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Influence of arbuscular mycorrhizal fungi and different salinity levels on growth enhancement and nutrient uptake of *Gossypium arboreum* L.

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ABSTRACT

A pot experiment was conducted to see the effect of Arbuscular Mycorrhizal (AM) fungi, i.e., *Glomus mosseae* and *Acaulospora laevis* on cotton (*Gossypium arboreum* L.) in the presence of different salinity levels, i.e., 4 dSm^{-1}, 8 dSm^{-1} and 12 dSm^{-1} with five replicates resulted in effective plant height, shoot and root biomass, root length, leaf area, root colonization, AM spore number, stomatal conductance, chlorophyll content, phosphorus content, nitrogen content, potassium content, sodium content, fibre yield and acidic and alkaline phosphatase activity. Under saline conditions, mycorrhizal inoculation significantly increased growth parameters as well as nutrient uptake of cotton plants over control. All growth parameters were found to be highest in dual combination of *Glomus mosseae* + *Acaulospora laevis* at 4 dSm^{-1} salinity level whereas sodium content was found to be highest in dual combination of *Glomus mosseae* + *Acaulospora laevis* at 8 dSm^{-1} salinity level. Overall results showed that AM colonization improves host plant mineral concentration and thereby increases the growth, yield and nutrient uptake of cotton plants by ameliorating the harmful effect of salinity stress.

Keywords: *Glomus mosseae, Acaulospora laevis*, *Gossypium arboreum*, growth, mineral uptake, salinity stress

INTRODUCTION

Soil salinity is a serious problem increasing steadily in many parts of the world, particularly in arid and semiarid regions (Giri *et al*., 2003; Al-Karaki, 2006). Saline soil occupy 7% of the total earth's land surface (Ruiz-Lozano *et al*., 2001) and is expected to have devastating and deleterious global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang *et al.*, 2003; Tavakkoli *et al*., 2011). The drastic, antagonistic and harmful impacts of salinity on plant growth and metabolism are associated with (1) low osmotic potential of soil solution reducing the amount of water available to the plant (2) nutritional imbalance (Adiku *et al*., 2001) (3) specific ion effect or toxicity of excessive Na^{+} and Cl^{2-} ions towards the cell the toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of photosynthesis, respiration and protein synthesis (Feng *et al*., 2002), and (4) a combination of all these factors (Turan *et al.*, 2010).

Salinity may directly or indirectly inhibit cell division and enlargement and finally the growth, productivity and yield of the whole plant. To counteract these problems, many strategies have been proposed to overcome salt detrimental effects such as searching for new salt-tolerant crops, genetically engineered plants, use of plant growth regulators, osmoprotectants and inorganic nutrients, removing excessive salt accumulation in groundwater and desalinizing water for irrigation (Flowers, 2004; Ashraf *et al*., 2008). Although these strategies appear efficient, yet they are costly and out of reach for developing countries that are the most affected. In this respect, biological processes such as Arbuscular Mycorrhizal application to alleviate salt stress would be a better option resulting in better increment, vigorous growth and consequently higher yield and biomass of plants. AM fungi are ubiquitous among a wide array of soil microorganisms inhabiting the rhizosphere. Symbiotic association of a plant with AM fungi makes it able to access immobile nutrients in nutrient-poor soils (Marschner and Dell, 1994). AMF have been shown to promote salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition (Al-Karaki and Al-Raddad, 1997), producing plant growth hormones, improving rhizospheric and soil conditions (Lindermann, 1994), altering the physiological and biochemical properties of the host and defending roots against soil-borne pathogens (Dehne, 1982).

Gossypium arboreum L. commonly known as desi cotton or tree cotton is under cultivation from time immemorial and is an important cash crop worldwide. *G. arboreum* as the perennial shrub grows up to a height of 25 feet. The plant is tropical in nature and it grows best in warm temperature. The plant is annually growing shrub native to tropical and subtropical regions around the world including America, Africa and India. Cotton plant is indispensable for its valuable fibre-yielding properties and also finds speciality applications in medical and hygienic uses. Most notably, the fibre is used to manufacture hydrophile cotton (cotton wool) compress, guaze bandages, tampons or sanitary towels, and cotton swabs. Arboreum cottons have wide adaptability and are relatively tolerant to biotic and abiotic stresses (Singh and Mohan, 2005). Cotton cultivation has always been a challenge all over the world with regards to insect pest infestation (Chandra and Sreenivassan, 2011).

To meet the increasing demand, the farmers apply huge quantity of chemical fertilizers with an aim to increase the production and without knowing its detrimental effects on soil property which leads to accumulation of toxic salts in the soil and hampers plant growth. Considering the beneficial effect of AMF on other crops, more attention should be paid towards the combining of appropriate mycorrhizal fungi to get good yield of cotton plant under salinity stress.

Keeping the above in view, trials were carried out in polyhouse to determine the beneficial effects of AM fungi on growth, biomass, mineral nutrition and yield of cotton

plants in conferring salinity tolerance.

MATERIALS AND METHODS

Physico-chemical characteristics of experimental soil

The soil used in the present investigation have the pH - 6.8, sand - 64.2%, silt - 21.81%, clay - 3.90%, phosphorus content - 7.30 Kg/acre, total nitrogen content - 0.042%, organic carbon - 0.40%, potassium - 88Kg/acre, sulphur - 14.80 ppm and electrical conductivity (EC) of 0.25 dSm^{-1}.

Collection of soil sample

Composite soil sample from rhizospheric soil of *G. arboreum* was collected. It was done by digging out a small amount of soil close to the plant roots up to the depth of 1530 cm and kept in sterilized polythene bags at 10 ºC for further processing.

Source of AM spores and their isolation

Two native predominant AM fungi, *Glomus mosseae* (T. H. Nicolson & Gerd.), C. Walker & A. Schüßler and *Acaulospora laevis* Gerd & Trappe were isolated from the rhizosphere of cotton plants by the wet sieving and decanting technique of Gerdemann and Nicolson (1963). In this technique, 50 g of soil was soaked in 500 ml of water for 24 h. The supernatant was then passed through a gradient of sieves with pore size ranging from 150 to 45 µm arranged one above the other in an ascending order. Each sieve was then washed in water and filtered through Whatmann No. 1 filter paper. This filter paper was then observed under stereobinocular microscope for the presence of various kinds of spores and mounted on polyvinyl lactic acid (PVLA) for further studies.

Quantification and identification of AM spores

Quantification was done by Gaur and Adholeya "Grid Line Intersect Method" (1994). Spores were counted under stereobinocular microscope by using a counter to record their morphological features, namely, colour, size, shape, wall structure, surface ornamentation, nature and size of subtending hyphae, bulbous suspensor, and the number and arrangement of spores in sporocarp. These AM spores (*G. mosseae* and *A. laevis*) were identified by using the identification manual of Walker (1983); Schenck and Perez (1990); Morton and Benny (1990) and Aggarwal *et al*. (2012).

Mycorrhizal root colonization

Roots were washed from the soil, blotted dry for determination of root fresh and dry weight, P content and mycorrhizal root colonization. Mycorrhizal root colonization was done by rapid clearing and staining method (Phillips and Hayman, 1970). The percentage of AM root colonization was determined as:

$$\text{Percentage root colonization} = \frac{\text{Number of root segments colonized}}{\text{Number of root segments studied}} \times 100$$

Quantification was done by new objective measure of colonization given by McGonigle *et al*. (1990).

Mass Production and maintenance of AM spores

Dominant AM spores *G. mosseae* and *A. laevis* were mass multiplied by using wheat as a host plant (Mangla *et al.,* 2010a).

Preparation of pot mixture

Top soil (0-3 cm) from experimental site was first collected, air dried, pulverized and then sieved through 2 mm sieve and mixed with the sand to form sand: soil ratio 1:3. This soil was sterilized in autoclave at 121 ºC for two consecutive days at 15 lb pressure for 15 minutes. There were no AM mycelia and spores in the soil. Three kg sterilized soil was put into the earthen pots (25 cm × 25 cm). The starter inoculum of each selected dominant AM fungus was prepared by "Funnel Technique" of Menge and Timmer (1982) using maize as a host plant. Ten per cent inoculum having AM spores and chopped colonized AM root pieces were added to each pot. Control pots were filled with sterilized sand: soil (1:3) only. For each fungus, around 870-890 AM spores and colonized root fragments of test plants with an infection level of around 94% were used as inoculum. The inoculum was applied as a band below the top soil layer in the pot. Surface-sterilized seeds of cotton plant were grown in earthen pots under polyhouse conditions having temperature (25±5 ºC) and humidity (50-70%). Fifteen days old plants were treated with different saline concentrations, i.e. 4, 8, and 12 dSm^{-1} EC (sodium chloride, calcium chloride and sodium sulphate, 7:2:1 w/v as per Richards (1954). The experiments were carried out alone and in combined inoculations as per following details. In control, no inoculum was added.

1- Uninoculated control (having no AM fungi) (C)

2- Inoculated with *G. mosseae* (G)

3- Inoculated with *A. laevis* (A)

4- Inoculated with both *G. mosseae* and *A. laevis* (GA)

Five replicates of each treatment were taken.

Harvest and analysis

Plants were harvested after 120 days and then plant height as well as root length was measured with the help of a scale. For root and shoot fresh and dry weight, roots and shoots were harvested after 120 days, weighed for their fresh weight and then, oven dried at 70 ºC for dry weight. Leaf area was measured by using Leaf Area Meter 211 (Systronics Ltd., Ahmedabad, India) and stomatal conductance was measured by using porometer (AP4-Delta T devices, Cambridge, UK). Amount of chlorophyll a, chlorophyll b, and total chlorophyll was estimated by method of Arnon (1949). Estimation of phosphorus was done by "Vanadomolybdo phosphoric yellow colour method" (Jackson, 1973). Fresh roots were used for extraction of acidic and alkaline phosphatases, assayed by using *p*-nitrophenyl phosphate as the substrate, which is hydrolyzed by the enzyme to *p*-nitrophenol. For this procedure, 1 g of fresh, washed roots were homogenized in 5 ml of ice-cold sodium acetate buffer (0.05 M with pH 4.8) for acid phosphatase and sodium carbonate bicarbonate buffer (0.05 M with pH 10) for alkaline phosphatase activity using a pre-chilled pestle and mortar. The resulting homogenate was centrifuged at 10,000 revolutions min^{-1} for 15 min and the supernatant thus obtained was referred to as crude enzyme extract and was

used for the assay of acid phosphatase activity, measured in international units per gram of fresh weight ($IUg^{-1}FW$; Tabatabai and Bremner, 1969). Fibre yield is the weight of fibre per plant in grams. When the capsule was mature, it opened into four parts showing the cotton fibre. The cotton fibre was removed manually and then weighed with the help of a weighing balance (Citizen balance with model number CY 104 having maximum weighing capacity 120 g and minimum weighing capacity 10 mg). Total nitrogen was calculated by Kjeldahl method (Kelplus nitrogen estimation system, supra-LX). Half gram digested plant sample was distilled using micro-Kjeldahl unit, and the liberated ammonia was trapped in boric acid containing mixed indicator and titrated against 0.01 N H_2SO_4. The nitrogen content in the shoot and root was expressed in per cent. Analysis of sodium and potassium content was done by inductively coupled plasma analyzer- Mass spectrometry (ICP-MS).

Statistical analysis

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

RESULTS

Plant height

AM colonization significantly enhanced the growth of cotton plant under different salinity levels. **Table 1** showed a gradual reduction in plant height with increased salinity levels. Highest increment in plant height was observed in dual inoculation of GA (134.5±2.40 cm) at 4 dSm^{-1} salinity level followed by single inoculation of *G. mosseae* (122.9±2.10 cm) at same salinity level. At 8 dSm^{-1} salinity level, maximum results were found in plants inoculated with dual combination of GA *(*114.8±2.26 *cm).*

Fresh and dry shoot and root weight

It is envisaged from **Table 1** that a significant increment in shoot (39.90±1.23, 4.18±0.196 g) and root (3.872±0.025, 0.986±0.003 g) biomass was observed in the dual inoculation of GA at 4 dSm^{-1} salinity level. Single inoculation of *G. mosseae* at same salinity level also showed better results for both shoot (34.50±1.47, 3.84±0.063 g) and root (3.475±0.191, 0.840±0.009 g) biomass.

Root length

Results of present investigation showed that total root length of mycorrhizal inoculated plants decreased as salinity level increased. Maximum root length was observed in plants treated with dual combination of GA (21.08±0.86 cm) at 4 dSm^{-1} salinity level followed by the same combination (19.58±0.87 cm) at 8 dSm^{-1} salinity level. Similarly, at 12 dSm^{-1} salinity level, root length was found to be more effective in the same treatment i.e. GA in comparison to control ones.

Root colonization and AM spore number

Salinity not only affects the host plant but also hamper the root colonization capacity, spore germination and growth of hyphae of the AM fungi. AM inoculated cotton plants showed higher percentage of root colonization and AM spore number at various salinity levels. Highest per cent mycorrhizal root colonization (85.60±1.38 %) and AM spore number (93.0±4.58) were found at lowest salinity level i.e. 4 dSm^{-1} in dual inoculation of GA followed by single inoculation of *G. mosseae* at same salinity level. Dual inoculation of GA was found to be more effective in comparison to control at both 8 dSm^{-1} (57.37±1.68 %) (62.4±4.03) and 12 dSm^{-1} (50.84±2.40 %) (40.6±5.81) salinity levels.

Leaf area and stomatal conductance

Leaf area and stomatal conductance were found to be higher in all AM inoculated plants as compared to control. Maximum increment in leaf area was found in double inoculation of GA *(*95.50±1.41 sq. cm) followed by the single inoculation of *G. mosseae* (92.12±2.25 sq. cm) at lowest i.e. 4 dSm^{-1} salinity level. Stomatal conductance was also found maximum in dual combination of GA for both lower and upper epidermis in both morning (311.80±4.81, 50.62±1.50 $mmol^{-2}s^{-2}$) and evening time (217.80±4.32, 33.24±0.81 $mmol^{-2}s^{-2}$) in comparison to control at 4 dSm^{-1} salinity level. Similarly, at 8 and 12 dSm^{-1} salinity levels, both leaf area and stomatal conductance enhanced in dual combination of GA in comparison to control.

Chlorophyll content

Table 1. Efficacy of AM fungi on various morphological parameters and mycorrhization of *Gossypium arboreum* under different levels of salinity stress after 120 days

Salinity level (DS/m)	Parameters? Treatments?	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Root length (cm)	Leaf area (cm^2)	AM Spore number/10 g of soil	AM Root colonization (%)
	Control	81.4±1.52[e]‡	12.79±0.58[e]	1.01±0.048[f]	0.966±0.022[e]	0.124±0.005[ef]	12.58±1.04[e]	41.60±1.61[e]	25.4±4.82[e]	28.52±1.38[e]
4 (DS/m)	G†	122.9±2.10[ab]	34.50±1.47[ab]	3.84±0.063[ab]	3.475±0.191[b]	0.840±0.009[b]	16.78±0.84[b]	92.12±2.25[b]	81.2±5.31[b]	71.58±1.68[ab]
	A	101.1±2.64[c]	22.69±1.40[c]	2.84±0.045[c]	2.216±0.161[cd]	0.601±0.001[cd]	15.44±0.68[c]	86.44±1.51[b]	70.6±5.94[c]	64.10±1.86[b]
	GA	134.5±2.40[a]	39.90±1.23[a]	4.18±0.196[a]	3.872±0.025[a]	0.986±0.003[a]	21.08±0.86[a]	95.50±1.41[a]	93.0±4.58[a]	85.60±1.38[a]
	Control	078.0±1.81[f]	10.74±1.25[e]	0.90±0.036[f]	0.860±0.024[ef]	0.116±0.005[ef]	11.22±0.84[e]	36.72±1.75[ef]	19.0±4.94[f]	21.45±1.52[ef]
8 (DS/m)	G†	109.4±1.49[b]	28.57±1.27[b]	3.42±0.157[b]	2.848±0.049[c]	0.724±0.005[c]	17.72±0.83[b]	63.86±1.95[c]	55.2±4.65[d]	50.54±1.53[c]
	A	096.3±2.35[cd]	21.09±0.84[c]	2.59±0.079[d]	2.070±0.051[cd]	0.542±0.008[d]	15.98±0.68[b][c]	59.52±2.32[d]	51.2±4.32[d]	42.84±1.52[d]
	GA	114.8±2.26[b]	31.29±1.08[ab]	3.67±0.077[b]	3.173±0.083[b]	0.799±0.005[c]	19.58±0.87[b]	78.90±2.09[c]	62.4±4.03[c]	57.37±1.68[c]
	Control	074.5±2.59[g]	09.78±0.59[f]	0.81±0.060[g]	0.787±0.017[f]	0.092±0.002[f]	10.32±0.67[f]	34.22±2.15[f]	12.2±3.83[f]	14.07±1.68[f]
12 (DS/m)	G†	091.6±2.53[d]	17.55±0.54[d]	2.15±0.107[e]	1.669±0.026[d]	0.414±0.003[e]	13.96±0.66[d]	50.00±2.02[d][e]	33.8±3.83[de]	42.47±1.39[d]
	A	086.0±2.06[e]	14.47±0.44[d]	1.65±0.050[e]	1.457±0.050[e]	0.307±0.003[e]	13.32±0.72[d]	46.40±1.49[e]	30.4±5.22[de]	35.62±1.62[e]
	GA	102.6±2.31[c]	26.61±1.53[b]	3.08±0.060[c]	1.849±0.051[d]	0.492±0.007[d]	14.56±0.71[c]	54.24±1.96[d]	40.6±5.81[de]	50.84±2.40[c]
ANOVA ($F_{11,24}$)	Treatments(t)	576.793	1715.753	1055.310	3038.230	4449.330	101.566	1523.857	1000.926	1410.176
($F_{11,24}$)	Salinity (S)	842.776	1397.411	2772.101	963.444	3527.141	144.462	3363.605	129.228	2357.524
($F_{11,24}$)	t × S	60.402	44.012	50.705	191.410	1593.297	19.183	103.129	30.278	85.869

G†- *Glomus mosseae*, A- *Acaulospora laevis*, AM - Arbuscular Mycorrhiza, ‡- Each value is a mean of five replicates, ± - standard deviation, values in columns followed by the same alphabet are not significantly different, (P=0.05), least significant difference test

Table 2. Efficacy of AM fungi on various physiological parameters of *Gossypium arboreum* under different levels of salinity stress after 120 days

Salinity level (DS/m)	Parameters? Treatments?	Chlorophyll content (mg g^{-1}FM)			Stomatal Conductivity ($mmol^2s^{-2}$)			
					Morning ($mmol^2s^{-2}$)		Evening ($mmol^2s^{-2}$)	
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Lower	Upper	Lower	Upper
	Control	0.593±0.011^{c}‡	0.338±0.014^{c}	0.931±0.013^{f}	63.80±6.53^{ef}	15.16±0.83^{f}	50.60±4.61^{f}	10.06±0.78^{f}
4 (DS/m)	G†	0.992±0.009^{ab}	0.818±0.013^{b}	1.810±0.018^{b}	291.60±5.12^{b}	47.82±0.85^{b}	182.00±4.63^{b}	30.24±0.87^{ab}
	A	0.819±0.012^{c}	0.698±0.015^{c}	1.517±0.013^{c}	278.80±6.37^{b}	43.84±1.79^{c}	156.80±3.83^{c}	28.48±1.69^{b}
	GA	1.047±0.011^{a}	0.999±0.014^{a}	2.046±0.019^{a}	311.80±4.81^{a}	50.62±1.50^{a}	217.80±4.32^{a}	33.24±0.81^{a}
	Control	0.553±0.007^{e}	0.255±0.013^{f}	0.809±0.020^{f}	52.20±5.31^{ef}	13.68±0.71^{f}	45.40±5.31^{f}	08.72±0.81^{f}
8 (DS/m)	G†	0.883±0.010^{b}	0.517±0.018^{d}	1.400±0.008^{de}	210.80±5.40^{c}	36.48±0.57^{d}	121.20±4.91^{d}	20.46±1.46^{c}
	A	0.801±0.009^{c}	0.650±0.008^{c}	1.452±0.013^{d}	180.80±4.81^{d}	31.66±1.38^{d}	108.60±4.82^{de}	18.22±0.81^{c}
	GA	0.962±0.007^{b}	0.760±0.013^{b}	1.722±0.008^{b}	246.00±6.08^{c}	40.36±1.56^{cd}	135.00±4.63^{cd}	24.16±1.04^{b}
	Control	0.475±0.009^{f}	0.239±0.017^{g}	0.714±0.023^{g}	41.80±5.11^{f}	11.70±0.70^{g}	40.20±4.60^{g}	08.36±1.19^{g}
12 (DS/m)	G†	0.764±0.009^{cd}	0.505±0.009^{d}	1.270±0.019^{e}	120.60± 6.54^{de}	19.28±0.49^{ef}	84.40±5.59^{e}	14.82±0.78^{d}
	A	0.644±0.009^{d}	0.462±0.011^{de}	1.107±0.017^{ef}	98.60± 7.30^{e}	24.20±0.66^{e}	67.00±3.16^{ef}	11.64±1.01^{e}
	GA	0.713±0.008^{d}	0.378±0.014^{e}	1.091±0.019^{f}	145.80±5.80^{d}	28.36±0.64^{e}	91.80±4.81^{e}	16.52±1.04^{d}
ANOVA ($F_{11,24}$)	Treatments(t)	3920.807	19047.447	3004.397	5828.847	2240.864	7169.375	3400.490
($F_{11,24}$)	Salinity (S)	3059.217	7304.642	1015.319	2154.515	1506.818	4746.739	277.104
($F_{11,24}$)	t × S	183.832	258.391	445.384	301.180	118.981	502.459	155.635

G†- *Glomus mosseae*, A - *Acaulospora laevis*, AM- Arbuscular Mycorrhiza, FM - Fresh matter, ‡- Each value is a mean of five replicates, ±- standard deviation, values in columns followed by the same alphabet are not significantly different, (P=0.05), least significant difference test

AM inoculated plants having different salinity levels showed increased level of chlorophyll content as compared to control (**Table 2**). Chlorophyll content was found to be increased in dual inoculation of GA (2.046±0.019 mg g^{-1}FM) followed by single inoculation of *G. mosseae* (1.810±0.018 mg g^{-1}FM) at lowest salinity level i.e. 4 dSm^{-}.

Phosphatase activity

In addition to changes in the mycorrhization of the plants, there were also changes in the functioning of the system, as evaluated by measuring the plant enzymatic activity. Phosphatase activity represents a broad range of intracellular as well as soil accumulated activity that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Speir and Ross, 1978). **Table 3** showed that alkaline phosphatase activity was found higher as compared to acidic phosphatase activity. Maximum increment in both acidic (0.264±0.009 $IUg^{-1}FW$) and alkaline (0.408±0.012 $IUg^{-1}FW$) phosphatase activity was observed in the plants inoculated with GA followed by single inoculation of *G. mosseae* at 4 dSm^{-1} salinity level. Similarly, at 8 and 12 dS m^{-1} salinity level, GA was found to be more effective as compared to control.

Phosphorus content

Both shoot and root phosphorus content was significantly higher in AM inoculated plants as compared to control (**Table 3**). Most effective results in both shoot (2.015±0.016 %) and root (2.195±0.009 %) P content was found in dual inoculation of GA at 4 dSm^{-1} salinity level followed by single inoculation of *G. mosseae* for both shoot (1.900±0.012 %) and root P (2.156±0.014 %) at same salinity level.

Nitrogen content

Root and shoot nitrogen content was found to be increased in AM inoculated plants at all salinity levels tested (**Table 4**). The results revealed that maximum concentration of N in shoot (2.63±0.03 %) and root (2.17±0.02 %) was found at lowest salinity level i.e. 4 dSm^{-1} in dual combination of GA. Second most effective results for N content in both shoot (2.05±0.02 %) and root (2.00±0.02 %) were found in single inoculation of *G. mosseae* at same salinity level.

Sodium content

The maximum concentration of Na^{+} in shoot (1.78±0.03

Table 3. Efficacy of AM fungi on phosphorus uptake and yield of *Gossypium arboreum* under different levels of salinity stress after 120 days

Salinity level (DS/m)	Parameters? Treatments?	Phosphatase activity ($IUg^{-1}FW$)		Phosphorus uptake (%)		Yield (Fibre weight) (g)
		Acidic Phosphatase ($IUg^{-1}FW$)	Alkaline Phosphatase ($IUg^{-1}FW$)	Shoot Phosphorus (%)	Root Phosphorus (%)	
	Control	0.028±.006^{ef}‡	0.088±.009^{e}	0.636±0.012^{f}	0.686±0.015^{d}	-
4 DS/m	G†	0.237±.008^{b}	0.385±.013^{b}	1.900±0.012^{b}	2.156±0.014^{b}	2.801±.218^{b}
	A	0.179±.009^{c}	0.337±.012^{c}	1.672±0.012^{c}	1.973±0.014^{bc}	1.254±.098^{cd}
	GA	0.264±.009^{a}	0.408±.012^{a}	2.015±0.016^{a}	2.195±0.009^{a}	3.240±.097^{a}
	Control	0.020±.006^{ef}	0.076±.011^{e}	0.470±0.010^{f}	0.652±0.009^{e}	-
8 DS/m	G†	0.154±.007^{d}	0.304±.011^{c}	1.554±0.014^{cd}	1.835±0.013^{c}	0.809±.053^{d}
	A	0.131±.010^{d}	0.260±.012^{cd}	1.208±0.008^{d}	1.773±0.014^{bc}	0.989±.076^{d}
	GA	0.197±.007^{c}	0.359±.012^{b}	1.797±0.015^{c}	2.008±0.009^{b}	1.854±.060^{c}
	Control	0.015±.005^{f}	0.046±.009^{f}	0.440±0.013^{g}	0.501±0.012^{f}	-
12 DS/m	G†	0.102±.012de	0.176±.012^{d}	0.907±0.012^{e}	1.537±0.010^{c}	-
	A	0.068±.007de	0.117±.015^{d}	0.791±0.013^{e}	1.341±0.015^{d}	0.624±.044^{f}
	GA	0.044±.009^{e}	0.208±.017^{cd}	1.106±0.013^{de}	1.566±0.011^{c}	0.748±.040^{e}
ANOVA ($F_{11,24}$)	Treatments(t)	1720.880	3480.625	1667.209	22867.909	13211.151
($F_{11,24}$)	Salinity (S)	8667.17	1957.229	6245.488	29701.562	22709.26
($F_{11,24}$)	t × S	115.613	83.449	1242.092	595.920	1617.14

G†- *Glomus mosseae*, A - *Acaulospora laevis*, AM - Arbuscular Mycorrhiza, FW - Fresh weight, (-) - absent, ‡- Each value is a mean of five replicates, ±- standard deviation, values in columns followed by the same alphabet are not significantly different, (P=0.05), least significant difference test.

Table 4. Efficacy of AM fungi on mineral/ nutrient uptake of *Gossypium arboreum* under different levels of salinity stress after 120 days

Salinity level (DS/m)	Parameters? Treatments?	Nitrogen content (%)		Potassium content (%)		Sodium content (%)	
		Shoot	Root	Shoot	Root	Shoot	Root
	Control	0.69±0.02^{i}	0.61±0.03^{g}	1.28±0.03^{h}	1.41±0.03^{h}	0.64±0.03^{i}	0.52±0.02^{i}
4 DS/m	G†	2.05±0.02^{b}	2.00±0.02^{ab}	2.27±0.03^{b}	2.48±0.04^{b}	1.31±0.03^{d}	1.26±0.02^{d}
	A	1.62±0.02^{d}	1.42±0.02^{c}	2.01±0.04^{c}	2.16±0.03^{d}	1.08±0.04^{e}	1.05±0.03^{ef}
	GA	2.63±0.03^{a}	2.17±0.02^{a}	3.45±0.03^{a}	3.97±0.02^{a}	1.45±0.03^{c}	1.38±0.03^{c}
	Control	0.61±0.03^{i}	0.54±0.03^{h}	1.21±0.04^{i}	1.27±0.02^{i}	0.73±0.02^{h}	0.61±0.03^{h}
8 DS/m	G†	1.48±0.02^{e}	1.21±0.02^{d}	1.81±0.04^{d}	2.09±0.03^{d}	1.63±0.02^{b}	1.52±0.03^{b}
	A	1.31±0.02^{f}	1.16±0.02^{d}	1.64±0.03^{e}	1.83±0.03^{e}	1.26±0.03^{d}	1.12±0.03^{e}
	GA	1.85±0.02^{c}	1.71±0.03^{b}	2.09±0.04^{c}	2.30±0.04^{c}	1.78±0.03^{a}	1.68±0.03^{a}
	Control	0.47±0.02^{j}	0.31±0.02^{i}	1.09±0.04^{j}	1.13±0.03^{j}	0.52±0.02^{j}	0.48±0.03^{j}
12 DS/m	G†	1.12±0.02^{g}	1.01±0.03^{e}	1.53±0.03^{ef}	1.64±0.03^{f}	0.95±0.02^{f}	0.91±0.03^{f}
	A	0.90±0.03^{h}	0.73±0.03^{f}	1.35±0.03^{g}	1.52±0.03^{g}	0.81±0.02^{g}	0.75±0.02^{g}
	GA	1.03±0.02^{g}	0.92±0.02^{e}	1.42±0.04^{f}	1.69±0.02^{f}	1.01±0.02^{e}	0.97±0.03^{f}
ANOVA ($F_{11,24}$)	Treatments(t)	398.235	549.107	109.488	39.354	1006.948	71.380
($F_{11,24}$)	Salinity (S)	817.429	139.966	165.978	73.172	2103.819	155.014
($F_{11,24}$)	t × S	38.088	20.698	13.787	35.633	92.272	20.325

G†- *Glomus mosseae*, A - *Acaulospora laevis*, AM- Arbuscular Mycorrhiza, ‡- Each value is a mean of five replicates, ± - standard deviation, values in columns followed by the same alphabet are not significantly different, (P=0.05), least significant difference test

%) and root (1.68±0.03 %) was found at medium salinity level (8 dSm^{-1}) in dual inoculation of GA followed by single inoculation of *G. mosseae* (1.63±0.02 %) (1.52±0.03 %), respectively. Higher salinity concentration i.e. 12 dSm^{-1} decreased the sodium uptake.

Potassium content

Out of different major essential nutrients, potassium (K^+) plays a vital role in plant growth and regulates various metabolic reactions (Taiz and Zeiger, 2010). The maximum uptake of K^+ concentration was found in both root (3.97±0.02 %) and shoot (3.45±0.03 %) in dual combination of GA followed by single inoculation of *G. mosseae* at lowest salinity level i.e. 4 dS m^{-1} (2.48±0.04 %) for root and (2.27±0.03 %) for shoot.

Yield

According to results, it was found that mycorrhizal colonization significantly enhanced the yield in salt stressed cotton plants. Maximum yield was found in dual combination of GA (3.240±0.097 g) followed by single inoculation of *G. mosseae (*2.801±0.218 g) at lowest salinity level i.e. 4 dSm^{-1}.

DISCUSSION

AM fungi have been reported to be involved in the improvement of plant growth by enhancing accumulation of plant nutrients through greater soil exploration through mycorrhizal hyphae (Tanwar *et al*., 2013). Higher salinity markedly reduced or decreased the plant growth parameters. This inhibitory effect at higher salinity level may be due to accumulation of soluble salts at higher concentrations in growth medium causing hyperosmolality and imbalancing of nutrients that harmfully decline plant growth (Zhu, 2003; Turan *et al.*, 2010). Yadav *et al. (*2013) observed that *G. mosseae* is an important growth promoting mycorrhizal fungus which help in increment of side branches of plant resulted in better plant biomass in soybean plants. The other reason of increased plant biomass can be that cumulative effect of both growth promoting mycorrhizal fungi, i.e., *G. mosseae* and *A. laevis* provide more nutrients and growth promoting substances which cause better growth of side branches resulted in shoot biomass increment. Inhibitory effect on plant biomass was observed at higher salinity level. Many studies have shown that growth index and fresh and dry weight of the shoot and root system (Abdul Jaleel *et al.*, 2007; Ashraf and Ali, 2008; Shahbaz *et al.*, 2010) were found to be decreased by increased salinity concentration.

It can be said that as a result of AMF treatment, root elongation was observed which ultimately absorb more nutrients especially away from the P depletion zone and resulted in the better growth of the plant in comparison to the uninoculated plants (Torrisi *et al*., 1999). Increased salinity level reduces the hyphal length resulting in inhibition of per cent mycorrhizal root colonization and symbiotic capability of AMF. Cantrell and Linderman (2001) observed a reduction in per cent mycorrhizal root colonization at higher salinity level in *Allium cepa* plants inoculated with different AM spores. A significant promoting effect on mycorrhizal colonization density and frequency was observed in cotton plants when inoculated with *Glomus* spp. (Long *et al*., 2008). There are reports of early colonization of *L. usitatissimum* by AM spores (Thompson, 2002).

Koide (2000) suggested that the increased stomatal conductance and transpiration rate in AM plants could be due to P-mediated improvement in photosynthetic capacity. Numerous studies showed the negative influence on leaf area by using different concentrations of NaCl (Zhao *et al.*, 2007; Yilmaz and Kina, 2008; Rui *et al.*, 2009) because of higher accumulation of toxic ions such as Na^+ and Cl^- in the chloroplast under saline conditions (Jain *et al.*, 2001; Alvarez *et al.*, 2003; Munns *et al.*, 2006). AM inoculated plants under different salinity stress levels were superior to those of non-mycorrhizal plants showing that mycorrhization is capable of counter balancing the stress (Zuccarini, 2007). Similarly, *Capsicum annuum* mycorrhizal treated plants showed higher chlorophyll content over control under salt stress (Demir, 2004). Increased chlorophyll content in mycorrhiza-treated plants indicates an increment in rate of photosynthesis, which can be due to more absorption of nutrients (Mangla *et al*., 2010b). Higher salinity level causes decrement in both acidic and alkaline phosphatase activity in cotton plants. Plants with higher mycorrhizal root colonization had maximum phosphatase activity (alkaline and acidic). Garcia-Gomez *et al*. (2002) also reported that root acid

phosphatase activity (RAPA) was higher in *Carica papaya* when plants were inoculated with *Glomus claroideum.*

AM symbiosis positively influence the composition of mineral nutrients of plants under salt stressed conditions (Al-Karaki and Clark, 1998). Higher salinity level showed a significant reduction in both shoot and root phosphorus content. These results are in consonance with those of Shokri and Maadi (2009) who observed that as soil salinity increased, shoot phosphorus content decreased in non-mycorrhizal *Trifolium alexandrium* plants as compared to inoculated one. Improved P uptake by AM fungus in plants grown under saline conditions may also reduce the negative effects of Na^+ and Cl^- ions 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). 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. Salts interfere with nitrogen acquisition and utilization by influencing different stages of N metabolism, such as NO^{-3} uptake and reduction in protein synthesis (Frechill *et al*., 2001). The results of present investigation are in close agreement with those of Zuccarini (2007) who found an increment in Na^+ uptake in shoot as compared to root in lettuce plant inoculated with *G. mosseae, G. intraradices* and *G. coronatum*. Likewise, Kaya *et al.* (2013) also observed a significant increase in sodium uptake up to a certain salinity level in maize plants. Higher salinity stress inhibits the uptake of K^+ in both shoot and root in *Gossypium arboreum*. These results confirm the findings of Kaya *et al*. (2013) who observed a significant decrement in K^+ uptake in salt stressed maize plants. Porras-Soriano *et al*. (2009) reported the efficacy of *G. intraradices* in maintaining favourable K^+: Na^+ ratio. Higher salinity level reduced or decreased the yield in cotton plants. Similar results were observed by Satti *et al*. (1995) who observed a significant reduction in yield of tomato plants under salt stressed conditions.

CONCLUSIONS

The present study showed that AM inoculation increased the morphological, physiological and biochemical performance and tolerance of the mycorrhizal plants under saline conditions. AM fungi alleviate the detrimental effect of salinity through improved water and nutrient uptake especially P through AM hyphae and colonized roots of plants. Greater effectiveness of combined inoculation may be due to synergistic interaction between both AM fungal species and variation in efficacy among fungal species. So, the present findings conclude a better practice to reduce the use of chemical fertilizers which are much expensive, hence the best combinations must be recommended to the farmers for better crop improvement and yield.

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