ARBUSCULAR MYCORRHIZAL FUNGI ENHANCE GROWTH, PHYSIOLOGICAL PARAMETERS AND YIELD OF SALT STRESSED *PHASEOLUS MUNGO* (L.) HEPPER

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ABSTRACT

A pot experiment was conducted in a greenhouse to investigate the effect of two dominant indigenous arbuscular mycorrhizal fungi, viz. *Funneliformis mosseae* (F) and *Acaulospora laevis* (A), on the growth of *Phaseolus mungo* subjected to salinity levels of 4, 8 and 12 dS m^{-1}. Mycorrhizal fungi alone and in combination improved the growth of the plants at all the salinity levels over that of the untreated control plants. However, a combination of *F. mosseae* and *A. laevis* resulted in maximum root and shoot length, biomass, photosynthetic pigments, protein content, mycorrhization, nodulation, phosphatase activity, phosphorus uptake and yield at the 8 dS m^{-1} salinity level. Peroxidase activity and electrolyte leakage were minimum at the 8 dS m^{-1} salinity level due to improved water absorption as a result of the highest mycorrhization occurring at this level of salinity. Nitrogen and potassium uptake decreased with increase in salinity and highest uptake of these nutrient elements was recorded in the treatment with both mycorrhizal fungi at a salinity level of 4 dS m^{-1}. The results of the present experiment indicate *P. mungo* inoculated with *F. mosseae* and *A. laevis* can be successfully cultivated of at salinity level of 8 dS m^{-1}. Saline soils with an electrical conductivity of nearly 12 dS m^{-1} were not suitable for growing this legume.

Keywords: mycorrhizal fungi, *Phaseolus mungo*, salinity stress, nutrient uptake, peroxidase

Introduction

The ability of soil to provide necessary nutrients for plants determines its sustainable productivity. The scarcity of micronutrients is one of the factors limiting the stability, productivity and sustainability of soil (Bell and Dell 2008). Soil salinization is a major and increasing problem in different parts of world, especially in dry and semi-arid areas. Nearly 7% of the land surface in the world is occupied by salt affected soils (Sheng et al. 2011). The most common reasons for increasing land salinization include; excessive use of chemical fertilizers, inadequate drainage as well as irregular irrigation particularly in protected cultivation. Increased concentration of salts in soil disrupts its basic structure causing a reduction in soil porosity and consequently decreased aeration and water conductance (Cucci et al. 2015). In saline soils, plants suffer from different physiological disorders, which affect their overall growth and productivity due to increased osmotic pressure and the harmful effects of Na^+ and Cl^- ions. Increased accumulation of Na^+ as well as Cl^- ions in saline soils causes nutrient imbalance as excess of Na^+ restrains uptake of K^+ while excess of Cl^- ions slows down NO_3^- uptake (Turkmen et al. 2005). Salinity stress in plants is associated with increased production of reactive oxygen species, which causes oxidative damage resulting in the oxidation of lipids, proteins and chlorophyll causing membrane leakage as well as damage to nucleic acids. In response to this plants have complex antioxidant system including enzymes like catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) and some non-enzymatic molecules like glycine, proline, betaine, sorbitol and mannitol to protect them from oxidative damage due to salinity stress (Parvaiz and Satyawati 2008).

To cope up with the increasing problem of soil salinity, development of inbred crop plants that are tolerant of salinity stress and other physiochemical methods have been tried but have failed because of physiological or genetical trait complexity (Flowers and Flowers 2005; Munns 2005). The use of plant growth promoting microorganisms as a useful and practical way to ameliorate salinity stress has received much attention in recent years. Among the plant growth promoting microorganisms, the role of arbuscular mycorrhizal fungi in improving soil structure and alleviating salinity stress is well established (Ahanger et al. 2014). Mycorrhizal associations are widely recorded in saline soils and are able to utilize water and mineral salts more efficiently than roots of plants. These symbiotic fungi act as bio-alleviators of salinity stress by improving nutrient uptake, chlorophyll content, antioxidant enzyme activity, membrane stability, vegetative growth and phosphatase activity thus reducing the damage to plants caused by salinity stress (Sheng et al. 2011; Beltrano et al. 2013). Since legumes establish a tripartite association with rhizobacteria and AMF, it is recommended that legumes are inoculated with these microbes, which may assist phosphorus and nitrogen uptake resulting in improvement of growth and productivity under salinity stress.

Among the different food legumes, *Phaseolus mungo* (L.) Hepper pulses are highly nutritious containing 60% carbohydrate, 24% protein and 1.3% fat, plus minerals like calcium, phosphorus, potassium and vitamins like A, B and C (Sarwar et al. 2004). It is one of the most highly prized pulses in India. This country is the largest producer of *P. mungo* in the world. The hazardous effect of salt in the soil on the productivity of legumes is the major problem confronted by the farmers throughout

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the world. Thus the ability of AMF to ameliorate salinity stress and improve the tolerance of *P. mungo* of salt could have important practical applications. The present experiment aimed to investigate the growth response of *P. mungo* grown at different levels of salinity and the role of AMF in improving growth and yield when grown in saline soils.

Materials and Methods

Growth Conditions

A pot experiment was conducted in a glass house at the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India. The temperature was maintained at (30 ± 5 °C) and the relative humidity at 60–70%. Apart from sunlight, light was also provided for 16 hours each day by cool white fluorescent lamps. Soil used in this experiment consisted of 64.2% sand, 21.81% silt and 3.90% clay.

Mass Multiplication of Bioinoculants

In this experiment, two arbuscular mycorrhizal species viz. *Funneliformis mosseae* and *Acaulospora laevis* were used. They were isolated from the rhizosphere of *P. mungo* grown in the botanical garden of Kurukshetra University, Kurukshetra. After preparation of a starter inoculum using the Funnel Technique of Menge and Timmer (1982), these species were propagated using maize growing in standard pot culture as a host. Mass multiplication of *Trichoderma viride* was done using a modified wheat bran-saw dust medium (Mukhopadhyay et al. 1986) and the *Rhizobium* sp. (*Bradyrhizobium japonicum*) culture (procured from Department of Microbiology, CCS Haryana Agricultural University, India) was multiplied using a nutrient broth medium. Seeds of *Phaseolus mungo* were procured from CCS Haryana Agricultural University, Hisar, Haryana, India. The seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite for 10 minutes and then washed with sterilized distilled water. Before seeds were sown in pots, 10 ml of a liquid suspension of *Bradyrhizobium* sp. was applied to each pot. Ten days after emergence the number of plants was reduced to 5 per pot.

Experimental Setup

The experiment was laid out in a randomized block design, with five replicates of each treatment. Soil from the experimental site was collected and mixed with sand in a ratio of 3:1 (soil : sand). This mixture was then sieved through 2-mm sieve and autoclaved at 121 °C for two hours for two consecutive days to render it free of naturally occurring microbes, including mycorrhizal fungi. After sterlization, the soil was then tested for the presence of microbes and it was found to be completely free of microbes. This was done to avoid the effect of other microbes on the growth response of *L. culinaris* under salinity stress. The Earthenware pots (24.5 × 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 dS m^{-1} (sodium chloride, calcium chloride and sodium sulphate in the ratio 7:2:1 w/v) as per Richards (1954). Then, pieces of maize root with 85% colonization by AM were chopped up and along with soil containing AM spores (620–650 per 100 g inoculum) were used as the AM inoculum. To each pot 10% (w/w), i.e. 200 g/pot inoculum of AM fungi alone and in combination was added to the soil before sowing the seeds. Pots were watered regularly with saline solution to maintain the required salinity level and were fertilized with a nutrient solution after 15 days (Weaver and Fredrick 1982), which contained half the recommended level of phosphorus and no nitrogen. For each level of salinity there were 4 treatments as outlined below:

1. Uninoculated (without AM inoculum but with *Bradyrhizobium* sp.)
2. *Funneliformismosseae* (F) with *Bradyrhizobium* sp.
3. *Acaulospora laevis* (A) with *Bradyrhizobium* sp.
4. F + A with *Bradyrhizobium* sp.

Plant Harvest and Analysis

After 120 days, plants were harvested by uprooting them and then various morphological and physiological parameters were measured. Plant height and root length were measured. For determining fresh and dry weight, roots and shoots were weighed after uprooting and then oven dried at 70 °C until a constant dry weight was obtained. Chlorophyll content was determined by using the method of Arnon (1949). Root and shoot phosphorus content was estimated using the 'Vanado-molybdo-phosphoric yellow colour method' (Jackson 1973) and nitrogen (N) content was determined using the Kjeldahl method (Kelplus nitrogen estimation system, supra-LX, Pelican Equipments, Chennai, India). Potassium content was analyzed using inductively coupled plasma analyzer-mass spectrometry (ICP-MS). Phosphatase activity was estimated using p-nitrophenyl phosphate (PNPP) as a substrate, which is hydrolyzed by the enzyme to p-nitrophenol (Tabatabi and Bremner 1969). Total protein was estimated using Bradford's (1976) method. Peroxidase activity was determined using Maehly's method (1954). Leaf area was measured using a leaf area meter (Systronics 21, Ahmedabad, India). Nodulation and yield in terms of number and weight of pods (g) per pot was recorded after 120 days.

Identification and Quantification of the Number and Colonization by AM Spores

Identification of AM spores (*F. mosseae* and *A. laevis*) was done using the identification manuals of Walker (1983); Scheneck and Perez (1990); Morton and Benny (1990) and Mukerji (1996). Quantification of AM spores was done using the Adholeya and Gaur 'Grid Line Intersect Method' (1994). 'Rapid Clearing and Staining' technique of Phillips and Hayman (1970) was used to estimate mycorrhizal colonization of roots. The latter was

calculated using the formula: (Number of root segments colonized / number of root segments studied) × 100.

Electrolyte Leakage

To determine electrolyte leakage, fresh leaf samples (200 mg) were cut into small discs (i.e. 5 mm in diameter) and placed in test tubes containing 10 ml of distilled and deionized water. The tubes sealed 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 an electrical conductivity meter. Afterwards the samples were autoclaved at 121 ± 8 °C for 20 minutes to kill the tissues 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 calculated using the formula of Dionisio-Sese and Tobita:

$EL = EC1/EC2 \times 100$

Statistical Analysis

Data were subjected to an 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

Growth

Plant Height and Shoot Biomass

In the present investigation, mycorrhizal plants were taller than non-mycorrhizal control plants (Table 1). Maximum height was recorded for the plants treated with the dual combination of F + A and growing in soil with a 8 dS m^{-1} (medium) salinity level followed by the same treatment but with plants growing in soil with a 4 dS m^{-1} (low) salinity level. In this experiment, untreated control plants subjected to a 12 dS m^{-1} salinity level were the smallest. This was reflected in the fresh and dry weights of the shoots. Highest shoot weight was recorded for F + A plants grown in soil with a medium salinity level (8 dS m^{-1}) followed by the same treatment at the low salinity level (4 dS m^{-1}). The shoot fresh weight of the control plants was the same when grown in soil with both a 12 dS m^{-1} and 4 dS m^{-1} salinity level. Inoculation of plants with *F. mosseae* proved to be more beneficial for increasing shoot fresh weight at a high salinity (12 dS m^{-1}) than a low salinity level (4 dS m^{-1}). In the un-inoculated plants, dry shoot weight decreased with increase in salinity while among the treated plants, inoculation with F + A at a medium salinity level (8 dS m^{-1}) gave the best results, as shown in Table 1.

Root length and root biomass

Root length and fresh and dry weights were highest for plants subjected to a medium salinity level (8 dS m^{-1}) followed by those subjected to a low salinity level (4 dS m^{-1}) and treatment F + A was the best of all the treatments (Table 1).

Leaf Area

Maximum leaf area was recorded for plants grown at the highest salinity level (12 dS m^{-1}) followed by those grown at the lowest salinity level (4 dS m^{-1}) when they

Table 1 Effect of AM fungi on the growth of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Plant Height (cm)	Shoot weight (g)		Root Length (cm)	Root weight (g)	
			Fresh	Dry		Fresh	Dry
	C	31.58 ± 2.140^{f}	0.69 ± 0.019^{gh}	0.29 ± 0.006^{h}	04.74 ± 0.350^{g}	0.35 ± 0.121^{ef}	0.15 ± 0.044^{f}
4 dS m^{-1}	F	59.46 ± 1.611^{d}	6.37 ± 0.304^{d}	2.12 ± 0.246 ^{d}	12.76 ± 0.371^{d}	0.69 ± 0.114^{cd}	0.42 ± 0.277b^{cd}
	A	38.44 ± 1.849^{ef}	3.70 ± 0.248^{ef}	1.23 ± 0.027^{ef}	10.02 ± 0.370^{f}	0.55 ± 0.090^{de}	0.33 ± 0.167^{cde}
	F + A	78.10 ± 1.063^{b}	8.45 ± 0.043^{b}	2.90 ± 0.027^{c}	15.62 ± 0.238^{b}	0.82 ± 0.159^{bc}	0.45 ± 0.085^{bc}
	C	33.38 ± 1.583^{f}	0.84 ± 0.002^{g}	0.11 ± 0.003^{i}	05.28 ± 0.334^{g}	0.59 ± 0.231^{d}	0.18 ± 0.356^{ef}
8 dS m^{-1}	F	69.38 ± 1.344^{c}	7.92 ± 0.311^{c}	3.42 ± 0.340^{b}	14.16 ± 0.304^{c}	0.97 ± 0.246^{ab}	0.55 ± 0.211^{bf}
	A	37.70 ± 0.113^{ef}	4.43 ± 0.266^{e}	1.41 ± 0.024^{e}	11.24 ± 0.288^{e}	0.73 ± 0.090^{cd}	0.33 ± 0.137^{cde}
	F + A	86.40 ± 1.414^{a}	9.40 ± 0.218^{a}	4.14 ± 0.030^{a}	16.34 ± 0.397^{a}	1.07 ± 0.105^{a}	0.85 ± 0.071^{a}
	C	28.00 ± 1.046^{g}	0.69 ± 0.002^{gh}	0.06 ± 0.003^{i}	03.96 ± 0.336^{h}	0.18 ± 0.005^{f}	0.10 ± 0.036^{f}
12 dS m^{-1}	F	29.68 ± 1.948^{fg}	4.28 ± 0.173^{e}	1.16 ± 0.023^{ef}	11.68 ± 0.238^{e}	0.56 ± 0.245^{de}	0.15 ± 0.066^{f}
	A	37.86 ± 1.292^{ef}	2.16 ± 0.028^{f}	0.92 ± 0.003^{g}	09.30 ± 0.412^{f}	0.33 ± 0.156^{f}	0.12 ± 0.044^{f}
	F + A	40.04 ± 1.275^{e}	7.62 ± 0.023^{c}	1.58 ± 0.040^{e}	13.88 ± 0.303^{c}	0.64 ± 0.109^{cd}	0.27 ± 0.049^{def}
	L.S.D (P ≤ 0.05)	906.3620	163.5300	488.9330	803.3010	13.9140	14.4890
	ANOVA $F_{(11,24)}$	1.9185	0.2239	0.1697	0.4238	0.1984	0.1631
F values	Salinity (S)	1271.0250	649.4820	507.2890	189.3410	35.8350	30.9380
	Treatments (T)	1799.3160	5.3780	1233.8870	2809.0000	26.8620	23.6350
	S × T	341.9970	91.9030	110.3380	5.1210	0.1330	4.4330

Legend: F†: *Funneliformis mosseae*, A: *Acaulospora laevis*, ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

were treated with F + A (Table 5). Among the single inoculation treatments and control, the maximum leaf areas were recorded for plants grown in soil with a medium salinity level (8 dS m^{-1}).

Chlorophyll Content

Content of photosynthetic pigments recorded in AM treated plants grown in soils with different levels of salinity were higher than in un-inoculated control plants (Table 2). However, the highest total chlorophyll content was recorded for plants treated with a combination of *F. mosseae* and *A. laevis* followed by a single inoculation with *F. mosseae* and grown in soil with a salinity level of 8 dS m^{-1}. Further increase in salinity to 12 dS m^{-1} resulted in a decrease in chlorophyll content. Lowest concentration of photosynthetic pigments was recorded in untreated plants grown in soils with a 12 dS m^{-1} salinity level.

Protein Content

Regardless of mycorrhizal treatments, a salinity level of 8 dS m^{-1} resulted in remarkable increase in leaf protein content (Table 2). Further, increase in salinity to 12 dS m^{-1} had an adverse effect on leaf protein content. The protein content increased with increase in soil salinity up to 8 dS m^{-1}. Although, further increase in soil salinity resulted in a decrease in the content of protein in leaves; inoculation with *F. mosseae* and *A. laevis* increased protein content to the maximum level, followed by treatment with *A. laevis* alone, indicating its stimulatory effect on protein synthesis at all the levels of salinity used.

Mycorrhization

As evident from Table 2, the plants subjected to a medium salinity level (8 dS m^{-1}) had a greater number of AM spores and % root colonization as compared to higher and lower salinity levels. Beyond the medium salinity level, i.e. at (12 dS m^{-1}), there was a negative correlation between salinity and mycorrhization. Maximum mycorrhization was recorded in the combined treatment F + A of plants grown in soil with a salinity level of 8 dS m^{-1} followed by that recorded for plants treated only with *F. mosseae* , which indicates that *F. mosseae* is more tolerant of salinity than *A. laevis*.

Nodulation

In un-inoculated control plants, nodulation decreased with increase in salinity level but in plants treated with AM fungi, nodulation increased up to maximum level at a salinity level of 8 dS m^{-1} and was much lower at a salinity of 12 dS m^{-1} (Table 5).

Peroxidase Activity

Data presented in Table 3 reveals that peroxidase activity at a salinity level of 8 dS m^{-1} was less than at a salinity level of 4 dS m^{-1} due to more mycorrhization at a medium salinity level, which improved the water status of plants, which resulted in less osmotic stress. At salinity

Table 2 Effect of AM fungi on some physiological parameters and mycorrhization of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Chlorophyll content (mg/g FW)			Protein content (mg/g FW)	AM spore number / 10 g of soil	AM Root colonization (%)
		Chl a	Chl b	Total Chl			
	C	0.714 ± 0.004^{k}	0.358 ± 0.019^{j}	1.0718 ± 0.012^{k}	0.191 ± 0.003^{k}	06.2 ± 2.86^{g}	1.98 ± 2.81^{i}
4 dS m^{-1}	F	1.067 ± 0.004^{d}	0.910 ± 0.006^{d}	1.978 ± 0.014^{d}	0.302 ± 0.002^{g}	63.8 ± 3.56^{c}	34.4 ± 4.72^{e}
	A	0.934 ± 0.003^{g}	0.681 ± 0.010^{g}	1.616 ± 0.014^{h}	0.466 ± 0.002^{d}	51.2 ± 3.70^{e}	29.9 ± 3.49^{f}
	F + A	1.264 ± 0.056^{c}	1.040 ± 0.005^{c}	2.304 ± 0.011^{c}	0.581 ± 0.004^{b}	72.6 ± 4.03^{c}	53.2 ± 2.86^{b}
	C	0.827 ± 0.005^{i}	0.411 ± 0.007^{h}	1.239 ± 0.023^{i}	0.206 ± 0.003^{j}	14.8 ± 3.83^{f}	03.0 ± 2.23^{i}
8 dS m^{-1}	F	1.627 ± 0.007^{b}	1.317 ± 0.006^{b}	2.944 ± 0.010^{b}	0.329 ± 0.002^{f}	77.6 ± 3.64^{a}	48.6 ± 3.49^{c}
	A	0.983 ± 0.006^{e}	0.921 ± 0.008^{d}	1.905 ± 0.013^{e}	0.574 ± 0.002^{c}	65.0 ± 4.00^{c}	37.6 ± 3.97^{de}
	F + A	1.753 ± 0.006^{a}	1.374 ± 0.007^{a}	3.126 ± 0.011^{a}	0.605 ± 0.006^{a}	78.6 ± 4.27^{a}	63.4 ± 3.84^{a}
	C	0.676 ± 0.004^{l}	0.356 ± 0.005^{j}	1.031 ± 0.010^{l}	0.179 ± 0.002^{l}	00.0 ± 0.00^{h}	00.0 ± 0.00^{i}
12 dS m^{-1}	F	0.880 ± 0.005^{h}	0.785 ± 0.011^{f}	1.665 ± 0.011^{g}	0.224 ± 0.003^{i}	56.4 ± 4.27^{d}	24.4 ± 3.20^{g}
	A	0.778 ± 0.006^{j}	0.376 ± 0.011^{i}	1.154 ± 0.004^{j}	0.286 ± 0.002^{h}	47.8 ± 3.34^{e}	16.8 ± 3.03^{h}
	F + A	0.957 ± 0.004^{f}	0.868 ± 0.012^{e}	1.826 ± 0.015^{f}	0.408 ± 0.007^{e}	59.2 ± 3.49^{d}	40.2 ± 4.32^{d}
	L.S.D ($P \leq 0.05$)	0.007	0.0138	0.0181	0.003	4.8759	4.596
	ANOVA $F_{(11,24)}$	185.540	625.5300	135.3400	176.240	291.9640	192.735
F values	Salinity (S)	36225.831	8310.8940	22637.2480	17434.234	128.7240	148.113
	Treatments (T)	33582.465	15861.2330	30146.8350	48110.463	979.4900	583.236
	S × T	5148.889	766.3310	2183.4160	2442.499	2.6230	12.358

Legend: F†: *Funneliformis mosseae*, A: *Acaulospora laevis*, ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, FW: fresh weight, values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test.

levels greater than 8 dS m^{-1} there was a marked increase in peroxidase activity associated with the stress induced damage. Single inoculation with *A. laevis* was less effective than a single inoculation with *F. mosseae* at all the salinity levels. Of the different treatments, the F + A combination resulted in the highest peroxidase activity at low and medium salinity levels, but inoculation with *F. mosseae* alone resulted in the maximum activity being recorded at the highest salinity level used.

Phosphatase Activity

Both in mycorrhizal and non-mycorrhizal control plants the maximum values of acid and alkaline phosphatase activity was recorded at a salinity level of 8 dS m^{-1}. At a salinity level of 12 dS m^{-1} the activity of both these enzymes decreased, however, the dual treatment F + A resulted in a greater increase in enzyme activity than the single treatment with either of the mycorrhizal fungi at all salinity levels (Table 3).

Electrolyte Leakage

In this study, increase in salt concentration in the soil above a salinity level of 8 dS m^{-1} resulted in a decrease in membrane stability. The double inoculation treatment with F + A at all the levels of salinity improved membrane stability, followed by a single treatment with *F. mosseae*. Less electrolyte leakage was recorded from mycorrhizal plants than non-mycorrhizal control plants at all the salinity levels used (Table 3).

Nutrient uptake

Phosphorus, Potassium and Nitrogen

In the present investigation, highest root and shoot phosphorus (P) content was recorded at the medium salinity level i.e. 8 dS m^{-1} in the double inoculation treatment F + A (Table 4). The combination of *F. mosseae* and *A. laevis* at all the salinity levels resulted in better root and shoot phosphorus contents than in the controls. Potassium (K) and nitrogen (N) uptake decreased with increase in salt concentration in the soil (Table 4). Mycorrhizal treatment increased potassium and nitrogen content in roots and shoots regardless of salt stress levels. Among the treated plants, treatment with *A. laevis* resulted in the least root and shoot potassium content at all salinity levels. Root potassium content was greater than that recorded in the shoots. Maximum shoot and root nitrogen uptake was recorded at 4 dS m^{-1} salinity level in treatment F + A. Shoots of *P. mungo* accumulated more nitrogen than the roots at all of the different levels of salinity used.

Yield

Since *P. mungo* is cultivated for its seeds, the effect of mycorrhizal inoculation on number and weight of pods under saline conditions is important. Mycorrhizal inoculation significantly increased yield of *P. mungo* compared to un-inoculated control plants at all the different levels of salinity used. At the 8 dS m^{-1} salinity level, maximum yield in terms of number and weight of pods per plant was recorded in the F + A treatment, followed that re-

Table 3 Effect of AM fungi on some biochemical parameters of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Phosphatase activity (IU/g FW)		Peroxidase activity (mg protein / 10 min)	Electrolyte leakage (%)
		Acidic	Alkaline		
	C	0.030 ± 0.006^{c}	0.076 ± 0.008^{h}	0.242 ± 0.011^{j}	39.74 ± 0.476^{b}
4 dS m^{-1}	F	0.138 ± 0.010^{abc}	0.295 ± 0.010^{c}	0.599 ± 0.004^{d}	33.36 ± 0.192^{g}
	A	0.066 ± 0.006^{bc}	0.158 ± 0.007^{e}	0.486 ± 0.013^{f}	36.98 ± 0.503^{d}
	F + A	0.194 ± 0.008^{ab}	0.321 ± 0.005^{b}	0.812 ± 0.002^{b}	29.74 ± 0.252^{h}
	C	0.038 ± 0.007^{c}	0.082 ± 0.006^{gh}	0.161 ± 0.020^{k}	35.34 ± 0.315^{e}
8 dS m^{-1}	F	0.195 ± 0.007^{ab}	0.328 ± 0.008^{b}	0.315 ± 0.009^{h}	28.13 ± 0.208^{i}
	A	0.125 ± 0.006^{abc}	0.258 ± 0.007^{d}	0.243 ± 0.011^{j}	29.85 ± 0.194^{h}
	F + A	0.240 ± 0.008^{a}	0.387 ± 0.006^{a}	0.405 ± 0.006^{g}	25.76 ± 0.742^{j}
	C	0.024 ± 0.005^{c}	0.045 ± 0.006^{i}	0.297 ± 0.018^{i}	42.82 ± 1.550^{a}
12 dS m^{-1}	F	0.075 ± 0.006^{bc}	0.116 ± 0.008^{f}	0.856 ± 0.001^{a}	38.73 ± 0.396^{c}
	A	0.043 ± 0.008^{c}	0.090 ± 0.005^{g}	0.520 ± 0.008^{e}	40.29 ± 0.277^{b}
	F + A	0.153 ± 0.007^{abc}	0.288 ± 0.006^{c}	0.764 ± 0.004^{c}	34.62 ± 0.251^{f}
	L.S.D ($P \leq 0.05$)	0.1231	0.0101	0.015	0.733
	ANOVA $F_{(11,24)}$	3.7830	132.6300	233.530	499.604
F values	Salinity (S)	1.4230	1530.1650	4828.379	1485.808
	Parameter (T)	11.7500	3459.0210	4474.442	806.357
	S × T	0.5850	191.0380	433.999	17.493

Legend: F†: *Funneliformis mosseae*, A: *Acaulospora laevis*, ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, FW: Fresh Weight, values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test.

corded for plants inoculated with same combination at the 4 dS m^{-1} salinity level (Table 5). Single treatment with *F. mosseae* also resulted in an increase in yield at all the different salinity levels compared to *A. laevis*. Soil salinity levels of 12 dS m^{-1} had a significant adverse effect on yield as the number and weight of pods were lower.

Discussion

Our results indicate that plant height, root length and biomass increased with increase in soil salinity up to 8 dS m^{-1}, but were all less at the 12 dS m^{-1}salinity level. In *Vicia faba* increase in plant height at medium and low sa-

Table 4 Effect of AM fungi on nutrient uptake of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Phosphorus content (%)		Nitrogen content (%)		Potassium content (%)	
		Root	Shoot	Root	Shoot	Root	Shoot
	C	0.676 ± 0.011^{i}	0.449 ± 0.008^{j}	0.301 ± 0.0030^{def}	0.444 ± 0.0348^{h}	0.974 ± 0.038^{h}	0.708 ± O.0587^{g}
4 dS m^{-1}	F	1.269 ± 0.005^{de}	0.694 ± 0.007^{f}	1.212 ± 0.4022^{b}	1.678 ± 0.018^{b}	1.906 ± 0.0288^{c}	1.035 ± 0.0587^{c}
	A	1.133 ± 0.008^{fg}	0.643 ± 0.003^{g}	0.616 ± 0.0320^{c}	1.300 ± 0.0029^{c}	1.660 ± 0.0223^{e}	0.972 ± 0.0192^{d}
	F + A	2.181 ± 0.007^{b}	1.257 ± 0.005^{b}	1.430 ± 0.0246^{a}	1.931 ± 0.00273^{a}	2.324 ± 0.0207^{a}	1.232 ± 0.1041^{a}
	C	0.806 ± 0.007^{h}	0.507 ± 0.007^{i}	0.206 ± 0.0288^{fg}	0.298 ± 0.0225^{i}	0.828 ± 0.0238^{i}	0.576 ± 0.0240^{h}
8 dS m^{-1}	F	1.337 ± 0.008^{d}	0.846 ± 0.006^{d}	0.720 ± 0.0254^{c}	0.820 ± 0.0269^{e}	1.748 ± 0.0414^{d}	0.920 ± 0.0316^{de}
	A	1.295 ± 0.006^{de}	0.713 ± 0.006^{e}	0.448 ± 0.0319^{d}	0.670 ± 0.0247^{f}	1.538 ± 0.0319^{f}	0.840 ± 0.0314^{f}
	F + A	2.431 ± 0.286^{a}	1.400 ± 0.002^{a}	0.744 ± 0.0384^{c}	1.017 ± 0.0599^{d}	2.026 ± 0.1040^{b}	1.124 ± 0.0288^{b}
	C	0.623 ± 0.008^{i}	0.395 ± 0.005^{k}	0.124 ± 0.0230^{g}	0.148 ± 0.0258^{j}	0.644 ± 0.6270^{j}	0.432 ± 0.0286^{i}
12 dS m^{-1}	F	1.187 ± 0.008^{ef}	0.630 ± 0.011^{h}	0.389 ± 0.0320^{de}	0.524 ± 0.0303^{g}	1.636 ± 0.0270^{e}	0.898 ± 0.0238^{e}
	A	1.063 ± 0.007^{g}	0.513 ± 0.006^{i}	0.228 ± 0.0356^{efg}	0.4722 ± 0.0030^{h}	1.220 ± 0.6254^{g}	0.724 ± 0.0304^{g}
	F + A	1.858 ± 0.017^{c}	0.975 ± 0.006^{c}	0.684 ± 0.0336^{c}	0.791 ± 0.0028^{e}	1.946 ± 0.0201^{c}	1.044 ± 0.0384^{c}
	L.S.D (P ≤ 0.05)	0.113	0.009	0.152	0.224	0.055	0.052
	ANOVA $F_{(11,24)}$	233.006	103.540	56.818	2112.724	795.512	133.440
F values	Salinity (S)	58.545	5894.968	109.260	5500.719	372.964	105.774
	Treatments(T)	800.891	32949.820	116.998	3412.981	2645.806	410.992
	S × T	7.218	532.687	10.913	333.264	11.217	3.894

Legend: F†: Funneliformis mosseae, A: Acaulospora laevis, ‡: each value is the mean of five replicates, ± : standard deviation AM: Arbuscular mycorrhizae, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

Table 5 Effect of AM fungi on leaf area, nodules and yield of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Leaf Area	No of nodules (per pot)	Yield (per plant)	
				No. of pods	Weight of pods (g)
	C	08.22 ± 1.522^{fg}	07.8 ± 2.387^{e}	03.0 ± 1.581^{d}	1.294 ± 0.343^{fg}
4 dS m^{-1}	F	18.31 ± 1.598^{c}	12.8 ± 2.432^{cd}	05.6 ± 2.302^{abcd}	1.916 ± 0.379^{de}
	A	13.76 ± 1.842^{d}	10.2 ± 1.923^{de}	03.6 ± 2.073^{cd}	1.556 ± 0.438^{ef}
	F + A	25.50 ± 2.214^{b}	17.0 ± 2.915^{b}	07.8 ± 1.923^{ab}	3.040 ± 0.436^{b}
	C	10.34 ± 2.226^{f}	03.8 ± 2.387^{fg}	04.0 ± 2.549^{cd}	1.510 ± 0.395^{ef}
8 dS m^{-1}	F	20.78 ± 2.554^{c}	15.6 ± 3.209^{bc}	06.6 ± 2.408^{abc}	2.390 ± 0.382^{cd}
	A	15.45 ± 2.019^{d}	13.6 ± 3.209^{bcd}	04.6 ± 2.701^{bcd}	1.868 ± 0.366^{e}
	F + A	23.47 ± 1.917^{b}	21.0 ± 2.738^{a}	09.0 ± 3.162^{a}	3.772 ± 0.346^{a}
	C	06.76 ± 2.214^{g}	02.2 ± 1.788^{g}	02.6 ± 2.408^{d}	0.858 ± 0.277^{g}
12 dS m^{-1}	F	13.09 ± 1.770^{de}	09.0 ± 3.535^{e}	05.0 ± 3.162^{bcd}	1.228 ± 0.445^{fg}
	A	10.86 ± 2.245^{ef}	06.8 ± 2.387^{ef}	02.8 ± 1.303^{d}	1.034 ± 0.201^{fg}
	F + A	29.26 ± 2.414^{a}	14.2 ± 2.364^{bc}	05.2 ± 3.492^{bcd}	2.600 ± 0.351^{bc}
	L.S.D (P ≤ 0.05)	2.7441	3.5661	3.3833	0.503
	ANOVA $F_{(11,24)}$	63.6630	21.8740	3.2330	28.063
F values	Salinity (S)	7.8950	22.3980	3.6940	33.374
	Treatments(T)	206.5570	60.0710	8.7870	79.060
	S ×T	10.8060	2.6010	0.3030	0.793

Legend: G†: *Funneliformis mosseae*, A: *Acaulospora laevis* ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

linity level is recorded by Amira and Qados (2010) while in ornamental Purslane, Alam et al. (2015) records an increase in fresh and dry shoot weight at a salinity level of 8 dS m^{-1}. Our results confirm the findings of Pessarakli et al. (2015) who note an increase in root biomass of the *Distichlis spicata* at medium salinity levels compared to that recorded in high and low salinity level treatments. Under salinity stress, plant growth and biomass is limited by a lower availability of nutrients and the energy expenditure necessary to nullify the toxic effects of NaCl and other salts. Mycorrhization increases growth and biomass of the host plant due to AM mediated enhanced nutrient acquisition, especially a better P nutrition (Sharifi et al. 2007; Colla et al. 2008). Salinity stress lowered the concentration of photosynthetic pigments due to the toxic effects of salt on nitrogen and magnesium absorption, which are vital constituents of chlorophyll (Kaya et al. 2009). Another reason could be the increased activity of chlorophyllase due to salinity stress, which resulted in the destruction of photosynthetic pigments. The greater chlorophyll content of plants inoculated with mycorrhizal fungi could be due to the increased uptake of magnesium and nitrogen by AM hyphae (Abdel Latef and Chaoxing 2011) or an increase in the activity of enzymes required for the synthesis of chlorophyll (Murkute et al. 2006). Due to the higher concentration of photosynthetic pigments, photosynthesis in mycorrhizal plants subjected to salinity stress is higher than in un-inoculated stressed plants (Abdel Latef and Chaoxing 2011), which resulted in increased growth.

Salinity stress up to salinity level 8 dS m^{-1} resulted in an increase in leaf protein content. The reason may be due to an accumulation of salt stress proteins, which help in establishing a proper cellular ion and osmotic homeostasis (Amini et al. 2007; Garcia et al. 2008). These proteins act as nitrogen reserves for plants, which can be utilized later. Further, the decrease in leaf protein content with increase in salinity up to 12 dS m^{-1} is due to a decrease in uptake and utilization of nitrogen, which is an essential element for protein synthesis (Kusano et al. 2011). Mycorrhizal inoculation of plants improved leaf protein content regardless of the salinity. Our findings are in agreement with those of Datta and Kulkarni (2014) who also report an increase in protein content in mycorrhizal plants subjected to salinity stress.

A high soil salinity may not reduce mycorrhization, as increased mycorrhization under high saline conditions is reported by Aliasgharzadeh et al. (2001) and Yamato et al. (2008). The upper limit of the salinity tolerance of the AMF used in the experiment was 12 dS m^{-1}, the level at which spore number and mycorrhization were drastically reduced. Decreased mycorrhization in *P. mungo* plants at salinities above 8 dS m^{-1} could be due to the high pH associated with high salt concentrations inhibiting the germination of fungal spores. Even though high salinity caused a decrease in mycorrhization subsequent mycorrhizal dependency increased, which indicates that the symbiosis between roots and AM fungi strengthens once the association is established, which indicates the importance of this symbiosis for plant production under saline conditions (Rabie and Almadini 2005). There was a direct correlation between mycorrhization and nodulation in the present experiment indicating the stimulatory role of mycorrhizae on nodulation. In this experiment, mycorrhizal plants growing at all of the salinity levels used were less affected in terms of nodulation parameters than the control plants because the root exudation pattern was modified both quantitatively and qualitatively by AMF, which results in an increase in nodulation (Garg and Manchanda 2009).

Salinity stress in plants results in an increase in the production of ROS (Reactive Oxygen Species) and hence, oxidative stress, which has toxic effects on different biomolecules. As different antioxidant enzymes nullify the effect of damage induced by ROS, it is possible that this accounts for the high activity of peroxidase recorded at the highest salinity level i.e. 12 dS m^{-1}. Increase in antioxidant enzyme activity with increase in salinity is confirmed by Hashem et al. (2015). At all the salinity levels used, there was a higher peroxidase activity in the treatment inoculated with mycorrhizal fungi, which support the findings of Alqarawi et al. (2014) and Abd Allah et al. (2015). Estimates of phosphatase activity in plants help to assess phosphorus metabolism in mycorrhizal plants as this enzyme is present in the vacuoles of AM hyphae (Tisserent et al. 1993). Mycorrhizal inoculation positively affected phosphatase activity. Our results confirm the findings of Peng et al. (2011) who report an increase in alkaline phosphatase activity in mycorrhizal *Astragallus sinicus* under saline conditions. The major organelle in plants adversely affected by soil salinity stress is the cell membrane, as peroxidation of lipids causes the solutes to leak through the membrane decreasing its stability (Kaya et al. 2009). Mycorrhizal inoculation of plants improved membrane stability due to higher antioxidant activity and phosphorus uptake. The decrease in electrolyte leakage in mycorrhizal plants recorded in this experiment confirms the findings of Abd Allah et al. (2015).

Phosphorus uptake was negatively affected at a soil salinity level of 12 dS m^{-1} because of the precipitation of phosphate (H_2PO_4) ions by calcium, magnesium and zinc ions, which adversely affects the uptake of this element (Marshner 1994). Mycorrhizal fungi are able to solubilize the precipitated phosphorus, thus increasing the availability of this immobile element under saline conditions (Srividya et al. 2010). Another reason for the improved P uptake by mycorrhizal plants is the greater soil volume penetrated by their extra radical mycelium, which extends beyond nutrient depleted zones in the soil. In the present experiment, mycorrhizal inoculation of plants also improved potassium uptake under different salinity levels. Our results confirm the findings of Patel et al. (2010) and Abd Allah et al. (2015). The decrease in the uptake of potassium, with increase in salinity recorded in this experiment is due to a high concentration of so-

dium within the root zone, which has an antagonistic effect. The elevated concentration of sodium and chloride ions interfere with potassium ion channels in the plasma membrane of root cells causing a decrease in the uptake of this nutrient. A possible reason for the increased K uptake by mycorrhizal plants is their ability to store sodium in vacuoles of root cells as well as intra-radical hyphae (Cantrell and Linderman 2001). Increase in the tolerance of mycorrhizal plants to saline conditions may be attributed to their increased biomass due to enhanced nutrient uptake, which results in the dilution of the toxic effects of ions (Campanelli et al. 2012). Like potassium, nitrogen content in the plants also decreased with increase in soil salinity. Increased nitrogen uptake by mycorrhizal plants could be attributed to the ability of the extra-radical mycelium of mycorrhizal fungi to absorb nitrate and ammonium and translocate nitrogen in the form of arginine (Guether et al. 2009). Another reason could be the AM mediated increase in activity of urease in the soil, which may help in breaking down urea and in the liberation of NH_3^+ or NH_4^+ ions (Zhao et al. 2010). Higher nitrogen uptake by the mycorrhizal plants helps them to maintain a greater concentration of photosynthetic pigments, proteins and other non-protein amino acids like proline, which are important in osmotic adjustment as osmoprotectants (Evelin et al. 2009).

Maximum yield at the medium salinity level was recorded in this experiment. Highest mycorrhization at the 8 dS m^{-1} salinity level helped the plants to cope up with the deleterious effects of the salinity, as it resulted in an increase in growth, P uptake, phosphatase activity, chlorophyll and protein content and decrease in electrolyte leakage. A 12 dS m^{-1} soil salinity level had an adverse effect on yield, as the number and weight of pods produced was significantly lower. The positive effect of mycorrhizal inoculations on yield under saline conditions is confirmed by the results of Hajiboland et al. (2010).

Conclusion

With increase in soil salinity levels up to 12 dS m^{-1} electrolyte leakage and peroxidase activity increased, whereas, photosynthetic pigments, nutrient uptake, leaf protein content, phosphatase activity, mycorrhization, nodulation and all the morphological parameters measured decreased. Although, mycorrhization decreased at high salinity levels, the AM treatment positively affected photosynthetic pigments, nutrient uptake, leaf protein content, phosphatase activity, mycorrhization, nodulation, peroxidase activity and growth and decreased membrane damage. The results of the present experiment indicate that the growing of *P. mungo* at a 8 dS m^{-1} salinity level after inoculation with a combination of F + A should be recommended. The cultivation of this pulse legume, however, should be discouraged if the salinity level of the soil is nearly 12 dS m^{-1} or above.

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