf* plants had soluble carbohydrate contents in the ranges of 9.507 to 15.742, 10.136 to 18.026 and 9.846 to 17.128 mg/g respectively (Figure 5).

Furthermore as compared to *Gm* and *Gf* plants, mixed mycorrhizae (*Gm* + *Gf*) treated plants had greater carbohydrate content and was determined in the range of 10.518 to 18.943 mg/g while *A. arabica* was allowed to grow at control and soil salinity treatments (Figure 5).

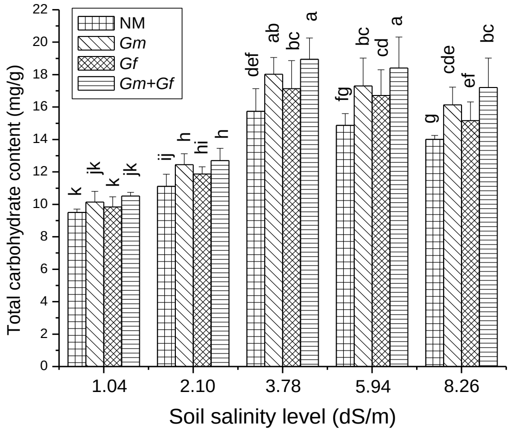

Figure 5. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on carbohydrate content of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

Reducing sugar content

In the present study, reducing sugar content was significantly enhanced with increase in soil salinity from 1.04 (control) to 3.78 dS/m and then a significant reduction of this content was noticed with further increment in salinity stress from 5.94 to 8.26 dS/m regardless of mycorrhizal treatments (Figure 6). NM plant contained reducing sugar in the range of 6.195 to 10.868 mg/g when exposed to control (1.04 dS/m) and soil salinity levels of 2.10 to 8.26 dS/m. However, higher soil salinity (5.94 to 8.26 dS/m) decreased reducing sugar content in test plant but following mycorrhizal colonizations, *Gm*, *Gf* and *Gm+Gf* plants had considerably higher amount of reducing sugar and were detected in the ranges of 6.566 to 12.029, 6.429 to 11.547 and 6.676 to 12.623 mg/g respectively at provided salt stress treatments (1.04 to 8.26 dS/m) (Figure 6).

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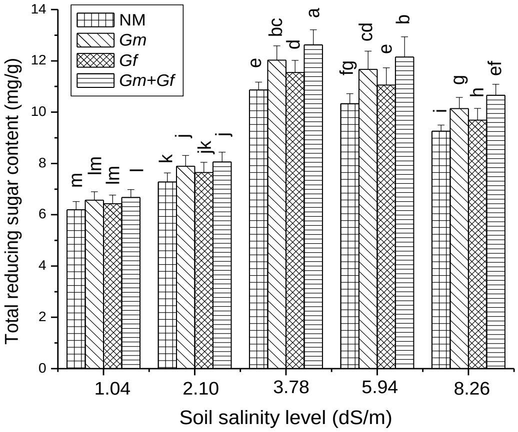

Figure 6. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on reducing sugar content of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

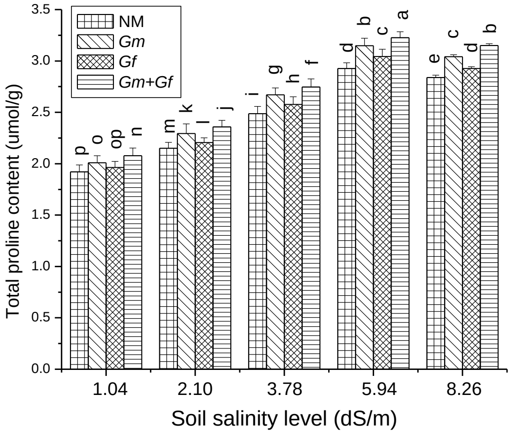

Figure 7. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on proline content of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

Proline content

A. arabica grown at control treatment (1.04 dS/m) had less proline content and the value was significantly increased with increase in soil salinity up to the fourth level (5.94 dS/m) followed by a significant reduction at the highest level of salt stress irrespective of mycorrhizal treatments (Figure 7).

Non-mycorrhizal *A. arabica* grown at control treatment (1.04 dS/m) and at soil salinity gradients of 2.10 to 8.26 dS/m had proline concentration in the range of 1.921 to 2.926 µmol/g. As compared to NM counterpart, individual and mixed mycorrhizal colonizations in test plant significantly improved free proline content while growing under provided salt stress. *Gm*, *Gf* and mixed mycorrhizae inoculated plants showed proline contents in the ranges of 2.009 to 3.149, 1.963 to 3.043 and 2.077 to 3.228 µmol/g respectively at control (1.04 dS/m) and soil salinity gradients of 2.10 to 8.26 dS/m (Figure 7).

Electrolyte leakage

At control treatment (1.04 dS/m), NM plant had leaf electrolyte leakage of 9.627 % and the value was increased to 39.46 % at the highest level of soil salinity (8.26 dS/m) (Figure 8).

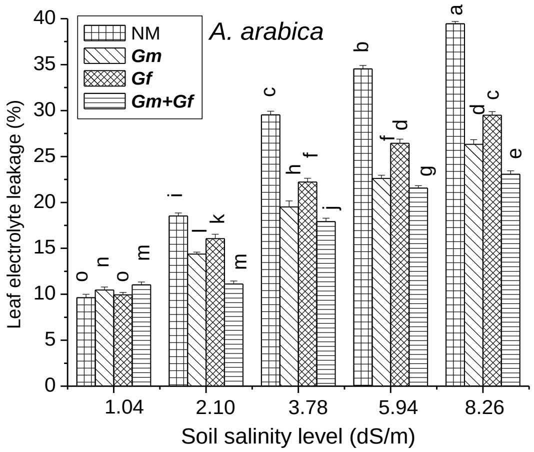

Figure 8. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on leaf electrolyte leakage of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

However, as compared to NM counterpart, *Gm* and *Gf* counterparts had significantly less percent electrolyte leakage at each level of soil salinity ranging from 2.10 to 8.26 dS/m

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and *Gm*+*Gf* treatment worked better in this regard. *Gm* and *Gf* plants had electrolyte leakage of 10.473 and 9.933% respectively and these values were enhanced up to 26.32 and 29.49% respectively at the highest soil salinity level (8.26 dS/m). However, mixed mycorrhizae inoculated plant had relatively less amount of electrolyte leakage and was in the range of 11.03 to 23.07% at control and salt stress gradients (Figure 8).

AM colonization

NM plant did not show any AM colonization in the roots however, colonization was noticed in *Gm*, *Gf* and *Gm*+*Gf* plants grown under control and salt stress treatments. Even though, at each level of soil salinity AM root colonization was noticed in mycorrhizae inoculated plants but the extent of percent colonization was decreased with increase in soil salinity up to the highest level (8.26 dS/m). Due to individual and mixed inoculations with *Gm* and *Gf* isolates in test plant, AM colonizations were of 44.17, 38.5 and 45.3% respectively at control treatment (1.04 dS/m). However at the highest level of soil salinity (8.26 dS/m), the percent root colonizations were progressively reduced to 30.67, 24.83 and 31.5% respectively (Figure 9).

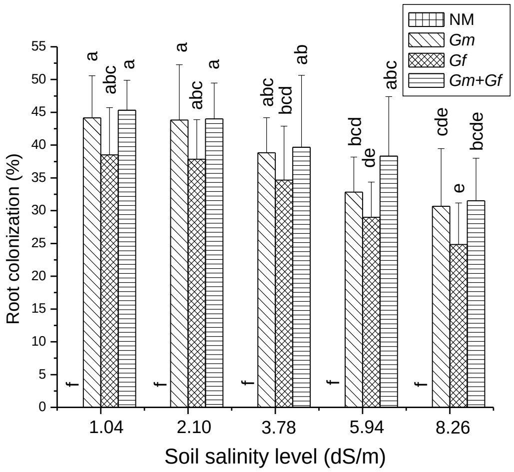

Figure 9. *Effects of various soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m] on root colonization of mycorrhizae (Gm, Gf and Gm+Gf) inoculated and non-mycorrhizal plants [Bars of each treatment followed by different letters indicate statistically significant difference (P<0.05) by Duncan's Multiple Range Test after performing ANOVA]*

Increment (%) in growth and biochemical parameters due to mycorrhizal colonizations over NM plant

Mycorrhizal inoculations with *Gm* and *Gf* isolates (individual and mixed) in test plant enhanced growth parameters and amount of various biochemical constituents over respective non-mycorrhizal counterpart grown at control and salt stress gradients and hence, the percent enhancement in dry biomass, plant height as well as biochemical accumulation due to mycorrhization over non-mycorrhizal counterpart was determined (Table 1). *A. arabica* inoculated with *Gm* and *Gf* isolates individually and in mixed, showed maximum accumulation of protein, carbohydrate and reducing sugar content at soil salinity of 5.94 dS/m over corresponding NM plant. However, individual and mixed mycorrhizal inoculations enhanced dry biomass, proline, and chlorophyll concentrations maximally at soil salinity either of 3.78, 5.94 or 8.26 dS/m (Table 1).

Probabilities of significance

Salinity and mycorrhizal treatment when considered individually produced significant effects on growth and various biochemical parameters of *A. arabica*. However, their interaction was found significant only on plant height, reducing sugar, proline content and on leaf electrolyte leakage (Table 2).

Discussion

The result of present study indicates that, *A. arabica* height was reduced prominently as soil salinity increased gradually. Plant height reduction during the event of salt stress is mainly related to the fact that, excess salt content in soil produces osmotic and ionic imbalances and also concentration of soluble salt is reported to have direct relation to plant growth suppression (Benlloch-González et al., 2005; Pascal et al., 2005). However, AM inoculations with *Gm* and *Gf* isolates reduced negative impact of salt stress on plant height and AM colonized plants were found to be taller as compared to non-mycorrhizal plants while exposed to various levels of soil salinity. Similar type of result was obtained when *Glomus intraradices* colonized *Trifolium alexandrinum* was irrigated with different sources of salt solutions (NaCl, Na_2SO_4 and $MgSO_4$) (Gharineh et al., 2009).

Salinity induced stress progressively reduced plant dry biomass and this result is in good agreement with previously published work by Al-Karaki et al. (2001) and Essa (2002**)**. The reason behind dry biomass reduction is partly related to

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less nutrient availability to plant due to NaCl toxicity in soil (Evelin et al., 2009). But, mycorrhizal inoculations reduced adverse salt effect on dry biomass content and prominently increased the amount in test plant than that of non-mycorrhizal plant at various soil salinity gradients. Dry biomass increment in mycorrhizal pepper, tomato, soybean plants grown in saline condition has already been reported by several researchers (Al-Karaki et al., 2001; Sharifi et al., 2007; Kaya et al., 2009).

In the present study, soil salinity drastically reduced total chlorophyll content in *A. arabica* regardless of mycorrhizal treatments and may be because, NaCl is known to have some negative effects on absorption of mineral elements (mainly magnesium), it suppresses chlorophyllase enzyme activity, inhibits chlorophyll synthesis (Feigin et al., 1991; Evelin et al., 2009). But, *Gm* and *Gf* colonizations in plant roots significantly enhanced chlorophyll concentration and reduced the adverse salt effect on chlorophyll biosynthesis and this result is in agreement with other reports where, various *Glomus* species significantly improved chlorophyll contents in *Zea mays*, *Capsicum annum* and *Solanum lycoperiscum* under various soil salinity stresses (Feng et al., 2002; Kaya et al., 2009; Hajiboland et al., 2010).

Table 1. *Percentage wise improvement of growth and biochemical parameters in mycorrhizal A. arabica over non-mycorrhizal counterpart under various soil salinity stress levels*

Parameter	**Soil salinity (dS/m)**														
	1.04			**2.10**			**3.78**			**5.94**			**8.26**		
	Gm	***Gf***	***Gm* + *Gf***	***Gm***	***Gf***	***Gm* + *Gf***	***Gm***	***Gf***	***Gm* + *Gf***	***Gm***	***Gf***	***Gm* + *Gf***	***Gm***	***Gf***	***Gm* + *Gf***
Height	6.05	3.15	18.31	11.22	**7.30**	21.06	**15.23**	6.94	**23.96**	12.59	6.44	21.54	10.29	5.02	20.45
Biomass	9.58	3.98	13.93	12.52	7.85	16.03	13.98	9.24	18.01	15.24	9.52	18.73	**15.93**	**12.39**	**20.35**
Chlorophyll	10.32	6.43	16.09	11.54	7.07	17.46	15.04	**8.33**	19.21	**15.37**	8.30	**20.94**	13.17	6.07	15.60
Protein	8.62	4.11	11.43	10.70	4.68	13.20	12.95	5.85	17.86	**14.18**	**6.31**	**19.72**	13.10	5.79	18.89
Carbohydrate	6.62	3.57	10.63	11.94	6.73	14.25	14.51	8.80	20.33	**16.35**	**12.39**	**23.83**	15.11	8.18	22.81
Reducing sugar	5.99	3.78	7.76	8.44	5.08	10.72	10.68	6.25	16.15	**12.94**	**7.04**	**17.60**	9.51	4.63	15.11
Proline	4.58	2.19	8.12	6.70	2.65	9.72	7.40	3.58	10.45	**7.62**	**4.00**	10.32	7.19	3.14	**10.99**

Table 2. *Probabilities of significance for ANOVA for the effect of individual variables (salinity and AM status) and their interaction on A. arabica growth and biochemical parameters [***P<0.001, **P<0.01, *P<0.05, NS non-significant]*

Parameter	**Soil salinity**	**AM status**	**Salinity X AM**
Height	***	***	***
Biomass	***	*	NS
Chlorophyll	***	***	NS
Protein	***	***	NS
Carbohydrate	***	***	NS
Reducing sugar	***	***	***
Proline	***	***	**
EL	***	***	***
Root colonization	***	***	NS

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Data from present study indicate that, increasing soil salinity (up to soil EC of 2.10 dS/m) protein content in all mycorrhizal and non-mycorrhizal plants was increased and may be because during salt stress salt-induced proteins are normally synthesized *de novo* for maintaining osmotic balance and subsequently provided salt tolerance to plants (Pareek et al., 1997). However, higher salt level in soil reduced protein content when salt interferes with nitrogen uptake and impaired protein synthesis (Aslam et al., 1984; Frechill et al., 2001). Comparatively higher soluble protein content was observed in mycorrhizae inoculated *A. arabica* over non-mycorrhizal plant as extra-radical mycelia of AM fungi helped in utilization of inorganic nitrogen (Govindarajulu et al., 2005). *Glomus intraradices* colonized tomato had more protein content than un-colonized counterpart under saline conditions (Hajiboland et al., 2010).

Increasing soil salinity up to 3.78 dS/m enhanced soluble carbohydrate concentration in *A. arabica* regardless of mycorrhizal treatments and is mainly because, carbohydrate plays a crucial role in maintaining osmotic balance in plant exposes to salt stress and hence protects plant from adverse salt effect (Murakeozy et al., 2003). Comparing with non-mycorrhizal plant, higher carbohydrate content was noticed in all the mycorrhizae inoculated plants and this result is supported by previous work where, *Glomus fasciculatum*, *Glomus intraradices* colonizations in *Phragmites australis* and *Glycine max* improved soluble carbohydrate contents under stress conditions (Porcel & Ruiz-Lozano, 2004; Al-Garni, 2006). Higher carbohydrate content in mycorrhizal plant than that of non-mycorrhizal counterpart might be related to the fact that, during mycorrhizal development in host root system they increase photosynthetic rate as they demand carbon compound from host. Also, mycorrhization helps in frequent conversion of starch to sugar and increases its accumulation in host (Nemec, 1981; Finlay & Söderström, 1992).

Under salt stress *Gm* and *Gf* colonizations in *A. arabica* roots increased reducing sugar accumulation which is required for better osmoprotection and this kind of data is supported by Kerepesi & Galiba (2000), Khatkar & Kuhad (2000) and Singh et al. (2000).

In this study, increasing soil salinity (up to 5.94 dS/m) enhanced proline concentration in *A. arabica* irrespective of mycorrhizal treatments and mainly proline accumulates in plant to adapt adverse salt effect (Sharma et al., 1990; Jindal et al., 1993). Moreover, comparatively higher proline content was observed in mycorrhizal *A. arabica* over non-mycorrhizal plant and this finding is consistent with the previous observations (Jindal et al., 1993; Sharifi et al., 2007).

A significant enhancement in leaf electrolyte leakage concentration was observed with increasing soil salinity and it implies loss in membrane stability and its integrity due to salt effect (Lutts et al., 1996; Kaya et al., 2009). Whereas, individual and mixed mycorrhizal colonizations (with *Gm* and *Gf* isolates) in plant roots lowered electrolyte leakage concentration in *A. arabica* at soil salinity gradients of 2.10 to 8.26 dS/m and it signified higher amount of electrolytes in AM plants. Hence it can be suggested that, mycorrhizal associations in *A. arabica* helped to improve membrane structure and its stability under salt stress condition. Similar type of finding was observed when mycorrhizal tomato, maize and pepper plants were allowed to grow under saline condition and had less membrane permeability over non-mycorrhizal plants (Feng et al., 2002; Zhong Qun et al., 2007; Kaya et al., 2009).

Increasing soil salinity reduced the amount of AM colonizations in roots of *A. arabica* and it may be because salt stress in soil solution reduces spore germination, arbuscule formation, inhibits hyphae enlargement and their spreading following infection in host roots (Hirrel, 1981; Pfeiffer & Bloss, 1988; McMillen et al., 1998). This observation is in consistent with other study in which AM root colonization was decreased at high soil salinity level (Al-Karaki, 2000).

Conclusion

Various growth parameters of test legume were improved under salt stress when *A. arabica* was symbiotically associated with *Gm*, *Gf* and *Gm+Gf*. This was also true for total chlorophyll content. In other word, the negative impact of soil salinity on chlorophyll biosynthesis was reduced by mycorrhizal action. Moreover, mycorrhization reduced electrolyte leakage in test plant and it may be presumed that, *Gm* and *Gf* colonizations (either individual or mixed) in plant roots enable host to maintain higher electrolyte concentration than non-mycorrhizal plant and subsequently to maintain membrane integrity. Enhanced growth, higher chlorophyll concentration in mycorrhizal plants grown under saline soil conditions has been partly related to greater accumulation of compatible solutes (protein, carbohydrate, reducing sugar and proline) which act as osmolytes and protect plant from adverse salt effects by providing salt tolerance to plants.

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In short, better plant height, higher dry biomass, total chlorophyll, protein, proline, carbohydrate, reducing sugar concentrations and lower electrolyte leakage concentration in mycorrhizal *A. arabica* at soil salinity levels like 2.10 to 8.26 dS/m over non-mycorrhizal plant implied that, *Gm* and *Gf* isolates in individual and in mixed inoculations could alleviate adverse salt effect on plant growth and biochemical profiling and moreover mixed AM inoculation was found to be more efficient in this regard.

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