

# Comparative study of cowpea genotypes under salinity stress with Arbuscular Mycorrhizal Fungi

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## **Abstract**

*The aim of present study was to examine the effect of arbuscular mycorrhizal fungi (AMF) on the morphological and biochemical attributes in cowpea (*Vigna unguiculata* [L.] Walp.) subjected to salt stress. Salt stress (0.25%, 0.35% and 0.45% NaCl) reduced germination, shoot length, no. of leaves and biomass in cowpea leaves. AMF ameliorated the negative impact of salinity on the growth parameters studied. The activity of antioxidant enzymes such as superoxide dismutase (SOD) and total soluble protein (TSP) enhanced under salt stress and AMF inoculation further enhanced their activity, thus strengthening the plant's defense system. AMF-inoculated plants providing efficient protection against salt stress. AMF also increased uptake of mineral elements of plant. The present study shows that AMF possesses the potential to enhance salt tolerance of cowpea.*

## **Introduction**

Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. The accumulated salts include sodium, potassium, magnesium and calcium, chloride, sulphate, carbonate and bicarbonate

(mainly sodium chloride and sodium sulphate). In general, abiotic stress often causes a series of morphological, physiological, biochemical and molecular changes that unfavorably affect plant growth, development and productivity. The total global area of salt-affected soils has recently been estimated to be approximately 830 million hectares (Martinez-Beltran et. al., 2005). All over the world, particularly in arid and semiarid regions salinity is one of the important problems and is increasing day by day. Increased use of saline water for irrigation purposes continuously adds to the salt affected soils thereby resulting in conversion of arable land into salinized waste land. Around 5 to 7% of global land is salt affected (Egamberdieva et al., 2015; Ruiz-Lozano et al., 2012).

Salt stress consists of two components: osmotic and ionic. The first is a result of the high concentration of salts in the root environment that leads to decreased in soil water potential and reduces the availability of water for the plant. The ionic component arises from the accumulation of certain ions (usually Na<sup>+</sup> and Cl<sup>-</sup>) and can cause nutritional imbalance,

toxicity or both (Greenway and Munns, 1980; Munns, 2002; Munns and Tester, 2008). Plants expose to high salinity its produces toxic reactive oxygen species (ROS) ultimately resulting in oxidative stress. Excess accumulation of ROS like superoxide, hydroxyl, and peroxide radicals is detrimental to normal metabolism and growth (Wu *et al.*, 2014). ROS accumulate in leaves and leads to oxidation of various cellular molecules including lipids, proteins and chlorophylls. Hence peroxidation of membrane lipids, protein oxidation, and damage to nucleic acids are the immediate effects of the excess ROS (Wu *et al.*, 2014).

The legumes are very important both ecological and agriculturally because of their ability to fix nitrogen in the root nodules in a symbiotic interaction with soil rhizobia. Cowpea (*Vigna unguiculata* (L.) Walp.) is well adapted on different environmental conditions and could be used an alternative crop for salt affected soil (Murillo-Amado *et al.*, 2002). Cowpea (*Vigna unguiculata* L.) belongs to family Fabaceae, also known as black eye pea, is an important legume grown as cash crop throughout the world. Cowpea is rich in protein and is normally cultivated for green pods and dry beans (Hadi *et al.*, 2012). The species *Vigna unguiculata* (L.) Walp has as main characteristics be rustic, it presently high protein tenor in grain, besides of

this, the crop is used in the agricultural areas under environmental stress.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous and have proved to posses the potential to improve soil structure and plant growth under normal as well as stressful environments (Tang *et al.*, 2009; Navarro *et al.*, 2013). AMF colonization brings morphological, nutritional and physiological changes and has also been reported to enhance resistance of plants to abiotic stresses (Hameed *et al.*, 2014). Under salt stress conditions arbuscular mycorrhizal fungi (AMF) are believed to act as essential bio-ameliorators of stress and hence helping plants in alleviating stress induced damage (Ahanger *et al.*, 2014). AMF colonization has been reported to enhance plant growth and vigour (Ahanger *et al.*, 2014; Alqarawi *et al.*, 2014a; Hashem *et al.*, 2014b). Mycorrhizal inoculation not only affects root morphology but also physiological status in host plants (Alqarawi *et al.*, 2014a; Hashem *et al.*, 2014a).

### **Material and Method**

**Seeds-** The cowpea [*Vigna unguiculata* (L.) Walp.] belongs to the family Fabaceae is one of the important pulses of India. The plant material (seeds) will be procured from the gene bank of Indian Grassland and Fodder Research Institute (IGFRI), Jhansi, Uttar Pradesh and National

Bureau of Plant Genetic Resources (NBPGR), Delhi. Four genotypes mainly IC-7832, IC-300039, BL-1 and EC-4216 of cowpea genotypes were used. Seeds with uniform size, colour and weight were chosen for experimental purpose.

**Pot culture experiments-** The selected mycorrhiza *Glomus intraeadices* inoculum was inoculated to the experimental soil. The inoculum consisted of spores, hyphae and colonized root fragments with mycorrhizal fungi. The mycorrhizal inoculum was added to the experimental soil as 10 g of trap soil culture (approx. 100 spores/g trap soil, M = 80%)/ pot (1Kg). Non-mycorrhizal inoculated soil was used as control (Abeer et al., 2015). Four genotypes IC-7832, IC-300039, BL-1 and EC-4216 of cowpea plants were grown in 12 pots, 3 pots of each genotype. 5 seeds were sown in each pot after 20 days 4 plants were leaved in each pots and rest of the plants transplanted in other pots. Each genotype was grown in triplicate. One pot was taken as control where as rest three pots were taken as test in which 0.25%, 0.35% and 0.45% concentration of NaCl was given.

**Total Soluble Protein (TSP) -** Freshly prepared 15 µl protein extract was taken in appendrop tubes. In this 85µl distilled water was added in each appendrop tube. 33µl samples from

appendrop tube transfer in test tubes and add 1 ml distilled water. After that 5 ml of potassium tartarate mixed solution were added in each tube and mixed properly. 0.5 ml folin reagent were added in each tube (solution appears yellowish at bottom), mixed properly with the help of vortex. Placed in dark room for 30 minutes and take OD at 660 nm (Lowary et. al., 1951).

**SOD (Superoxide dismutase) -** The assay of SOD was done according to Giannopolitis and Ries (1977). The 3 ml assay mixture consisted of 79.2 mM EDTA, 10.8 mM tetraethylene diamine, 0.0033 % bovine serine albumin, 6 mM nitroblue tetrazolium (NBT), 600 µM riboflavin in 5 mM KOH and 0.2 ml enzyme extract. The reaction was initiated by placing the glass tubes in between fluorescent bulbs. By switching the light on and off, the reaction was started and terminated, respectively. The increase in absorbent due to formazen formation was read at 560 nm (Giannopolitis et. al., 1977).

## **Results and Discussion**

### **Percentage germination**

Analysis of data revealed control (AMF) treatment had maximum 93.33% and control (NI) had 90.21% germination while under 0.45% salinity decreased to 73.33% and 66.32% germination respectively. The germination decreased significantly with increasing salinity levels. The germination of AMF inoculated

plants enhancement was more pronounced i.e. 53.21%, 53.33%, 80% and 73.33%, while germination of non-inoculated plants was 46.66%, 48.66, 60% and 66.32% respectively. BL-1 showed overall maximum germination as compared to BL-2 and IC-300039 and IC-7832 had minimum germination. The AMF inoculated plants percent germination was increased as compared to the control (NI) as well as salt stressed plants. Zia & Khan (2002)

have also reported reduced germination under saline conditions in some medicinal plants that also strengthen our findings. Rajper & Sial (2002) and Gulzar et al., (2003) reported that increasing salinity caused reduction in percentage seedling emergence of wheat and *Aeluropus lagopoides*. However, our results do not agree with Muhammad & Hussain (2010) who stated that there was no effect on % germination of tested plants (see in table no. 1.).

**Table No. 1- Percentage germination (%) of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	73.33	56.63	52.31	46.66
	AMF	76.43	60	58.76	53.21
IC-300039	NI	71.22	60	52.33	48.66
	AMF	73.33	66.66	60	53.33
BL-1(Lobia)	NI	86.66	80	72.23	60
	AMF	93.33	93.33	86.66	80
BL-2 (Lobia)	NI	90.21	84.23	73.33	66.32
	AMF	93.33	86.75	81.36	73.33

NI = non inoculated, AMF = Presence of mycorrhiza

### Plant height

The plant height of control (AMF) treatment had maximum 30.86 cm and control (NI) had 30.46 cm height while under 0.45% salinity it was decreased to 26.83 cm and 21.83 cm respectively. The plant height decreased with increasing salinity levels. The AMF inoculated plant height was

increased i.e. 25.83, 27.3, 30.5 and 26.16 cm respectively as compared to non-AMF treated plants height was 24, 26.77, 26.38 and 21.83 cm under higher salinity stress condition. BL-2 showed overall maximum germination as compared to BL-1 and IC-300039 and IC-7832 had minimum germination. The AMF inoculated plants height was increased as

compared to the control (NI) as well as salt stressed plants. The increment in the growth parameters as a result of AMF was in corroboration with the results of Tang *et al.*, (2009) who reported amelioration of salinity

stress by AMF in maize. Ameliorative role of AMF under salt stressed plants is ascribed to role of AMF in increasing water and nutrient uptake (Abeer *et al.*, 2015). (see in table no. 2.).

**Table No. 2- Plant height(cm) of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	27.61	26.1	24.96	24
	AMF	28.7	26.75	25.58	25.83
IC-300039	NI	28.83	28.25	28.56	26.77
	AMF	29.48	28.56	28.41	27.3
BL-1(Lobia)	NI	33.97	32.38	29.22	26.38
	AMF	35.16	33.61	31.25	30.5
BL-2 (Lobia)	NI	30.46	27.35	26.06	21.83
	AMF	30.86	28.9	26.81	26.16

NI = non inoculated, AMF = Presence of mycorrhiza

### Number of Leaves

The experimental data revealed control (AMF) treatment had maximum no. of leaves was 31.83 and control (NI) had 30 while under 0.45% salinity it was decreased to 27.58 and 25.02 respectively. The number of leaves decreased with increasing salinity levels. The AMF inoculated plant leaves was increased to 17.67, 19.41, 25.83 and 27.58 respectively as compared to non-AMF treated plant leaves was 16.88, 18.25, 24 and 25.02 cm under higher

salinity stress condition. BL-2 showed overall maximum no. of leaves as compared to BL-1 and IC-300039 and IC-7832 had minimum no. of leaves. The AMF inoculated plant leaves were increased as compared to the control (NI) as well as salt stressed plants. Our findings are supported by Hashem *et al* (2015) that inoculation of AMF improved the number of leaves per plant as compared to non treated plant with AMF under saline condition in cowpea (see in table no. 3.).

**Table No. 3- Number of Leaves of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	19.77	17.86	17.91	16.88
	AMF	20.69	19.13	18.63	17.67
IC-300039	NI	24.6	22.27	20.23	18.25
	AMF	25.13	22.65	20.91	19.41
BL-1(Lobia)	NI	24.27	26.1	24.96	24
	AMF	28.7	26.75	25.58	25.83
BL-2 (Lobia)	NI	30	28.85	28.07	25.02
	AMF	31.83	30.38	29.33	27.58

NI = non inoculated, AMF = Presence of mycorrhiza

### Shoot weight

Analysis of data revealed control (AMF) treatment had maximum shoot weight was 40.53 gm and control (NI) had 34.33 gm while under 0.45% salinity it was decreased to 34.84 gm and 26.86 gm respectively. Shoot weight was decreased with increasing salinity levels. The AMF inoculated shoot weight was increased to 20.27, 25.19, 31.94 and 34.84 gm respectively as compared to non-AMF treated plants shoot weight was 14.25, 20.66, 26.08 and 26.86 gm under higher salinity stress condition. BL-2

showed overall maximum weight as compared to BL-1 and IC-300039 and IC-7832 had minimum shoot weight. Bheema Reddy et al., (2011) investigated that decreased biomass and number of leaves in plants was observed under salinity and inoculation of AMF increased these attributes and also mitigated the salinity induced deleterious effects in *wheat* and Usha et al. (2005) also reported that AMF inoculated plants of *Vitis vinifera L*, maintained their growth due to efficient uptake of mineral elements (see in table no. 4.).

**Table No. 4- Shoot weight (gm) of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	20.57	18.87	16.79	14.25
	AMF	25.07	23.77	22.07	20.27
IC-300039	NI	26.83	25.15	23.33	20.66
	AMF	34.1	31.8	27.64	25.19
BL-1(Lobia)	NI	32.28	30.06	27.98	26.08
	AMF	38.31	36.44	34.87	31.94
BL-2 (Lobia)	NI	34.33	32.23	28.24	26.86
	AMF	40.53	38.53	36.69	34.84

NI = non inoculated, AMF = Presence of mycorrhiza

### Root weight

Analysis of data revealed control (AMF) treatment had maximum root weight was 4.42 gm and control (NI) had 4.008 gm while under 0.45% salinity it was decreased 2.75 gm and 2.65 gm respectively. Root weight was decreased with increasing salinity levels. The AMF inoculated root weight was increased to 1.88, 2.44, 2.59 and 2.75 gm respectively as compared to non-AMF treated plants root weight was 1.34, 2.17, 2.43 and 2.65 gm under

higher salinity stress condition. BL-2 showed overall maximum weight as compared to BL-1 and IC-300039 and IC-7832 had minimum root weight. Padder et al. (2012) and Babu et al. (2012) investigated that length, fresh biomass of shoot and root reduced with the increasing salinity in *Vigna radiate L*, and *tomato*. Bheema Reddy et al., (2011) also reported inoculation of AMF increased biomass and also mitigated the salinity induced deleterious effects in *wheat* (see in table no. 5.).

**Table No. 5- Root weight (gm) of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	2.33	2.04	1.87	1.34
	AMF	2.46	2.39	2.22	1.88
IC-300039	NI	3.45	3.01	2.57	2.17
	AMF	3.69	3.25	2.69	2.44
BL-1(Lobia)	NI	3.85	3.53	2.95	2.43
	AMF	4.01	3.66	2.96	2.59
BL-2 (Lobia)	NI	4.008	3.46	2.83	2.65
	AMF	4.42	3.77	3.2	2.75

NI = non inoculated, AMF = Presence of mycorrhiza

### Total Soluble Protein (TSP)

The study revealed that at different levels of NaCl TSP level was found to be decreased continuously but in presence of AMF TSP level was found to be decreased marginally, However AMF inoculated cowpea plants maintained higher TSP level as compared to control and also helped in reduced the salinity induced toxic

effects. BL-2 showed overall maximum TSP level as compared to BL-1 and IC-300039 and IC-7832 had minimum TSP. Protein concentration can be negatively or positively affected under saline condition. Khosravinejad et al. (2009) reported that the sodium chloride treatments reduced the protein concentration in the plant seedlings in *barley* but in contrast of

our result Kapoor et al. (2010) reported in *Vigna mungo* increase in protein concentration with

increasing salt concentration (see in table no. 6.).

**Table No. 6- Total Soluble Protein (mg/gm Fwt.) of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	23.33±0.072	21.71±0.023	19.71±0.042	16±0.082
	AMF	23.8±0.067	22±0.062	21.23±0.032	18.28±0.075
IC-300039	NI	26.85±0.074	23.42±0.051	20.57±0.556	18.28±0.043
	AMF	28.56±0.324	26.09±0.513	23.9±0.020	22.05±0.013
BL-1(Lobia)	NI	26.85±0.044	24.47±0.065	23.9±0.326	19.61±0.069
	AMF	27.61±0.421	26.47±0.448	24.19±0.083	23.7±0.138
BL-2 (Lobia)	NI	27.61±0.512	26±0.674	23.52±0.268	18.66±0.027
	AMF	30±0.047	27.71±0.037	26.57±0.030	23.8±0.019

NI = non inoculated, AMF = Presence of mycorrhiza

### Superoxide Dismutase Activity

Results about activities of antioxidant as influenced by salinity and AMF are depicted in table no.7. SOD activity at different salinity stress condition in genotype IC-7832 SOD activity was initially increased at 0.25% and 0.35% salinity which decrease later on at 0.45% salinity and in genotype IC-300039, BL-1 and BL-2 activity of SOD increased continuously. However inoculation of AMF to salt stressed plants (NaCl + AMF) resulted in marked

enhancement in activities of antioxidant enzymes examined and the increase being more as compared to the control as well as salt stressed plants. SOD activity increases in response to salinity have been reported in cowpea, sorghum, cotton (Freitas *et al.*, 2011) and *Cicer arietinum* (Rasool *et al.*, 2013). Increase in activity of antioxidant enzymes SOD as a result of AMF colonization coincide with the results of Tang *et al.*, (2009) for *Zea mays* and Hashem *et al.*, (2015) for tomato.

Genotype	Treatments	Control	0.25%	0.35%	0.45%
IC-7832	NI	1.064±0.032	1.731±0.231	2.374±0.129	2.175±0.116
	AMF	1.156±0.035	1.831±0.372	2.437±0.013	2.217±0.209
IC-300039	NI	1.093±0.020	1.617±0.034	2.215±0.050	3.058±0.160
	AMF	1.293±0.431	1.727±0.208	2.325±0.034	3.148±0.065
BL-1(Lobia)	NI	1.383±0.218	2.317±0.339	2.87±0.046	3.025±0.048



	AMF	1.473±0.442	2.437±0.110	2.998±0.246	3.225±0.226
BL-2 (Lobia)	NI	1.4±0.248	2.357±0.324	2.944±0.232	3.204±0.032
	AMF	1.521±0.139	2.467±0.226	3.124±0.221	3.314±0.043

**Table No. 7- Superoxide Dismutase activity (Unit/min) of four genotypes/ varieties of cowpea under different salinity levels of NaCl.**

NI = non inoculated, AMF = Presence of mycorrhiza

### CONCLUSION

Salinity resulted in altered growth of cowpea due to its effects on the various physiological and biochemical parameters studied. Salinity increased lipid peroxidation leading to membrane imbalance and at the same time also reduced the uptake of important mineral elements. However, in the present study, AMF ameliorated the negative impact of salinity on growth and biochemical parameters. AMF allayed the salt stress by preventing the excess uptake of Na<sup>+</sup> and at the same time causing further enhancement in activities of antioxidant enzymes thus ensuring better scavenging of ROS. Thus salinity induced toxic effects on growth, antioxidant enzymes and mineral nutrients in cowpea can be alleviated by AMF.

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