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Arbuscular Mycorrhizal Colonization Enhances Biochemical Status in and Mitigates Adverse Salt Effect on Two Legumes

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Abstract

Symbiotic association between arbuscular mycorrhizal (AM) species and host plant roots improves plant growth and protects them from several abiotic stress factors. In the present study, the effect of *Glomus mosseae* and *Glomus fasciculatum* as an individual inoculation and in combination was studied on two legumes (*Glycine max* and *Cyamopsis tetragonoloba*) under soil salinity stress gradient [1.04 (control) to 8.26 dS/m]. Individual and co-inoculation of both the AM fungi alleviated adverse salt effect, with improvement in plant dry weight matter and biochemical parameters. However, these two isolates worked better in combination with respect to higher accumulation of soluble carbohydrate, reducing sugar, protein, proline concentration etc. *C. tetragonoloba* showed better response as compared to *G. max* in relation to improvement in nutritional profile under salt stress after AM treatment. As compared to non-mycorrhizal counterparts, co-inoculation with *G. mosseae* and *G. fasciculatum* in *C. tetragonoloba* enhanced total chlorophyll (14.83% at soil salinity of 3.78 dS/m), soluble carbohydrate (17.26% at soil salinity of 5.94 dS/m), proline (8.79% at soil salinity of 3.78 dS/m) while exposed to different soil salinity levels. Also, co-colonization with both the isolates showed more root colonization (%) and may be responsible for the better effect in salt stress alleviation. Electrolyte leakage of mycorrhizal plants was lowered at soil salinity gradient of 2.10 to 8.26 dS/m and hence, maintained membrane stability. These two isolates can be utilized as bio-inoculant in alleviation of adverse salt effect in soil in association with the two test legume plants.

Keywords: *Cyamopsis tetragonoloba*, electrolyte leakage, *Glomus fasciculatum, Glomus mosseae, Glycine max*, proline, soil salinity stress

Introduction

Salinization of soil is becoming a severe agricultural constraint in arid and in semi arid regions of India. Presence of excess salt concentrations in soil solution arises mainly due to the heavy use of chemical fertilizers and use of low quality water (during irrigation of agricultural land) which contains soluble salts in large amount (Al-Karaki, 2000; Chartzoulakis and Klapaki, 2000). When salt concentration in soil reaches beyond the normal range, it makes the land unproductive as it results in nutrient imbalance in plants, hampers water availability followed by stunted growth and leads to reduced crop productivity (Al-Karaki *et al.*, 2001; Hopkins and Huner, 1999). Development of salt tolerant crop varieties and physico-chemical methods for removal of excess salts from agricultural soils have been tried (Cuartero and Fernandez-Munoz, 1998; Hamdy, 1990; Hamdy, 1990; Muralev *et al.*, 1997). These approaches have been successful but are costly and hence, a new alternative attempt has taken up to tackle the deleterious effects of saline soils which involve inoculation of salt tolerant arbuscular mycorrhizal (AM) fungi in agricultural crop.

AM fungi are considered as obligate symbionts and are naturally associated with roots of around 90% vascular plants (Allen, 1991). AM fungi improve plant health by providing essential mineral nutrients from soil. They maintain physiological processes of the host plant, as well as protect it from several biotic and abiotic stresses (Augé, 2000; Ruiz-Lozano, 2003; Sylvia and Williams, 1992). In salt stressed soil, phosphate ions usually precipitate along with Ca^{2+}, Mg^{2+}, Zn^{2+} and are less available to plants (Azcon-Aguiler *et al.*, 1979). But, AM symbiosis in plants enhances the uptake of less mobile phosphorus by extending their external hyphal network beyond nutrient depletion zone. In mitigation of salt stress, AM fungi also improve plant growth, hormonal status, increase nutrient acquisition, maintain osmotic balance, reduce ion toxicity etc. (Juniper and Abbott, 1993; Lindermann, 1994; Ruiz-Lozano, 2003).

With increasing world population, the demand for food, mainly cereal based protein rich diet is increasing, especially in developing countries. Due to scarcity of productive agricultural land and ever increasing population growth, the developing countries are facing the problem of food crisis and hence, protein-rich leguminous food may be one of the substitutes (Sadik, 1991; Weaver, 1994). In such scenario, cultivation of legume plants is promising considering protein content and presence of carbohydrate pool. It is reported that

under abiotic stress condition, improvement of biochemical and antioxidant profile of the host legume plant was observed following colonization with AM fungi (Porcel and Ruiz-Lozano, 2004). Several reports suggest that mycorrhizal inoculation with single AM isolate reduces the detrimental effects of salinity, but salt tolerance level of the plant as well as mycorrhizal efficiency to respond the host varies among different plant species and mycorrhizal fungi (Burke *et al*., 2003; Tian *et al*., 2004). Hence, in alleviation of salt stress for particular plant species, selection of specific AM fungi as an individual or in combination of two or more is essential. The interaction between legume plant and combined inoculation of two different AM fungi is not well studied before and hence, the present study was aimed at investigating the individual and combined effects of *Glomus mosseae* and *Glomus fasciculatum* on two different crop legumes under soil salinity stress. A comparison between two crop legumes *Glycine max* and *Cyamopsis tetragonoloba* was undertaken in relation to improvement in plant growth and biochemical status. *G. max* is considered as inexpensive source of proteins and is also valued for its high oil content. *C. tetragonoloba* is an annual legume and is an important source of guar gum. Guar gum is directly used in dairy industry and in textile industry after its derivatization. Salt tolerant AM fungi were used in this study as bio-inoculants for cultivation of legumes in salt stressed soil, as both the legumes have nutritional significance.

Materials and methods

Chemicals

All the chemicals used in this study were of analytical reagent grade.

Plant Material

Seeds of *G. max* and *C. tetragonoloba* used in this study were obtained from Naik Seeds Pvt. Ltd., Pune, Maharashtra (India). Seeds were surface sterilized using sodium hypochlorite solution (0.5% w/v): reverse-osmosis water (1:3), followed by rinsing with reverse-osmosis water. Surface sterilized seeds of *G. max* and *C. tetragonoloba* were directly placed on moist filter paper for germination. At first true leaf stage, seedlings of almost equal length were used for transplantation in pot.

Mycorrhizal treatment and experimental design

G. mosseae (Nicol and Gerd) and *G. fasciculatum* (Thaxt.) Gerd and Trappe used in this study were isolated in the laboratory of University of Pune, earlier from Lonar Crater Lake, Buldhana and Sakri, Dhule, Maharashtra respectively, by wet sieving and decanting method (Datta, 2012; Gerdemann and Nicolson, 1963; Schenck and Perez, 1990). Spores were propagated in *Zea mays* L. for three months. *G. mosseae* and *G. fasciculatum* soil based inocula contained 150 to 160spores/10 g air-dried soil with AM colonized roots (~75% colonization) and 100 to 110spores/10 g air-dried soil and roots of ~53% AM colonization respectively. These soil based inocula were used for further study.

Soil used for greenhouse study, contained sand (35%), slit (57%) and clay (8%) with properties of pH: 6.0, organic matter: 1.3%, available P: 0.75 mg/ 100 g, available N: 0.12 mg/ 100 g and available K: 0.45 mg/ 100 g and was mixed with river sand (particle size of <0.3 mm) (1:1 v/v). This sand-soil mixture was autoclaved (121 °C, 103.4 kPa, for 1 h) and used for further study.

The experiment was designed in a complete randomized design, consisted of four mycorrhizal treatments (NM: non-mycorrhizal, *Gm*: *G. mosseae*, *Gf*: *G. fasciculatum* and *Gm+Gf*: *G. mosseae* + *G. fasciculatum*), five soil salinity levels [1.04 (control), 2.10, 3.78, 5.94 and 8.26 dS/m], two test plants (*G. max* and *C. tetragonoloba*), with five replicates (three plants per replicate). In *Gm* and *Gf* treatment, 50 g of *G. mosseae* and 75 g *G. fasciculatum* soil based inocula were used. However, in *Gm+Gf* treatment, 25 g of *G. mosseae* and 40 g of *G. fasciculatum* were used. Variable amount of soil based inocula were applied to achieve almost similar number of spores per pot (~800spores/pot). Mycorrhizal treatments were provided by placing respective soil based inoculum 3 cm below the seedling prior to seedling transplantation. Plants without mycorrhizal inoculum served as non-mycorrhizal control. Sand-soil volume (3 kg) was kept constant in all mycorrhizae treated and untreated pots. On every alternate day, plants were irrigated with tap water (sieved through 105 μ sieve) and with Hoagland solution (X/10) twice a month (Hoagland and Arnon, 1940). Irrigation was done in such a way to make soil water content of around 40% field capacity. After one month of seedling transplantation, saline stresses were provided and the stress was increased gradually to prevent shock. Saline solution was continuously applied until the target salinity level reached. Plants were grown for four months in greenhouse (at 25-35 °C, relative humidity: 65-70%, photon flux intensity: 300-350 μmol/m^2/S).

Plant dry weight

After two months of seedling transplantation, plants from each treatment were harvested and washed in distilled deionized water. Fresh plants were kept in oven at 60 °C for 24h for drying and then dry weights were recorded.

Total chlorophyll content

The youngest fully expanded fresh leaves from each treatment were collected for estimation of total chlorophyll content following the method of Strain and Svec (1966). Fresh leaf samples were first extracted in 80% (v/v) acetone and then subjected to centrifugation. Concentrations of chlorophyll *a* and chlorophyll *b* of the supernatant were estimated by using spectrophotometer. Total chlorophyll content was determined and the value was expressed in terms of mg/g leaf samples.

Soluble protein content

Fresh plant material (both shoot and root tissues) from each treatment was used for determination of soluble protein content. Sample was extracted in phosphate-buffered saline and then soluble protein content was detected by dye binding assay using

Coomassie Brilliant Blue G 250 (Bradford, 1976). BSA was used as standard for the preparation of calibration curve.

Soluble carbohydrate content

Fresh plant material (both shoot and root tissues) from each treatment was subjected to acid hydrolysis and then total soluble carbohydrate content was estimated using anthrone method (Hedge and Hofreiter, 1962). Glucose was used as a standard for preparing the calibration curve.

Reducing sugar content

Fresh plant material (both shoot and root tissues) from individual treatment was extracted in hot ethanol for two successive times and total reducing sugar content was determined using 3,5-dinitrosalicylic acid method (Miller, 1972). Standard glucose solution was used for preparation of calibration curve.

Free proline content

Free proline content was determined by using Ninhydrin acid reagent (Bates *et al*., 1973). Fresh plant material (both shoot and root tissues) from each treatment was extracted in sulfo-salicylic acid (3% w/v) and filtered through Whatman No. 2 filter paper. Filtrate was then treated with glacial acetic acid and acid ninhydrin reagent and was kept for boiling (1 h). After boiling, toluene was added to the reaction mixture and vortexed. Toluene layer was separated and absorbance of sample was measured at 520 nm using UV/Vis spectrophotometer (Shimadzu UV 1601). Standard proline was used for preparation of calibration curve.

Electrolyte leakage (EL)

Fresh leaf tissue from each treatment was used for determination of electrolyte leakage. Leaf samples were cut into small pieces of almost equal length and used for EL measurement. 0.5 g sample was taken in a test tube containing distilled deionized water and then placed in water bath (at 32 °C) for 2 h. After incubation, tubes were cooled to 25 °C and initial electrical conductivity (EC) was measured using conductivity meter (Hanna, HI 8733). Then, the samples were autoclaved for 20 min and final EC was measured using conductivity meter (Hanna, HI 8733) after cooling the tubes at 25 °C (Dionisio-Sese and Tobita, 1998). EL of fresh leaf samples was calculated using the following formula:

EL = (Initial EC/ Final EC) × 100

where EL = Electrolyte leakage; EC = Electrical conductivity.

AM root colonization (%)

Root samples were washed with deionized water, followed by cutting it into 1 cm pieces. The root pieces were thoroughly mixed and a sub-sample (0.5 g) was cleared in hot KOH solution (10% w/v, at 90 °C) for 1 h. Cooled root samples were washed with deionized water and placed in HCl (10% v/v) for 3 min and stained with trypan blue (0.05% w/v) for 15 min at 90 °C (Phillips and Hayman, 1970). Percentage of AM colonization in
384 mples was estimated by gridline intersects method (Giovannetti and Mosse, 1980).

Statistical analysis

Data were analyzed by ANOVA followed by Duncan's test and values of each treatment followed by different letters indicate statistically significant difference at $P<0.05$ level. SPSS v. 9.0 was used for the statistical data analysis.

Results

In the present study, soil salinity hampered growth and biochemical status of both the test plants regardless of mycorrhizal treatment. However, it was observed that, mycorrhizal colonization in *G. max* and *C. tetragonoloba*, reduced the extent of deleterious salt effect and improved plant growth even under provided soil salinity. Although, both the mycorrhizal isolates showed positive response in enhancement of host plant growth, but combined inoculation of both the isolates was found superior in this respect, followed by individual inoculation with *Gm* and *Gf*.

Plant dry weight

G. max and *C. tetragonoloba* plants grown under control treatment (1.04 dS/m) had relatively more dry weight content than the corresponding plants from saline stress treatment (2.10 to 8.26 dS/m). The values were significantly declined as soil salinity was increased from 2.10 to 8.26 dS/m regardless of mycorrhizal inoculations (Tab. 1).

With increasing soil salinity gradient dry weights of test plants were reduced, but under control (1.04 dS/m) and at each level of soil salinity (2.10 to 8.26 dS/m) mycorrhizae inoculated plants had significantly higher dry weight than respective non-mycorrhizal plants. Moreover, inoculation with mixed mycorrhizal fungi was found to be superior over the individual inoculations. Individual effect of soil salinity and mycorrhizal fungi on dry weight was found significant for both the plants. However, their interaction was mainly significant with respect to dry weight content of *G. max* (Tab. 1).

Total chlorophyll content

Regardless of mycorrhizal inoculations, the test plants grown under control treatment (1.04 dS/m) had significantly higher chlorophyll content and the concentration was reduced with increase in salinity stress (Tab. 2).

However, at each level of soil salinity (2.10 to 8.26 dS/m) and in control treatment (1.04 dS/m), mycorrhizal colonization (individual and combined) in test plants resulted in significantly higher chlorophyll content as compared to respective non-mycorrhizal control plants (Tab. 2).

It was also observed that, regardless of salinity treatments, among the three types of mycorrhizal inoculations, combined mycorrhizae inoculated plants (*G. max* and *C. tetragonoloba*) showed higher chlorophyll content, than the corresponding plants inoculated with individual isolates (Tab. 2).

Soil salinity and mycorrhizal inoculations had significant effect on total chlorophyll content in *G. max* and *C. tetragonoloba* plants, and their interaction was

Tab. 1. Dry weight (g/plant) of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	
		G. max	*C. tetragonoloba*
1.04	NM	1.757±0.03^{d}	1.571±0.03^{c}
	Gm	1.836±0.02^{b}	1.633±0.03^{b}
	Gf	1.781±0.01^{c}	1.598±0.03^{c}
	Gm + Gf	1.871±0.02^{a}	1.663±0.02^{a}
2.10	NM	1.588±0.03^{hi}	1.45±0.03^{f}
	Gm	1.673±0.02^{f}	1.54±0.03^{d}
	Gf	1.627±0.02^{g}	1.492±0.03^{e}
	Gm + Gf	1.722±0.02^{e}	1.573±0.03^{c}
3.78	NM	1.439±0.02^{k}	1.238±0.03^{i}
	Gm	1.57±0.02^{i}	1.346±0.02^{g}
	Gf	1.517±0.02^{j}	1.301±0.03^{h}
	Gm + Gf	1.6±0.02^{h}	1.369±0.03^{g}
5.94	NM	1.187±0.03^{o}	1.107±0.04^{l}
	Gm	1.301±0.02^{m}	1.202±0.03^{j}
	Gf	1.251±0.02^{n}	1.168±0.04^{k}
	Gm + Gf	1.34±0.02^{l}	1.229±0.04^{ij}
8.26	NM	0.978±0.02^{s}	0.931±0.05^{o}
	Gm	1.049±0.01^{q}	0.997±0.03^{mn}
	Gf	1.02±0.02^{r}	0.971±0.03^{n}
	Gm + Gf	1.092±0.02^{p}	1.022±0.04^{m}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity × Mycorrhizae		0.000	0.453

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

Tab. 2. Total chlorophyll content (mg/g) of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	A inoculation	Chlorophyll content (mg/g)	
		G. max	*C. tetragonoloba*
1.04	NM	1.668±0.01^{c}	1.401±0.02^{e}
	Gm	1.779±0.01^{b}	1.507±0.01^{b}
	Gf	1.737±0.01^{b}	1.485±0.01^{c}
	Gm + Gf	1.824±0.01^{a}	1.574±0.03^{a}
2.10	NM	1.246±0.01^{f}	1.268±0.01^{g}
	Gm	1.333±0.01^{de}	1.369±0.01^{f}
	Gf	1.309±0.01^{e}	1.369±0.02^{f}
	Gm + Gf	1.373±0.01^{d}	1.436±0.02^{d}
3.78	NM	1.03±0.01^{i}	1.095±0.02^{i}
	Gm	1.104±0.01^{gh}	1.216±0.01^{h}
	Gf	1.084±0.01^{h}	1.203±0.01^{h}
	Gm + Gf	1.153±0.01^{g}	1.257±0.01^{g}
5.94	NM	0.827±0.01^{l}	0.859±0.01^{m}
	Gm	0.894±0.01^{jk}	0.954±0.01^{k}
	Gf	0.869±0.01^{k}	0.935±0.02^{l}
	Gm + Gf	0.926±0.01^{ij}	0.98±0.02^{j}
8.26	NM	0.537±0.01^{o}	0.715±0.01^{q}
	Gm	0.573±0.01^{mn}	0.794±0.01^{o}
	Gf	0.548±0.01^{n}	0.774±0.02^{p}
	Gm + Gf	0.598±0.01^{m}	0.811±0.01^{n}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity × Mycorrhizae		0.000	0.000

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

also significant on total chlorophyll content of both the plants (Tab. 2).

Soluble protein content

Regardless of mycorrhizal inoculations, as compared to control (1.04 dS/m), salt stress (at salinity level of 2.10 dS/m) resulted in significant enhancement of soluble protein content of both the test plants, but with further increment in salinity stress level from 3.78 to 8.26 dS/m, a significant reduction was noticed (Tab. 3).

Although, higher levels of soil salinity had negative impact on plant protein content, test plants inoculated with *Gm* and *Gf* isolates (individual and in combination) showed a significantly higher protein content as compared to non-mycorrhizal plants grown under control (1.04 dS/m) and at each level of soil salinity (2.10 to 8.26 dS/m). Individual and combined effect of salinity and mycorrhizal inoculations were found significant on soluble protein contents of the test plants (Tab. 3).

Soluble carbohydrate content

In *C. tetragonoloba* plant, a steady, significant increment in soluble carbohydrate content was noticed, when the soil salinity was raised from control (1.04 dS/m) to 3.78 dS/m and with further increase in saline stress; the magnitude of carbohydrate content got reduced significantly irrespective of mycorrhizal inoculations. However, in *G. max* plant, the concentration of soluble carbohydrate significantly increased up to salinity stress level of 5.94 dS/m, followed by a decreasing trend regardless of mycorrhizal inoculations (Tab. 4).

With increasing soil salinity either up to third or fourth level (3.78 or 5.94 dS/m), soluble carbohydrate content increased steadily in mycorrhizae treated and untreated test plants; although comparatively higher concentration was found in plants following mycorrhizal inoculations (*Gm* and *Gf*). Combined inoculation of both the mycorrhizal isolates worked better in this regard. The individual and interactive effect of soil salinity and mycorrhizal inoculations were found significant on carbohydrate content of test plants (Tab. 4).

Reducing sugar content

During this study it was found that, regardless of mycorrhizal inoculations, reducing sugar content of *G. max* and *C. tetragonoloba* plants increased significantly

Tab. 3. Total soluble protein content (mg/g) of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	Soil salinity (dS/m)
		G. max	*C. tetragonoloba*
1.04	NM	10.892±0.36^{m}	9.32±0.32^{g}
	Gm	11.398±0.46^{j}	9.742±0.35^{e}
	Gf	11.228±0.51^{k}	9.591±0.26^{ef}
	Gm + *Gf*	11.831±0.35^{i}	10.086±0.22^{d}
2.10	NM	14.146±0.44^{f}	11.919±0.94^{c}
	Gm	14.926±0.44^{c}	12.527±0.21^{b}
	Gf	14.654±0.42^{d}	12.313±0.25^{b}
	Gm + *Gf*	15.727±0.48^{a}	13.254±0.25^{a}
3.78	NM	13.334±0.57^{h}	9.055±0.29^{h}
	Gm	14.554±0.49^{e}	9.792±0.25^{e}
	Gf	13.969±0.6^{g}	9.432±0.26^{fg}
	Gm + *Gf*	15.174±0.35^{b}	10.308±0.2^{d}
5.94	NM	9.382±0.87^{p}	4.974±0.34^{k}
	Gm	10.338±0.59^{n}	5.479±0.27^{j}
	Gf	9.877±0.55^{o}	5.189±0.24^{k}
	Gm + *Gf*	10.96±0.43^{l}	5.723±0.39^{i}
8.26	NM	6.327±0.68^{t}	3.972±0.43^{m}
	Gm	6.939±0.84^{r}	4.359±0.3^{l}
	Gf	6.583±0.56^{s}	4.084±0.28^{m}
	Gm + *Gf*	7.302±0.5^{q}	4.537±0.35^{l}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity x Mycorrhizae		0.000	0.002

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

Tab. 4. Total soluble carbohydrate content (mg/g) of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	Soil salinity (dS/m)
		G. max	*C. tetragonoloba*
1.04	NM	13.733±0.28^{r}	15.876±0.5^{s}
	Gm	14.586±0.32^{r}	16.804±0.35^{q}
	Gf	14.105±0.4^{s}	16.293±0.48^{r}
	Gm + *Gf*	15.05±0.39^{q}	17.297±0.56^{p}
2.10	NM	15.64±0.33^{p}	17.878±0.51^{o}
	Gm	16.865±0.37^{m}	19.406±0.25^{l}
	Gf	16.285±0.26^{o}	18.662±0.39^{m}
	Gm + *Gf*	17.435±0.28^{l}	19.766±0.5^{k}
3.78	NM	16.693±0.32^{n}	21.516±0.88^{h}
	Gm	18.612±0.42^{i}	24.105±0.47^{c}
	Gf	17.564±0.23^{k}	22.977±0.44^{e}
	Gm + *Gf*	18.981±0.43^{h}	24.552±0.54^{a}
5.94	NM	19.445±0.3^{f}	20.653±0.66^{j}
	Gm	21.914±0.38^{b}	23.601±0.52^{d}
	Gf	20.531±0.51^{d}	21.979±0.55^{f}
	Gm + *Gf*	23.107±0.28^{a}	24.217±0.37^{b}
8.26	NM	18.201±0.41^{j}	18.429±0.34^{n}
	Gm	20.431±0.33^{e}	20.954±0.46^{i}
	Gf	19.016±0.32^{g}	19.384±0.36^{l}
	Gm + *Gf*	21.536±0.44^{c}	21.582±0.4^{g}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity x Mycorrhizae		0.000	0.000

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

with increasing soil salinity stress from control (1.04 dS/m) to 3.78 dS/m. However, with further increment in saline stress, a significant reduction was noted (Tab. 5).

Even though, due to saline stress gradient (up to 3.78 dS/m), reducing sugar content was increased in mycorrhizae inoculated and non-mycorrhizal test plants, but *Gm* and *Gf* colonized (with individual and combined) test plants had significantly higher amount of reducing sugar over corresponding non-mycorrhizal plants under control (1.04 dS/m) and provided salinity stress gradient (2.10 to 8.26 dS/m) (Tab. 5).

Moreover, test plants colonized with both *Gm* and *Gf* isolates had reducing sugar contents to a little higher amount as compared to corresponding plants with individual *Gm* and *Gf* isolates. Individual effect of soil salinity and mycorrhizal colonization and their interaction were found significant on reducing sugar contents of test plants (Tab. 5).

Free proline content

Proline concentration in test plants was significantly improved under increasing soil salinity up to 5.94 dS/m, regardless of mycorrhizal inoculations. Then it was reduced (P<0.05) in mycorrhizal and non-mycorrhizal plants at the highest level of soil salinity (8.26 dS/m) (Tab. 6).

It is worth noting that, even though, increasing soil salinity gradient (up to 5.94 dS/m) resulted in enhancement of proline concentration, but as compared to non-mycorrhizal plants, mycorrhizal plants had significantly higher proline content at control (1.04 dS/m) and soil salinity gradient (2.10 to 8.26 dS/m) in case of both the plants. Plants with combined mycorrhizal treatment showed more proline content over the corresponding plants colonized with individual *Gm* and *Gf* isolates, when exposed to control (1.04 dS/m) and soil salinity stress gradient (2.10 to 8.26 dS/m) (Tab. 6).

Soil salinity and mycorrhizal inoculations when considered individually and in combination showed a significant effect on proline content of *G. max* and *C. tetragonoloba* plants (Tab. 6).

Electrolyte Leakage

Percent electrolyte leakage of mycorrhizae inoculated and non-mycorrhizal test plants grown under control (1.04 dS/m) and various levels of soil salinity is presented in Tab. 7.

Test plants subjected to control treatment (1.04 dS/m) had relatively less electrolyte leakage concentration (%) and the values increased rapidly with increase in soil salinity stress (2.10 to 8.26 dS/m), irrespective of mycorrhizal inoculations. Although salt stress increased electrolyte leakage in test plants, but mycorrhizal inoculation significantly lowered percent electrolyte leakage in all the plants grown at each level of soil salinity (2.10 to 8.26 dS/m) (Tab. 7).

Tab. 5. Total reducing sugar content (mg/g) of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	Soil salinity (dS/m)
		G. max	*C. tetragonoloba*
1.04	NM	7.624±0.3^{r}	13.163±0.31^{l}
	Gm	7.854±0.4^{o}	13.51±0.36^{j}
	Gf	7.727±0.21^{q}	13.259±0.26^{k}
	Gm + Gf	8.119±0.27^{n}	14±0.38^{i}
2.10	NM	8.59±0.35^{l}	14.37±0.36^{h}
	Gm	9.078±0.29^{j}	14.923±0.39^{f}
	Gf	8.774±0.27^{k}	14.623±0.38^{g}
	Gm + Gf	9.258±0.33^{i}	15.436±0.44^{e}
3.78	NM	11.155±0.25^{f}	15.746±0.36^{d}
	Gm	11.916±0.29^{b}	16.697±0.37^{b}
	Gf	11.539±0.3^{d}	16.106±0.5^{c}
	Gm + Gf	12.469±0.3^{a}	17.351±0.28^{a}
5.94	NM	10.393±0.21^{h}	11.474±0.48^{p}
	Gm	11.214±0.26^{e}	12.277±0.33^{n}
	Gf	10.85±0.26^{g}	11.79±0.27^{o}
	Gm + Gf	11.742±0.28^{c}	12.673±0.26^{m}
8.26	NM	7.415±0.25^{t}	7.247±0.5^{t}
	Gm	7.757±0.26^{p}	7.473±0.39^{r}
	Gf	7.517±0.26^{s}	7.353±0.32^{s}
	Gm + Gf	8.144±0.16^{m}	7.831±0.37^{q}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity x Mycorrhizae		0.000	0.000

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

Tab. 6. Total proline content (µmol/g) of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	
		G. max	*C. tetragonoloba*
1.04	NM	1.163±0.25^{t}	1.355±0.17^{t}
	Gm	1.211±0.18^{r}	1.414±0.18^{r}
	Gf	1.179±0.11^{s}	1.378±0.21^{s}
	Gm + Gf	1.232±0.11^{q}	1.443±0.21^{q}
2.10	NM	1.328±0.14^{p}	1.55±0.21^{p}
	Gm	1.391±0.12^{n}	1.633±0.24^{n}
	Gf	1.354±0.15^{o}	1.577±0.13^{o}
	Gm + Gf	1.421±0.1^{m}	1.661±0.24^{m}
3.78	NM	1.504±0.14^{l}	1.752±0.22^{l}
	Gm	1.581±0.1^{j}	1.858±0.23^{j}
	Gf	1.556±0.11^{k}	1.804±0.18^{k}
	Gm + Gf	1.618±0.09^{i}	1.906±0.26^{i}
5.94	NM	1.694±0.15^{g}	2.118±0.21^{g}
	Gm	1.79±0.12^{b}	2.258±0.34^{b}
	Gf	1.749±0.09^{d}	2.195±0.17^{d}
	Gm + Gf	1.849±0.11^{a}	2.3±0.26^{a}
8.26	NM	1.669±0.13^{h}	2.075±0.15^{h}
	Gm	1.737±0.13^{e}	2.176±0.13^{e}
	Gf	1.709±0.12^{f}	2.14±0.14^{f}
	Gm + Gf	1.779±0.1^{c}	2.217±0.13^{c}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity × Mycorrhizae		0.000	0.000

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

Even though, combined mycorrhizal inoculation with both *Gm* and *Gf* isolates reduced this to a greater magnitude. However, exact opposite trend was observed in control (1.04 dS/m) treatment, where mycorrhizae inoculated plants showed higher percentage of electrolyte leakage over non-mycorrhizal counterparts of both the test plants. Individual and combined effect of soil salinity and mycorrhizal inoculations were found significant with respect to percent electrolyte leakage of test plants (Tab. 7).

Root colonization

At the end of the whole study, non-mycorrhizal plants remained uncolonized whereas, AM colonization was detected in mycorrhizae inoculated test plants exposed to control (1.04 dS/m) and soil salinity gradient up to 8.26 dS/m (Tab. 8).

Due to individual and combined AM inoculations, the magnitude of root colonization was different and relatively higher AM root colonization was noticed in plants inoculated with combined isolates followed by individual colonization with *Gm* and *Gf* isolates under control (1.04 dS/m) and each soil salinity level (2.10 to 8.26 dS/m) (Tab. 8).

Soil salinity and mycorrhizal inoculation when considered individually a significant effect on AM root colonization was observed in case of both the test plants. However, their interaction was mainly non-significant on this parameter (Tab. 8).

Mycorrhizal response on plant dry weight and various biochemical parameters

The overall effect of AM inoculations with *Gm* and *Gf* isolates (individually and in combination) on test plant dry weight and accumulation of various biochemical constituents was determined over respective non-mycorrhizal plants grown under control (1.04 dS/m) and each level of soil salinity (2.10 to 8.26 dS/m) (Fig. 1a to 1f).

Tab. 7. Percent electrolyte leakage of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	
		G. max	*C. tetragonoloba*
1.04	NM	8.59±0.26^{o}	8.68±0.17^{q}
	Gm	9.06±0.04^{n}	9.03±0.26^{o}
	Gf	8.72±0.21^{o}	8.73±0.16^{p}
	Gm + *Gf*	9.73±0.15^{m}	9.79±0.1^{n}
2.10	NM	11.16±0.15^{k}	15.78±0.34^{j}
	Gm	9.68±0.12^{m}	11.95±0.18^{l}
	Gf	10.44±0.36^{l}	13.32±0.17^{k}
	Gm + *Gf*	9.53±0.27^{m}	11.6±0.25^{m}
3.78	NM	19.4±0.31^{h}	24.06±0.28^{d}
	Gm	16.91±0.16^{j}	17.89±0.2^{i}
	Gf	18.71±0.24^{i}	20.88±0.25^{g}
	Gm + *Gf*	16.77±0.17^{j}	17.72±0.31^{i}
5.94	NM	27.55±0.14^{b}	30.25±0.11^{b}
	Gm	20.92±0.09^{e}	21.12±0.15^{g}
	Gf	22.11±0.1^{d}	22.36±0.28^{e}
	Gm + *Gf*	20.43±0.24^{g}	20.34±0.21^{h}
8.26	NM	32.93±0.19^{a}	36.4±0.26^{a}
	Gm	21.13±0.15^{e}	21.73±0.2^{f}
	Gf	26.35±0.21^{c}	27.97±0.23^{c}
	Gm + *Gf*	20.82±0.4^{f}	21.05±0.19^{g}
Salinity		0.000	0.000
Mycorrhizae		0.000	0.000
Salinity × Mycorrhizae		0.000	0.000

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference (P<0.05) by Duncan's test after performing ANOVA

In *G. max* and *C. tetragonoloba* plants, AM inoculations (*Gm* and *Gf*) increased dry weight yield, chlorophyll, protein, proline, carbohydrate and reducing sugar content to a maximum amount at soil salinity either of 3.78 dS/m or of 5.94 dS/m.

At control (1.04 dS/m) and various soil salinity levels (2.10 to 8.26 dS/m), the effect of individual and combine mycorrhizal inoculations (with *Gm* and *Gf* isolates) was more pronounced in *G. max* over *C. tetragonoloba*, in response to higher accumulation of several biochemical constituents (protein and reducing sugar) and dry weight yield (Fig. 1a to 1f).

However, better accumulation of total chlorophyll, proline and carbohydrate content was noticed in *C. tetragonoloba* as compared to *G. max*, following individual and combined inoculation with AM isolates under various levels of soil salinity. Moreover, it was observed that, regardless of test plants and soil salinity, the acquisition of biochemical constituents was found to be higher following combined inoculation with *Gm* and

Tab. 8. Percent root colonization of mycorrhizal and non-mycorrhizal *G. max* and *C. tetragonoloba* grown under control (1.04 dS/m) and at increasing soil salinity levels (2.10 to 8.26 dS/m)

Soil salinity (dS/m)	AM inoculation	Chlorophyll content (mg/g)	
		G. max	*C. tetragonoloba*
1.04	NM	0^{f}	0^{f}
	Gm	18.17 ± 6.79^{bcd}	28.5 ± 8.38^{abc}
	Gf	15 ± 6.26^{cde}	27.83 ± 9.47^{abcd}
	Gm + Gf	26.33 ± 8.5^{a}	35 ± 7.82^{a}
2.10	NM	0^{f}	0^{f}
	Gm	16.67 ± 7.31^{cde}	27.67 ± 5.75^{abcd}
	Gf	13.33 ± 5.13^{de}	27.5 ± 13.4^{abcd}
	Gm + Gf	24.67 ± 7.23^{ab}	32.67 ± 9.93^{ab}
3.78	NM	0^{f}	0^{f}
	Gm	14.83 ± 6.18^{cde}	26.5 ± 12.44^{abcde}
	Gf	12.17 ± 5.78^{de}	24.17 ± 6.97^{bcde}
	Gm + Gf	22 ± 5.29^{abc}	29.5 ± 9.91^{abc}
5.94	NM	0^{f}	0^{f}
	Gm	15.5 ± 6.66^{cde}	19.33 ± 6.8^{cde}
	Gf	12 ± 5.69^{de}	18 ± 8.74^{de}
	Gm + Gf	19.33 ± 6.71^{abcd}	24 ± 7.51^{bcde}
8.26	NM	0^{f}	0^{f}
	Gm	12.35 ± 5.24^{de}	17.33 ± 5.54^{e}
	Gf	9.5 ± 3.89^{e}	17.17 ± 6.94^{e}
	Gm + Gf	16.33 ± 9.05^{cde}	22.5 ± 6.89^{bcde}
Salinity		0.019	0.000
Mycorrhizae		0.000	0.000
Salinity × Mycorrhizae		0.883	0.705

NM: non mycorrhizal, Gm: *Glomus mosseae*, Gf: *Glomus fasciculatum*, Gm+Gf: *Glomus mosseae* + *Glomus fasciculatum*, values are mean±SE of three replicates. Within column, different letters for each treatment indicate statistically significant difference ($P<0.05$) by Duncan's test after performing ANOVA

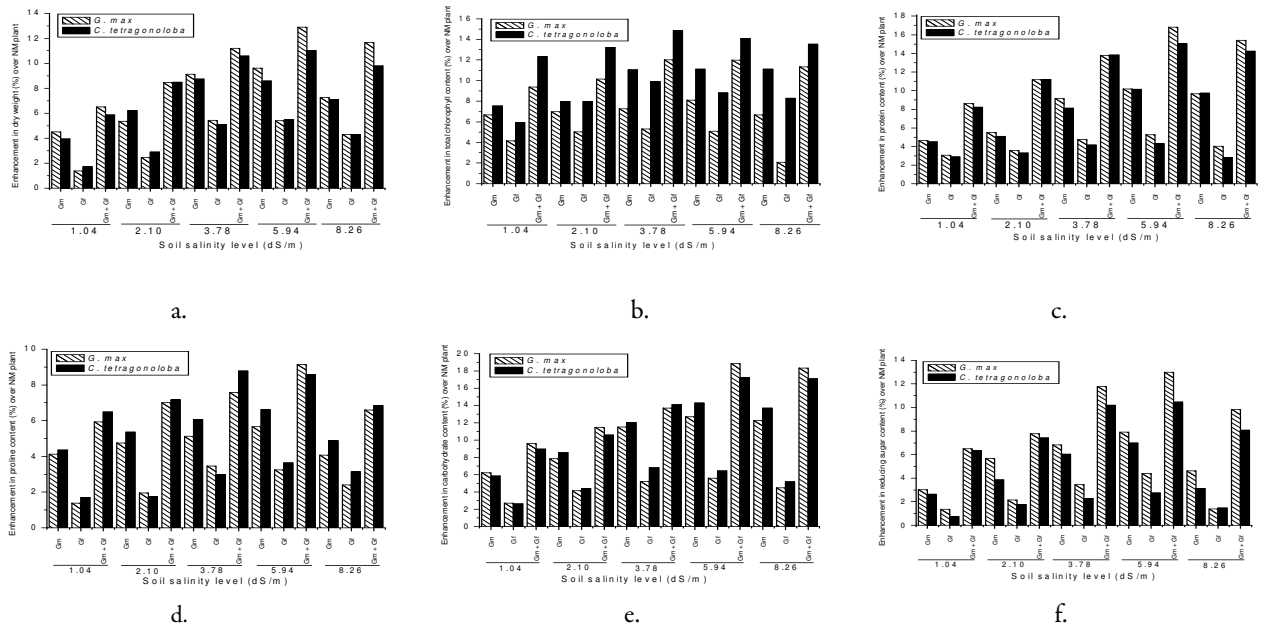

Fig. 1. Mycorrhizal response on enhancement of (a) dry weight yield, (b) total chlorophyll, (c) soluble protein, (d) free proline, (e) soluble carbohydrate, (f) reducing sugar content in individual and combined AM inoculated *G. max* and *C. tetragonoloba* over corresponding non-mycorrhizal plants exposed to control (1.04 dS/m) and increasing soil salinity gradient (2.10 to 8.26 dS/m)

Gf than their individual colonization.

Discussion

This study investigated the effect of two indigenous AM isolates in promoting growth in terms of dry weight matter and various biochemical parameters at different levels of salt stress.

Dry weight of mycorrhizae treated and untreated *G. max* and *C. tetragonoloba* reduced significantly with progressively increasing soil salinity stress, and this may be because of ion toxicity or indirect effect of osmotic imbalance between soil and plant caused by salt ions (Abdel-Latef, 2010). Also, the stunted plant growth under salt stress is normally occurred when high salt contents in soil prevent cell division directly or indirectly (Singh and Chatrath, 2001). However, mycorrhizal inoculation mitigated dry matter reduction in test plants under each salinity level as compared to respective non-mycorrhizal plants. Similar type of results were obtained in previous reports in which AM fungi improved plants dry biomass under salinity stress (Al-Karaki and Hammad, 2001; Gharineh *et al*., 2009). Present study indicated that individual mycorrhizal inoculation significantly improved dry matter accumulation in test plants as compared to respective non-mycorrhizal plant, but co-inoculation was found to be superior in this respect under salinity stress. It is already known that AM fungi are able to adapt edaphic environmental conditions and isolates from saline soil would have a better salt tolerance capacity (Copeman *et al*., 1996; Kaya *et al*., 2009). In our study, *Gm* was isolated from saline soil as a dominant spore and showed a greater positive response than the other isolate (*Gf*), but both of these worked better in co-inoculation treatment. Although, mycorrhizal inoculation resulted in enhanced dry weight in both the test plants, but, magnitude of dry weight increment due to mycorrhization was found better in *G. max*. Better growth of mycorrhizae treated plants has been correlated with more nutrient acquisition in AM mediated plants (Plenchette and Dupponois, 2005).

In the present study, increasing soil salinity gradually reduced chlorophyll content of mycorrhizae inoculated and uninoculated test plants. This result is in agreement with previous report where chlorophyll content in maize plant was declined under saline stress (Sheng *et al*., 2008). The reduction in chlorophyll content at higher salinity level is mainly because of suppression of chlorophyll synthesizing enzyme activity and less uptake of magnesium ion by plants (El-Desouky and Atawia, 1998; Murkute *et al*., 2006). But mycorrhizal inoculation can alleviate this problem to some extent and in our study, at each salinity level, significantly higher chlorophyll content was detected in leaves of *G. max* and *C. tetragonoloba* plants as compared with respective non-mycorrhizal plants. Thus, *Gm* and *Gf* association (individually and in combination) enhanced the photosynthetic ability of *G. max* and *C. tetragonoloba* under salinity stress, which is in agreement with earlier report (Sonnazzaro *et al*., 2006). Data from present study indicated that, regardless of host plant, maximum chlorophyll accumulation occurred by AM fungi in co-association as compared to their individual association. At each soil salinity level leaves of *C. tetragonoloba* showed better chlorophyll accumulation as compared to *G. max*.

When plants grow under saline conditions several types of salt stress proteins accumulate in plant to maintain osmotic balance, act as storage form of nitrogen and furthermore they can be re-utilized later once stress is over (Singh *et al*., 1987). In the present study, protein content followed an increasing trend at low range of salt stress however, higher soil salinity significantly reduced soluble protein contents in *G. max* and *C. tetragonoloba* plants irrespective of mycorrhizal inoculations. However at each salinity level protein content was improved significantly after mycorrhizal colonization as compared to corresponding non-mycorrhizal plants, even though maximum protein accumulation was observed in co-inoculated test plants. Reduction in protein concentration with increasing soil salinity was observed, because salt stress interferes with nitrogen acquisition and utilization by interrupting protein synthesis (Aslam *et al*., 1984; Frechill *et al*., 2001). In present study, mycorrhizal inoculation mitigated the reduction in protein content in plants grown under saline gradient (2.10 to 8.26 dS/m) and control (1.04 dS/m) treatment and may be because extra radical mycelia of AM fungi easily takes up inorganic nitrogen and transports it to intra radical mycelia. Hence, nitrogen can be easily transferred from fungus to plant in AM association (Govindarajulu *et al*., 2005). Moreover, percentage accumulation of soluble protein was found maximum in *C. tetragonoloba* plant, followed by *G. max*. This finding might be related to the fact that specific nitrogen containing compound accumulate in varying concentration in different plant species under salinity stress (Rabie and Almadini, 2005).

In this study, plants exposed to lower level of salt stress (1.04 to 3.78 dS/m) contained better carbohydrate content than the plants exposed at higher salt stress and may be because of the fact that, in response to soil salinity, soluble carbohydrates have been proved to accumulate in plants to adjust osmotic potential and also serving as a carbon storage compound (Murakeozy *et al*., 2003; Parvaiz and Satyawati, 2008). Soluble carbohydrate contents of both the test plants were reduced significantly at higher level of salinity stress. However, mycorrhization in all the test plants significantly improved soluble carbohydrate contents compared to respective non-mycorrhizal plants exposed to control (1.04 dS/m) and soil salinity stress (2.10 to 8.26 dS/m). It was also observed that better carbohydrate accumulation was achieved by combined AM inoculation irrespective of plant species. The positive correlation between mycorrhization of host plant and soluble sugar accumulation was also reported earlier (Al-Garni, 2006; Datta and Kulkarni, 2013). When both the test plants are compared, *C. tetragonoloba* plant accumulated better carbohydrate than *G. max* plant.

Increasing soil salinity (upto 3.78 dS/m) enhanced the content of reducing sugar in test plants and this may

be due to accumulation of several osmolytes (reducing sugar) in plants to remove osmotic disturbance which comes into effect under salt stress and this accumulated reducing sugar also acts as carbon storage (Parida and Das, 2005). Increment in reducing sugar content in various plant species (wheat, grapevine) has already been reported when they were exposed to salt stress (Kerepesi and Galiba, 2000; Khatkar and Kuhad, 2000, Singh *et al*., 2000). But mycorrhizal inoculations increased reducing sugar accumulation in test plants over the non-mycorrhizal plants at various soil salinity levels. Mycorrhizal inoculations with *Gm* and *Gf* isolates adjust osmotic balance in a better way and may help in higher osmo-protection.

With increasing soil salinity consistent increase in proline content in both the test plants (mycorrhizal and non-mycorrhizal treated) was observed. Under salinity stress exposure, proline (the protective osmolyte) accumulates for adaptation of salt stress, maintains osmotic balance and also acts as energy and nitrogen reservoir to be used by plant (Ashraf and Foolad 2007; Jindal *et al*., 1993). It was observed that, mycorrhizal treatment significantly improved proline concentration and their accumulation as compared to non-mycorrhizal plant under increasing soil salinity stress gradient. But a maximum proline accumulation was observed in co-colonized plant. More proline accumulation after AM colonization has already been reported earlier (Jindal *et al*., 1993). Better proline accumulation in *C. tetragonoloba* may be correlated with fact that more proline accumulates in less salt tolerant plant as a symptom of stress for maintaining osmotic adjustment (Wang *et al*., 2004).

Data of present study clearly indicate that electrolyte leakage can be positively correlated with provided salinity stress. In all the test plants, by increasing stress level (2.10 to 8.26 dS/m), a significant reduction in electrolyte leakage was observed in AM inoculated *G. max* and *C. tetragonoloba* plants as compared to non-mycorrhizal plants. Less electrolyte leakage in mycorrhizae treated test plants may be correlated with higher electrolyte concentration in AM plants and this helps to maintain membrane stability (Garg and Manchanda, 2008). Mycorrhization in host plants is responsible for lowering electrolyte leakage at 2.10 to 8.26 dS/m soil salinity stress and this finding is in agreement with the other report (He *et al*., 2007).

Root colonization percentage was reduced significantly in mycorrhizae treated test plants, when salinity level was increased gradually. At each stress level, *Gf* inoculated plants had the least colonization percentage, however, *Gm* and co-inoculation with both AM fungi colonized the test plants roots in higher amount. The reduction in magnitude of percentage colonization under increasing salt stress might be due to inhibition of AM spore germination, interruption in hyphal growth in soil or reduction in arbuscule formation etc. (Hirrel, 1981; McMillen *et al*., 1998; Pfeiffer and Bloss, 1988). Between the two test plants, percentage of mycorrhizal colonization (by individual isolate and in combination) was found to be higher in *C. tetragonoloba* than *G. max* under various levels of salt stress (2.10 to 8.26 dS/m) and control treatment (1.04 dS/m). More percentage of AM root colonization (individually and in combination) in *C. tetragonoloba* plant may be responsible for better accumulation of total chlorophyll, proline, carbohydrate contents etc. under salt stress and protected *C. tetragonoloba* plant from detrimental effect of salt stress in a better way.

Conclusion

G. mosseae and *G. fasciculatum* symbiotic interaction (as individually and in combination) with *G. max* and *C. tetragonoloba* plants, alleviated the deleterious effect of salt stress by improving various biochemical parameters. Even though, individual AM fungi showed positive effect in mitigation of salt effect, but co-inoculation with both *Gm* and *Gf* isolates worked better in this respect. Out of the two test plants, *C. tetragonoloba* showed better growth profile as compared to *G. max* in response to co-inoculation by both the AM isolates under salt stress. Mycorrhization reduced electrolyte leakage profoundly and could maintain osmotic balance by stabilizing membrane stability. Hence, these two AM fungi can be further utilized as bio-inoculant in salt affected soils.

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