Journal of Stress Physiology & Biochemistry, Vol. 10 No. 3 2014, pp. 134-152 ISSN 1997-0838

ORIGINAL ARTICLE

Application of AM Fungi with *Bradyrhizobium japonicum* in improving growth, nutrient uptake and yield of *Vigna radiata* L. under saline soil

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Received April 2, 2014

A pot experiment was conducted under polyhouse conditions, to evaluate the effect of two different arbuscular mycorrhizal fungi (*G. mosseae* and *A. laevis*) in combination with *Bradyrhizobium japonicum* on growth and nutrition of mungbean plant grown under different salt stress levels (4 dS m^{-1}, 8dS m^{-1} and 12 dS m^{-1}). It was found that under saline conditions, mycorrhizal fungi protect the host plant against the detrimental effect of salinity. The AM inoculated plants showed positive effects on plant growth, dry biomass production, chlorophyll content, mineral uptake, electrolyte leakage, proline, protein content and yield of mungbean plants in comparison to non-mycorrhizal ones but the extent of response varied with the increasing level of salinity. In general, the reduction in Na uptake along with associated increase in P, N, K, electrolyte leakage and high proline content were also found to be better in inoculated ones. The overall results demonstrate that the co-inoculation of microbes with AM fungi promotes salinity tolerance by enhancing nutrient acquisition especially phosphorus (P), producing plant growth hormones, improving rhizospheric and condition of soil by altering the physiological and biochemical properties of the mungbean plant.

Key words: Vigna radiata, Arbuscular mycorrhizal fungi, Bradyrhizobium japonicum, Soil salinity, mineral uptake, proline

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Key words: Vigna radiata, Arbuscular mycorrhizal fungi, Bradyrhizobium japonicum, Soil salinity, mineral uptake, proline

Soil salinity is worldwide problem of grave concern because it negatively affects plant productivity and yield of plants particularly in arid and semi-arid and tropical regions of the world. Excessive salts, decline soil water availability for plants, inhibit plants metabolism, nutrients uptake and is also responsible for osmotic imbalance (Evelin *et al.,* 2009). In the recent years, the consumption of chemical fertilizers

has increased exponentially throughout the world, causing serious environmental problems. Thus, exploitation of soil microorganisms in soil amendment is of considerable importance (Yadav *et al*., 2013). Among the various biological approaches to enhance the plant growth in saline conditions, the role of biofertilizers such as Rhizobia and Arbuscular Mycorrhizal (AM) fungi in tolerating environmental stress has been well established and steadily receiving increased recognition from scientists (Kadian *et al*., 2013a). This could be attributed to the fact that they pose no ecological threats having a long lasting effect and considered as bio ameliorators of saline soils (Kadian *et al*., 2013).

AM fungi are ubiquitous soil microorganisms inhabiting the rhizosphere and establish a symbiotic relationship with more than 90% of plant species of natural ecosystems and are also known to occur in saline soils. Symbiotic association of a plant with AMF results in higher ability for taking up the immobile nutrients in nutrient-poor soils as well as improvement of tolerance to salinity (Dixon *et al*., 1993).

Vigna radiata (L.) Wilczek commonly known as mung bean is an important grain legume crop in South East Asia and Africa, and a source food that has a high nutritive value (Kumar *et al*., 2002; Salunke *et al*., 2005). It is not only a rich and economical source of protein, phosphorus, carbohydrate, minerals and provitamin A, but also commonly used as fodder and green manure. Mung bean contains bioactive components with antioxidant, antimicrobial and insecticidal properties (Bounce 2002; Kaprelynts *et al*., 2003; Madhujith *et al*., 2004; Ahmad *et al*., 2008).

Researches in the past few decades on various aspects of root symbionts have shown that dual interaction of AM fungi and *Rhizobium* has improved the growth, nodulation and yield and also nutrient status in legumes. In the light of the above, the present study was carried out to study the efficiency of dual inoculation of AM fungus (*Glomus mosseae and Acaulospora laevis*) with *Bradyrhizobium japonicum* in alleviating the adverse effect of salinity stress of mungbean.

MATERIALS AND METHODS

Growth conditions

The experiment was carried out under poly house at Botany Department, Kurukshetra University, India at a temperature ($30^0C \pm 5^0C$) and humidity (50% -70%). Light was provided by cool white fluorescent lamps (8000 lux) under a 16-hour photoperiod. The glasshouse also received sunlight. The soil characteristics are as follows: sand-64.2%, silt-21.81%, clay-3.90%, pH-6.890, EC- 1.00 dS/m, organic carbon-0.40%, total N-0.042%, P-0.0018 Kg/m^2, K-0.022 Kg/ m^2, and S-14.80 ppm.

Mass multiplication of bio-inoculants

Mass multiplication of arbuscular mycorrhizal fungi (AMF)

The native predominant AM fungi *Glomus mosseae* (T.H. Nicolson and Gerd.) Walker and Schüβler and *Acaulospora laevis (*Gerdemann and Trappe) were isolated from the rhizosphere of mungbean plants. Both AM fungi were mass multiplied in sterilized soil and sand (3:1) substrate using maize as a suitable host in polyhouse conditions. The starter inoculum or pure culture of

each selected dominant AM fungus (*G. mosseae* and *A. laevis*) was raised by the funnel technique of Menge and Timmer using maize as a host.

Mass multiplication of Rhizobium sp.

The culture of *Bradyrhizobium japonicum* was procured from Department of Microbiology, CCS Haryana Agricultural University, Hisar, India and was used as a basal dose.

Plant material

The seeds of mungbean were surface sterilized with 0.5% (v/v) sodium hypochlorite for 10 minutes, subsequently washed with sterilized deionized water. Before sowing seeds 10 ml of a liquid suspension of *Bradyrhizobium* sp. with a density 10^8 cells/ml, was applied to each pot. After 10 days, emergence seedlings were thinned to 6 plants per pot.

Experimental setup

The experiment was laid out in a randomized complete block design, with five replicates of each treatment. Soil from experimental site was collected and mixed with sand in a proportion of 3:1 (soil: sand). This mixture was then sieved through 2-mm sieve and autoclaved at 121^0C for two hours for two consecutive days. Earthen pots (24.5 x 25 cm) were selected and filled with 2.5 kg soil. Initially, the pots were saturated with three different levels of saline solution, i.e. 4, 8, and 12 dSm^{-1}(sodium chloride, calcium chloride and sodium sulphate, 7:2:1 w/v as per Richards (1954). Then, chopped AM colonized root pieces of maize having 80%-85% of colonization along with the soil having AM spores (620-650 per100 g inoculum) were used as AM inoculum. To each pot 10% (w/w), i.e. 200g/pot inoculum of AM fungi alone and in combinations were added into the soil before plantation. Pots were watered regularly with saline solution to maintain the required salinity level and fertilized with a nutrient solution after 15 days (Weaver and Fredrick 1982), which contained half the recommended level of phosphorus and no nitrogen. The experiment had 4 treatments with a single inoculum, a combined inoculum or no inoculums as outlined below:

1. Uninoculated (autoclaved sterile sand: soil without AM inoculum but having *Bradyrhizobium* sp.)

2. *Glomus mosseae* (G) having *Bradyrhizobium* sp.

3. *Acaulospora laevis* (A) having *Bradyrhizobium* sp.

4. G + A having *Bradyrhizobium* sp.

Plant harvest, growth and nutrient analysis

Plants were harvested after 100 days by uprooting them from the soil and various morphological and physiological parameters were measured. For determining root and shoot fresh and dry weight, roots and shoots were harvested after 100 days, weighed and then, oven dried at 70 °C and weighed again. Amount of chlorophyll a, chlorophyll b and total chlorophyll was estimated using the method of Arnon (1949). Phosphorus concentration were determined using the 'Vanado-molybdo-phosphoric yellow colour method' (Jackson, 1973) and nitrogen (N) was calculated by Kjeldahl method (Kelplus nitrogen estimation system, supra-LX, Pelican Equipments, Chennai, India). Analysis of Sodium and Potassium was done by inductively coupled plasma analyzer-Mass spectrometry (ICP-MS). Phosphatase activity was assayed using p-nitrophenyl phosphate (PNPP)

as a substrate, which is hydrolyzed by the enzyme to p-nitrophenol (Tabatabi and Bremner, 1969).

Identification and quantification of the number and colonization by AM spores

AM spores (*G. mosseae* and *A. laevis*) were identified by using the identification manual used by Walker (1983), Scheneck and Perez (1990), Morton and Benny (1990) and Mukerji (1996). Quantification of the number of AM spores was done using the Adholeya and Gaur 'Grid Line Intersect Method' (1994). Mycorrhizal colonization of roots was determined using the 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). Percentage AM colonization of roots was: (Number of root segments colonized / number of root segments studied) × 100.

Electrolyte leakage

To resolve electrolyte leakage, fresh leaf samples (200 mg) were cut into small discs (i.e. 5mm in diameter) and placed in test tubes containing 10 ml distilled, deionized water. The tubes covered with cotton plugs were placed in a water bath at a constant temperature of 32±8°C. After 2 h the initial electrical conductivity of the medium (EC1) was measured using electrical conductivity meter. The samples were autoclaved afterwards at 121±8°C for 20 minutes to kill the tissues completely and release all electrolytes. The samples were then cooled to 25 ±8°C and final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was estimated using the formula of Dionisio-Sese and Tobita:

EL= EC1/EC2 X 100

Proline Determination

Proline was determined by the Bates *et al*. (1973). The proline content was estimated by using the formula:

Proline content = 34.11 x A_{520} x 10/ 2x 0.5

Protein content

Total protein was estimated by method of Bradford (1976).

Statistical analysis

Data were subjected to analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.11.5, Chicago, IL, USA).

RESULTS

Plant height

All the treatments resulted in increment in plant height over control at different salinity levels (Table 1). Maximum change in plant height was recorded in dual inoculation i.e. *G. mosseae* + *A. laevis* at 4 dS m^{-1} followed by single inoculation of *F. mosseae*.

Plant biomass

Biomass of all the inoculated plants of mungbean increased significantly in terms of fresh and dry shoot & root weight. Maximum increment in shoot biomass (fresh and dry) was recorded in single inoculation of *G. mosseae* followed by dual inoculation of *F. mosseae* + *A. laevis* at 4 dS m^{-1} and 8dS m^{-1} salinity levels (Table 1). According to the results root biomass was also found to be increased significantly irrespective of treatments over control. After 100 days, the increase in root biomass (both fresh and dry) was observed maximum in dual inoculation of *G. mosseae* + *A. laevis* followed by single inoculation of *G.*

mosseae at all salinity levels.

Root length

The study revealed a prominent increment in root length in all the treated plants at different salinity levels. However, the best results were observed in plants inoculated with *G. mosseae* followed by the single inoculation of *A. laevis* (Table1). It is clearly evident that increased level of salinity from 4 dS m^{-1} to 12 dS m^{-1} resulted in a reduction of root length.

AM association (AM spore number and % root colonization)

All the treated plants were found to harbor more AM association in comparison to control at all the salinity levels but the AM association also decreases as the salinity level increases (Table1). The results were found to be most significant in dual inoculation of *G. mosseae* + *A. laevis* having highest number of spores and percent root colonization at all the three different level of salinity followed by single inoculation of *G. mosseae*.

Chlorophyll content

Chlorophyll content was found to be increased in all treated plants over control (Table 2). The highest increase in total chlorophyll content was observed in single inoculation of *G. mosseae* followed by dual inoculation of *F. mosseae* + *A. laevis* at all the three different levels of salinity.

Electrolyte leakage

Salt stress caused a significant increase in electrolyte leakage compared to that in the non-stressed plants (Table 2). However, mycorrhizal inoculation significantly reduced the electrolyte leakage in the salt-stressed plants of mungbean. The dual inoculation of G+A was found to be effective in all the three levels of salt stress in lowering the uptake of electrolytes.

Proline content

In general, proline content in leaves of mycorrhizal mungbean plants was significantly higher than that of non-mycorrhizal plants grown in saline soil. Such increase in proline content was linked to the degree of mycorrhizal infection. Among all the treated plants, dual inoculation of G+A was the most efficient for their ability to improve proline content (Table 2).

Protein content

The protein content of mungbean plants was significantly reduced under salinity stress conditions in all the treated plants. It can also be seen that protein level in AM plants was higher than that of non-AM plants (Table 2). In all the treated plants, G+A was more effective for their ability to improve protein content.

Phosphatase activity

In saline soil, acid and alkaline phosphatases activities were significantly higher in mycorrhizal than in non-mycorrhizal mungbean plants. In all the treated plants alkaline phosphatase activity was found to be more than the acidic phosphatase activity and the effect were more pronounced in the plants treated with dual inoculation of G+A under different levels of salinity stress.

Mineral uptake

AM fungi have been shown a positive influence on the composition of mineral nutrients of plants grown in salt stressed conditions by enhancing selective uptake of nutrients. The concentration of N, K and P

were found to be increased in mycorrhizal inoculated plants as compare to non-inoculated ones. In case of Na uptake, concentration of Na was continuously increased in the leaves and root of non- AM inoculated plants as compare to inoculated ones with increasing levels of salinity. However, higher level of salinization resulted in decreased uptake of mineral elements from 4ds/m to 12ds/m (Table 3).

Table 1: Effect of AM fungi along with *Rhizobium* on growth parameters and mycorrhization of Mung bean plant under salinity stress

Salinity level	Treatments	Plant height (cm)	Shoot weight (g)		Root length (cm)	Root weight (g)		(%) Root colonization	AM spore number/10g of soil
			Fresh	Dry		Fresh	Dry		
4DS/m	Control	09.6±1.14^{g}	1.03±0.035^{i}	0.35±0.023^{h}	6.4±1.14^{a}	0.39±0.023^{fg}	0.08±0.004^{g}	2.0±0.70^{i}	3.4±1.67^{g}
	G	24.0±1.58^{b}	4.36±0.032^{a}	0.95±0.022^{a}	14.4± 1.81^{a}	0.62±0.044^{c}	0.16±0.025^{cd}	45.2±3.03^{b}	52.4±2.07^{b}
	A	19.4±1.94^{d}	2.95±0.041^{d}	0.68±0.025^{d}	9.6±1.14^{b}	0.54±0.025^{d}	0.13±0.021^{d}	33.6±4.03^{e}	45.2±2.86^{c}
	G+A	27.8± 1.92^{a}	4.03±0.039^{b}	0.72±0.019^{c}	9.2±1.64^{b}	0.87±0.025^{a}	0.40±0.025^{a}	57.6±3.04^{a}	65.0±2.54^{a}
8DS/m	Control	07.2±0.83^{h}	0.86±0.040^{k}	0.28±0.028^{j}	4.6±1.51^{fg}	0.30±0.026^{h}	0.07±0.002^{gh}	0.6±0.89^{j}	2.0±1.58^{h}
	G	19.6±1.81^{d}	3.05±0.030^{c}	0.78±0.028^{b}	8.8 ±0.83^{bc}	0.50±0.052^{d}	0.11±0.017^{e}	34.4±4.61^{d}	43.0±3.46^{c}
	A	14.0±1.58^{e}	2.13±0.034^{f}	0.42±0.031^{g}	7.2 ±1.64^{cde}	0.45±0.039^{e}	0.09±0.002^{ef}	29.4±1.94^{f}	38.2±2.04^{d}
	G+A	21.8± 1.78^{c}	2.77±0.037^{a}	0.61±0.039^{e}	6.2 ± 0.37^{ef}	0.71±0.054^{b}	0.25±0.033^{b}	40.8±2.68^{c}	51.2±2.28^{b}
12DS/m	Control	2.2±0.83^{i}	0.73±0.024^{f}	0.07±0.007^{i}	3.7±0.33^{g}	0.22±0.039^{i}	0.05±0.003^{h}	0.00±0.00^{i}	0.0±0.00^{i}
	G	9.0±1.58^{g}	1.76±0.026^{h}	0.49±0.034^{f}	8.4±1.81^{bc}	0.43±0.030^{ef}	0.08±0.003^{efg}	24.6±3.84^{g}	32.6±1.94^{e}
	A	4.4±2.70^{h}	0.98±0.025^{j}	0.32±0.035^{h}	6.8±1.09^{cde}	0.36±0.042^{g}	0.06±0.003^{gh}	17.0±3.46^{h}	26.2±1.78^{f}
	G+A	12.0±1.87^{f}	1.99±0.019^{g}	0.59±0.018^{e}	6.1 ± 0.08^{ef}	0.68±0.026^{b}	0.18±0.021^{c}	32.0±2.12^{ef}	42.4±2.07^{c}
	L.S.D ($P \leq 0.05$)	1.9768	0.042	0.0352	1.6027	0.0475	0.4423	3.6229	2.7568
	ANOVA $F_{(11,24)}$	135.067	6942.964	399.340	24.249	124.760	159.448	209.236	490.972
F values	Salinity (s)	449.049	14356.265	799.714	58.371	340.072	135.971	81.246	344.263
	Parameter (p)	331.756	11952.495	1844.220	52.625	827.780	1231.684	1026.997	1492.044
	s x p	6.868	1718.149	76.273	2.386	1.349	24.890	26.032	41.326

G: *Glomus mosseae*, A: *Acaulospora laevis* ; each value is a mean of five replicates.

±: standard deviation AM: Arbuscular mycorrhizal.

Values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test.

Table 2: Effect of AM fungi along with *Rhizobium* on physiological parameters of Mung bean plant under salinity stress

Salinity level	Treatments	Chlorophyll content			Proline content		Electrolyte leakage	Protein content
		Chl a	Chl b	Total Chl	Root	Shoot		
4DS/m	Control	0.93±0.004^{i}	0.63±0.011^{i}	1.57±0.009^{i}	0.10 ± 0.002^{k}	2.04 ±0.003^{h}	26.7 ± 1.42^{g}	11.3 ± 0.27^{h}
	G	1.51±0.003^{a}	1.22±0.007^{a}	2.74±0.008^{a}	0.34 ± 0.003^{h}	5.11± 0.003^{c}	20.8 ± 0.97^{h}	21.5 ± 0.94^{b}
	A	1.17±0.006^{e}	0.93±0.009^{e}	2.10±0.003^{e}	0.23 ± 0.003^{i}	4.09± 0.003^{e}	21.3 ± 1.26^{h}	16.0± 0.37^{e}
	G+A	1.41±0.002^{b}	1.11±0.008^{b}	2.53±0.010^{b}	0.40 ± 0.002^{g}	6.19± 0.003^{c}	19.8 ± 1.49^{h}	24.4± 0.31^{a}
8DS/m	Control	0.87±0.003^{j}	0.59±0.007^{j}	1.47±0.010^{j}	0.17 ± 0.003^{j}	2.72 ±0.004^{g}	40.2 ± 0.99^{c}	7.6 ± 0.24^{i}
	G	1.34±0.004^{c}	1.02±0.006^{c}	2.36±0.005^{c}	0.61 ± 0.005^{e}	4.77± 0.003^{d}	32.4 ± 1.01^{f}	17.7 ± 0.40^{d}
	A	1.11±0.004^{f}	0.84±0.008^{f}	1.96±0.006^{f}	0.44 ± 0.004^{f}	4.19 ± 0.003^{e}	34.5 ± 2.00^{ef}	15.0 ± 0.36^{f}
	G+A	1.29±0.003^{d}	0.99±0.005^{d}	2.29±0.009^{d}	0.64 ± 0.003^{d}	5.79 ± 0.003^{c}	32.9 ± 1.87^{ef}	19.1 ± 0.71^{c}
12DS/m	Control	0.85±0.003^{k}	0.51±0.007^{k}	1.36±0.010^{k}	0.30 ± 0.004^{i}	3.41 ± 0.002^{f}	48.8 ± 1.28^{a}	3.1 ± 0.00^{j}
	G	1.06±0.002^{g}	0.80±0.007^{g}	1.86±0.009^{g}	1.02 ± 0.003^{b}	7.16 ± 0.003^{b}	36.9 ± 2.89^{d}	13.6 ± 0.03^{g}
	A	0.95±0.003^{i}	0.66±0.005^{i}	1.61±0.008^{i}	0.68 ± 0.003^{c}	6.82 ± 0.004^{b}	42.7 ± 1.72^{b}	11.7 ± 0.46^{h}
	G+A	1.01±0.002^{h}	0.77±0.006^{h}	1.79±0.004^{h}	1.33 ± 0.002^{a}	7.84 ± 0.003^{a}	34.8 ± 1.43^{e}	14.0 ± 0.27^{g}
	L.S.D (P≤0.05)	0.0053	0.0142	1.3715	0.012	0.4915	2.0597	0.5564
	ANOVA F $_{(11,24)}$	14123.280	1919.234	13548.763	7315.701	110.411	158.721	873.160
F values	Salinity (s)	49339.958	8135.940	41069.469	16409.891	182.900	794.612	2153.312
	Parameter (p)	16359.285	3195.020	23227.069	15186.612	267.200	93.651	1793.629
	Salinity X P	2565.520	171.621	1578.953	1902.297	7.895	9.891	65.569

G: *Glomus mosseae*, A: *Acaulospora laevis* ; each value is a mean of five replicates.

±: standard deviation AM: Arbuscular mycorrhizal.

Values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

Table 3: Effect of AM fungi along with *Rhizobium* on nutrient uptake of Mung bean plant under salinity stress

Salinity level	Treatments	Nitrogen content (%)		Phosphorus content (%)		Potassium content (%)		Sodium content (%)	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
4DS/m	Control	0.38±0.024^{f}	0.55±0.025^{h}	0.50±0.038^{j}	0.37±0.039^{h}	1.00±0.035^{i}	0.77±0.038^{h}	1.45±0.047^{b}	1.59±0.040^{c}
	G	0.77±0.036^{b}	1.94±0.033^{b}	1.34±0.027^{b}	1.03±0.034^{b}	2.19±0.039^{a}	2.02±0.039^{a}	1.28±0.026^{d}	1.31±0.040^{f}
	A	0.70±0.030^{cd}	1.75±0.033^{c}	1.00±0.027^{e}	0.79±0.028^{d}	1.33±0.043^{f}	1.18±0.025^{e}	0.63±0.027^{h}	0.70±0.032^{j}
	G+A	1.00±0.053^{a}	2.10±0.038^{a}	1.69±0.027^{a}	1.17±0.030^{a}	1.67±0.032^{d}	1.35±0.049d	1.08±0.052^{f}	1.22±0.046^{g}
8DS/m	Control	0.25±0.031^{g}	0.37±0.033^{i}	0.24±0.036^{j}	0.22±0.038i	0.90±0.033^{j}	0.68±0.031i	1.79±0.022^{a}	1.82±0.057^{a}
	G	0.70±0.016^{cd}	0.90±0.043^{e}	1.08±0.046^{d}	0.81±0.020^{cd}	2.06±0.040^{b}	1.77±0.034^{b}	1.39±0.026^{c}	1.57±0.035^{c}
	A	0.50±0.043^{ef}	0.71±0.038^{f}	0.86±0.040^{f}	0.61±0.038^{f}	1.17±0.034^{g}	1.00±0.033^{f}	0.75±0.041^{g}	0.95±0.018^{h}
	G+A	0.75±0.041^{bc}	1.08±0.028^{d}	1.25±0.042^{c}	0.85±0.033^{c}	1.39±0.037^{e}	1.17±0.036^{e}	1.27±0.024^{d}	1.40±0.040^{e}
12DS/m	Control	0.13±0.027^{h}	0.17±0.029^{j}	0.19±0.021^{k}	0.12±0.018j	0.83±0.066^{k}	0.59±0.036^{j}	1.50±0.038^{b}	1.68±0.054^{b}
	G	0.53±0.056^{e}	0.66±0.024^{g}	0.74±0.027^{g}	0.49±0.042^{g}	1.89± 0.039^{c}	1.67±0.032^{c}	1.29±0.048^{d}	1.47±0.027^{d}
	A	0.43±0.031^{f}	0.65±0.041^{g}	0.68±0.050^{h}	0.40±0.038^{h}	1.08±0.041^{h}	0.92±0.037^{g}	0.72±0.041^{g}	0.92±0.059^{h}
	G+A	0.65±0.030^{d}	0.85±0.040^{e}	0.88±0.027^{f}	0.68±0.054^{e}	1.31±0.043^{f}	1.14±0.036^{e}	1.20±0.038^{e}	1.37±0.039^{e}
	L.S.D(P≤0.05)	0.2679	0.0442	0.0452	0.0463	0.0524	0.0464	0.0478	0.0542
	ANOVA(F11,24)	203.046	1610.916	774.780	387.885	612.873	749.173	427.687	308.827
F values	Salinity (s)	288.096	6945.698	334.48	602.424	855.117	1146.350	2163.875	165.958
	Parameter (p)	700.530	3212.258	1089.046	3356.748	14045.681	7060.287	1392.734	479.158
	Salinity x p	11.360	278.981	36.596	134.197	19.095	11.764	4.986	3.238

G: *Glomus mosseae*, A: *Acaulospora laevis*; each value is a mean of five replicates.

±: standard deviation AM: Arbuscular mycorrhizal.

Values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test.

Table 4: Effect of AM fungi along with *Rhizobium* on phosphatase activity, yield and nodulation of Mung bean plant under salinity stress

Salinity level	Treatments	Yield (per pot) No. of pods	Yield (per pot) Wt. of pods (g)	Number of nodules/pot	Phosphatase Activity Acidic	Phosphatase Activity Alkaline
4DS/m	Control	2.4 ± 1.14^{f}	1.02±0.019g	3.4 ± 1.14^{gf}	0.038 ± 0.007^{j}	0.082 ± 0.006^{j}
	G	9.6 ± 1.67^{b}	2.82±0.019b	18.0 ± 2.54^{b}	0.195 ± 0.007^{c}	0.328 ± 0.008^{c}
	A	6.2 ± 0.83^{d}	2.07±0.033e	13.4 ± 1.14^{c}	0.125 ± 0.006^{e}	0.258 ± 0.007^{d}
	G+A	12.8 ± 1.30^{a}	3.23±0.030a	24.6 ± 3.57^{a}	0.240 ± 0.008^{a}	0.387 ± 0.006^{a}
8DS/m	Control	0.0 ± 0.00^{g}	0.00 ± 0.00g	2.0 ± 0.70^{gf}	0.032 ± 0.007^{j}	0.061 ± 0.006^{k}
	G	6.2 ± 1.30^{d}	2.24±0.027d	15.0 ± 1.87^{c}	0.140 ± 0.008^{d}	0.245 ± 0.007^{e}
	A	03.8±1.30e	1.48±0.027f	9.8 ± 1.48^{d}	0.097 ± 0.008^{i}	0.218 ± 0.007^{f}
	G+A	8.0 ± 0.70^{c}	2.50±0.023c	18.4 ± 2.70^{b}	0.223 ± 0.007^{b}	0.346 ± 0.005^{b}
12DS/m	Control	0.0 ± 0.00^{g}	0.00 ± 0.00^{i}	1.0 ± 0.70^{f}	0.024 ± 0.005^{k}	0.045 ± 0.006^{l}
	G	0.00 ± 0.00g	0.00 ± 0.00^{i}	7.2 ± 2.16^{d}	0.075 ± 0.006^{g}	0.116 ± 0.008^{i}
	A	0.00 ± 0.00g	0.00 ± 0.00^{i}	4.6 ± 2.07^{e}	0.064 ± 0.008^{h}	0.154 ± 0.006^{g}
	G+A	2.2 ± 0.83^{i}	0.94±0.016h	9.2 ± 2.28^{d}	0.044 ± 0.009^{j}	0.144 ± 0.008^{h}
	L.S.D ($p \leq 0.05$)	1.223	0.0259	2.5775	0.0097	0.0092
	ANOVA F $_{(11,24)}$	99.984	17568.266	67.795	496.970	1285.755
F values	Salinity (s)	418.819	21538.190	107.684	680.865	6490.335
	Parameter (p)	92.917	44056.000	113.574	913.120	3194.482
	Salinity X p	20.305	4280.779	14.302	251.308	264.851

G: *Glomus mosseae*, A: *Acaulospora laevis*; each value is a mean of five replicates.
±: standard deviation AM: Arbuscular mycorrhizal.
Values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test.

Yield and nodulation

Data on yield indicate that bioinoculation of mungbean plants with either *G. mosseae* or *A. laevis* resulted in a significant increase in the weight of the

pods and consequently higher yields under different salinity levels (Table 4). Treatment with a mixture of *G. mosseae* and *A. laevis* at 4ds/m resulted in the greatest increase in yield in terms of the number of pods followed by those plants treated with *G. mosseae* alone. However, the yield was found to be decreased as the salinity level increases from 4ds/m to 8ds/m and 12ds/m. That is, the greatest yield was of plants inoculated with both *G. mosseae* and *A. laevis* which operated together more effectively in supplying their host plants with their nutrient requirements than when either operated on its own. Colonization of a legume by AM fungi also increased the number of nodules as evident from Table 4.

DISCUSSION

Salt stress adversely affects the morphology as well as physiology of plants grown under saline soil by increasing the osmotic stress, ion toxicity and nutrient deficiency. However, colonization of mungbean plant with AM fungi significantly increased growth response. The G+A treatments gave better results at all the three different salinity levels than other treatments because AM fungi counteract the toxic effects of salts by the better acquisition of nutrition especially phosphorus (Sharifi *et al*., 2007) and other elements by extraradical mycorrhizal hyphae, and transferring them to the root tissues (Wu *et al*., 2010; Giri and Mukerji 2004). The results of present investigation are in close conformity with those of Colla *et al*. (2008) who reported improved growth of *Cucurbita pepo* colonized with *Glomus intraradices* under salinity stress.

Giri and Mukerji (2003) also reported a significant increase in root and shoot dry weights of *Acacia auriculiformis* inoculated with mycorrhizal treated plants than non mycorrhizal plants. Similar results are also reported by Al karaki (2000) for tomato plant when inoculated with mycorrhizal fungi. This increment may be due to more absorption of nutrients especially P via an increase in root surface area through AM fungi (Prakash *et al.,* 2011) and ability of plants for replacement of K by Na (Haijiboland and Joudmand, 2009). Under saline soil, greater CO_2 assimilation could adequately provide carbohydrates for the fungal partner and results in more benefits to plants from AM association. These AM isolates from saline soils have a better ability to improve the survival, growth and ultimately biomass of host plants (Tain *et al.,* 2004).

The better results in root length was obtained when only the plants were inoculated with *F. mosseae* alone at different salinity levels may be due to space and nutrition for its multiplication and survival in sterilized soil resulting in absorption of more nutrients from the soil. Similarly, Quilambo (2000) and Shekoofeh and Sepideh (2011) also observed significant increment in root length with an indigenous AM fungi at various salinity levels. Correlation of root length with mycorrhizal inoculation amount of root is probably related to suitable ventilation of soil, that is the result of hypha network of mycorrhizal fungi that connects particles of soil and as result the root spreads into deep soil (Turk *et al*., 2006).

AM inoculated plants showed higher percentage of colonized roots as compared to control. On the basis of present investigation, it was found that root

colonization and AM spore number were greatly influenced by increasing soil salinity level. Similarly, Al-Khaliel (2010) reported that mycorrhizal colonization and spore density decreases under highest salinization level in peanut treated with *G. mosseae.* The suppressed spore number and colonization of arbuscular mycorrhizal under different salinity level may be attributed to the reduced spore germination and hyphal extension of AMF that were inhibited by salt (Belew *et al*., 2010).

The single inoculation of *G. mosseae* results in maximum increase in chlorophyll content at 4DS/m due to less interference of salt with chlorophyll synthesis in mycorrhizal than in non-mycorrhizal plants (Giri and Mukerji, 2004). Under saline conditions, mycorrhization helps in better absorption of Mg in plants and the antagonisitic effect of Na^+ on Mg^+ uptake is counter balanced and suppressed resulting in increased chlorophyll synthesis (Giri and Mukerji, 2003). In *Glomus etunicatum* inoculated maize plant, increase in photosynthesis speed, transpiration and chlorophyll a, b density was reported under stress (Zhu *et al*., 2010).

Mycorrhizal plants had significantly higher root P concentration than shoot. The phosphorus concentration in plant tissuses rapidly lowered under salt stress because phosphate ion precipitates with Ca, Mg and Zn, then being unavailable to plants (Evelin *et al*., 2009; Park *et al*., 2009). 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). Our results are in accordance with those of Shokri and Maadi (2009) who reported that the concentration of phosphorus in *Trifolium alexandrium* plants was found to be higher relative to non-inoculated ones but it decreases with the increasing level of salinity. Similar results were also obtained by Giri and Mukerji (2004) who reported maximum uptake of phosphorus in roots when the plants were inoculated with *Glomus macrocarpum*. Higher P uptake by mycorrhizal plants under salt stress increases the plant ability of reducing of negative effects of Na^+ and Cl^- ions (Feng *et al*., 2002) by maintaining vacuolar membrane integrity, which facilitates compartmentalization within vacuoles and selective ion intake, thereby preventing ions from interfering in metabolic pathways of growth (Cantrell and Lindermann, 2001).

AM fungi can function as a facilitator for N uptake through activation of a plant ammonium transporter (Guether *et al*., 2009) and salts interferes less with nitrogen acquisition and utilization by influencing different stages of N metabolism, such as NO^-_3 uptake and reduction and protein synthesis (Frechill *et al*., 2001). Thus, improved uptake of N in mycorrhizal plants under salt stress may be due to better nutrient uptake and maintenance of ionic balance and better acquisition of N (both nitrate and ammonium ions) from the soil. Garg and Manchanda (2008) also recorded highest accumulation of N in shoots of mycorrhizal *Cajanus cajan* than non mycorrhizal plants at all salinity levels.

AM induce a buffering effect on the uptake of Na^+ when the content of Na^+ is within the permissible limit

(Allen and Cunningham, 1983). This also indicates the possibility of a regulatory mechanism operating in the plant contain Na+ ions. The accumulation of Na is strongly influenced by the form of N available (NO_3 and NH_4) and it may also be influenced by the synthesis and storage of polyphosphate (Orlovich and Ahford, 1993) as well as by other cations, particularly K (Giri *et al*., 2003). The results of present investigation are in consonance with Giri *et al*., 2007 in *Acacia nilotica* when inoculated with *Glomus fasciculatum*. Similar results are also obtained by Tian *et al* (2004) when the plants of *Gossypium arboretum* were inoculated with *G. mosseae* under salt stress.

Sharifi *et al*. (2007) and Zuccarini and Okurowska (2008) also observed increase in K^+ content when inoculated with *Glomus etunicatum* under different levels of salt stress. Higher K^+ accumulation in mycorrhizal plant under salt stress conditions may help in maintaining a high K/Na ratio, thus preventing the disruption of various enzymatic processes and inhibition of protein synthesis. This capacity of plants to maintain a high cytosolic K^+: Na^+ is one of the important factor of plant salt tolerance (Maathuis and Amtmann, 1999).Our results are in accordance with the findings of Shokri and Maadi (2009), Porras-Soriano *et al*. (2009) who reported efficacy of *G. intraradices* in maintaining favourable K^+: Na^+ ratio.

In saline soil, acid and alkaline Phosphatase activities were significantly higher in mycorrhizal than non-mycorrhizal ones. Such increases in those activities were related to the degree of active mycorrhizal infection of each fungal species. Gianinazzi-Pearson and Gianinazzi (1978) and Ezawa and Yoshida (1994) detected mycorrhizal-specific Phosphatase (MSPase) only in the mycorrhizal root extract, and it was of fungal origin. The close relation between mycorrhizal growth responses and the active arbuscular phase of the infection supports the hypothesis that the Phosphatase enzyme is somehow involved in assimilation of phosphorus by arbuscular mycorrhizal fungi (Abdel-Fattah, 2001).

Under saline conditions, many plants accumulate proline as a non-toxic and protective osmolyte to maintain osmotic balance under low water potentials (Ashraf and Foolad, 2007; Parida *et al*., 2002). It also acts as a reservoir of energy and nitrogen for utilization during salt stress conditions (Goas *et al*., 1982). Proline levels were found to be increased significantly with salinity stress in mycorrhizal plants when compared to non-mycorrhizal plants. Sharifi *et al*. (2007) also reported a higher proline concentration in AM soybean than non-AM plants at different salinity level. This increment in proline could be due to the induction of proline biosynthesis enzymes and/or to the reduction of oxidation to glutamate (Stewart, 1981). Several roles have been attributed to this supraoptimal level of proline; for instance, osmoregulation and detoxification of free radicals (Kaul *et al*., 2008).

Salt stress caused a significant increase in electrolyte leakage compared to that in the non-stressed plants. Mycorrhizal treated plants have lower electrolyte leakage as compared to non-mycorrhizal plants by maintaining improved integrity and stability of membrane (Zhongoun *et al*., 2007; Manchanda, 2008). Mycorrhizal plants had much lower root plasma

membrane electrolyte permeability than the non-mycorrhizal plants (Kaya *et al*., 2009). The increased membrane stability has been attributed to mycorrhizal mediated enhanced P uptake and increased antioxidant production (Feng *et al*., 2002).

Higher protein concentration could be due to higher efficiency of the osmotic regulation mechanism in mungbean plants which in turn prevents protein reduction under salt stress (Flowers and Yeo, 1995; Kumar *et al*., 2010) and induces the synthesis of osmotin like protein structure. This protein increment lead to membrane stabilization and helps plants to grow and develop under saline conditions (Goudarzi and Pakniyat, 2009). Mycorrhizal and nodule symbioses often act synergistically on infection rate, mineral nutrition and plant growth (Patreze and Cordeiro, 2004; Rabie, 2005) which support the need for both N and P and increased tolerance of plants to salinity stress (Rabie and Almadini, 2005).

CONCLUSION

In conclusion, the results of the present investigation showed that the tripartite symbiosis of bacterial-AM-legume significantly alleviated the harmful effects of salt stress in legumes plant. However, many practical problems remain, such as the selection of better strain dosages, salinity tolerance of symbionts, choice of good symbionts to plant and appropriate time for inoculation that needs further studies.

ACKNOWLEDGMENT

The authors are grateful to Kurukshetra University, Kurukshetra, for providing laboratory facilities to carry out the research work.

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