Research Article
***Cameroon Journal of Biological and Biochemical Sciences* 2022, Vol 30, Serie 2, 74-86**
ISSN 1011-6451/*CJBBS*.2021. Submission (January 2022). Accepted and Published Online (April 2022) www.camjournals.com)

Effects of arbuscular mycorrhizal biofertilizer (*Scutellospora gregaria*) on some metabolites, mineral distribution, chlorophyll content, growth and agronomic parameters of black bean (*Phaseolus vulgaris* L.) under saline conditions.

Effet d'un biofertilisant mycorrhizien arbusculaire (*Scutellospora gregaria*) sur certains metabolites, distribution minérale, teneur en chlorophyll, paramètres de croissance et agronomic chez le haricot noir (*Phaseolus vulgaris* L.) sous contrainte saline.

NOUCK Alphonse Ervé[1*]; EKWEL SONDI Serge[2]; YONG Cecile KUOMAI[1]; NJOUONKOU André-Ledoux[1]; Erica WIRAGHAN SHANG[1] and TAFFOUO Victor Désiré[2]

[1]*Department of Biological Sciences, Faculty of Sciences, The University of Bamenda, P.O. Box 39 Bambili-Cameroon.*

[2]*Department of Botany, Faculty of Science, The University of Douala, P.O. Box 24157, Douala-Cameroon.*

***Corresponding author: Nouck Alphonse Ervé**. *Department of Biological Sciences, Faculty of Sciences, The University of Bamenda, P.O. Box 39 Bambili-Cameroon, Phone: 00237 677228724, Email: alphonseervenouck@yahoo.fr*

ABSTRACT

The effects of arbuscular mycorrhizal biofertilizer (AMF) on some metabolites, mineral distribution, chlorophyll content, growth and agronomic parameters of Black bean (*Phaseolus vulgaris* L.) under saline conditions were examined in the greenhouse. Seeds were planted in polythene bags that were previously filled with sand, 25 g of Biofertilizer and supplied with a nutrient solution during four weeks in a completely randomized design. Plants were subjected to NaCl treatments (0, 50, 100 and 200 mM) with 0 as control. 0 and 50 mM NaCl were tested in the farm during four months. Plots were arranged in a randomized block design with treatments NaCl only and NaCl + Biofertilizer. The flowering time, number of flowers per plant, number of pods per plant, pod yield and harvest index were evaluated. The results in the greenhouse showed that the supply of intake doses of NaCl in the culture medium significantly decreased the dry biomass, stem height, leaf area and chlorophyll content respectively from 100 mM NaCl. K, Ca and Mg significantly ($P<0.001$) decreased in the plant organs. The different metabolites (proline, Soluble carbohydrates, and total phenolic contents significantly ($P< 0.001$) increased from 50 mM NaCl. The findings indicate that AMF positively influenced the growth and agronomic parameters of common bean under salinity. The planting of common bean (Dor 701) in salt affected soils could be encouraged for better development. AMF can be encouraged for use to improve crop productivity in semi-arid, arid and coastal zones.
Keywords: *Phaseolus vulgaris,* growth parameters, osmolytes, salinity, pod yield.

RESUME

L'Effet d'un biofertilisant mycorrhizien arbusculaire (AMF) sur certains metabolites, la distribution minérale, teneur en chlorophyll, les paramètres de croissance et agronomic chez le haricot noir (*Phaseolus vulgaris* L.) sous contrainte saline a été étudié pendant quatre semaines en serre à quatre niveaux de salinité (0, 50, 100 et 200 mM de NaCl) avec 0 comme témoin. Les graines ont été plantées dans les sacs de pépinières remplis de sable et 25 g de AMF préalablement désinfecté et arrosé avec une solution nutritive. Le dispositif expérimental

mis en place en champ est celui d'un split-plot avec randomisation composé de 3 blocs par traitement (NaCl et NaCl+Bio) avec 0 et 50 mM NaCl. Le temps de floraison, nombre de fleur et de gousse par plante, rendement et l'indice foliaire ont été évalués. L'apport des doses de NaCl dans le milieu de culture a entrainé une baisse significative de la biomasse sèche, hauteur de la tige, surface foliaire et teneur en chlorophyll à partir de 100 mM NaCl. Le K, Ca et Mg connaissent une baisse significative ($P<0.001$). Une hausse significative ($P<0.001$) des différents metabolites (proline, sucre soluble, phenol totaux et flovonoids) a été observeé à partir de 50 mM NaCl. Les paramètres agronomiques ont montrés un apport positif des AMF. La culture de la variété Dor 701 et l'utilisation des AMF peut être conseillée dans les sols salés comme indicateur pour un bon développement des zones arides, semi-aride et Littorale.

Mot clés: *Phaseolus vulgaris*, paramètes de croissance, osmolites, salinité, rendement en graine.

INTRODUCTION

Salinity negatively impacts plant growth and consequently leads to a decline in the yield for most major crop plants growing in salty areas in the world (Ndouma et al., 2020, Nouck et al., 2021). Salt stress occurs in areas where soils are naturally over salted and precipitation is low or where irrigation brings salt to the surface soil that inhabit plants (Nasri et al., 2015). The main causes are saline parent bed rocks, mineral degradation, invasion of sea water in coastal regions and the over use of saline water for irrigation (Ndouma et al., 2020). More than 20% of irrigated land has suffered from salt problem (Ghassemi et al., 1995). Salinity induces an imbalance in water potential, cellular dehydration, (inhibits) intracellular enzyme activities, cell division and expansion, resulting in a reduction in growth and productivity (Kosovà et al., 2013, Nouck et al., 2021). The mineral uptake by plants under salt stress has been largely discussed by previous workers (Menguekam et al., 2014, Hand et al., 2017, Nouck et al., 2021). They showed that the inhibition of potassium by sodium is a result of K^+/Na^+ antagonism.

Many workers have proved that salt tolerance is determined by osmotic adjustment, maintenance of ion homeostasis, the control of ion and water flux, the specific protein and free radical enzymes involved in the protection of protoplast functions (Grigore et al., 2011, Meguekam et al., 2014, Nouck et al., 2016). Tolerant crop plants synthesized metabolites like proline, total soluble carbohydrates, total phenol and flavonoids in the salt stress conditions. Proline is the appropriate amino acid in the cytoplasm that contributes to the stability of the osmotic pressure of ions in the vacuoles under saline conditions. It is highly stored and has positive effects in the process of adaptations of cells to salt and water stress (Nouck et al., 2016). Numerous studies showed that the accumulation of soluble carbohydrates depends on genotypes and species and could be used as indicators to identify salt tolerant species (Khosravinejad et al., 2009). Total phenol acts as defense mechanism against biotic and abiotic stress (Taïbi et al., 2016) and flavonoids are the main subgroup of polyphenols with a wide array of biological functions including lipid peroxidation inhibition. Their accumulations during stress could be considered as cellular adaptive mechanisms for scavenging oxygen free radicals (Di Ferdinando et al., 2012).

Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts or biotrophs which acquire carbon solely from host plants. They absorb organic nutrients from the plant and in turn, supply the plants with water and mineral nutrients from the soil. They also provide resistance against soil pathogens and drought (Beltrano et al., 2013). Therefore, AMF increases leaf area and increases salinity resistance in several host plants, such as maize, tomato and pepper (Kaya et al., 2009, Beltrano et al., 2013). Previous researchers showed that Arbuscular mycorrhizal fungi could enhance the ability of plants to cope with salt stress by improving mineral nutrient absorption, maintaining ion balance, protecting enzyme activities, and facilitating water uptake (Colla et al., 2008, Beltrano et al., 2013). Some studies have presented an increase in proline accumulation in mycorrhizal plants subjected to salt stress (Kaya et al., 2009). The accumulation of sugars induced by the AM symbiosis is a positive response to salt stress, since it can prevent structural changes in soluble protein, maintain the osmotic equilibrium in plant cells, and protect membrane integrity (Beltrano et al., 2013).

Black bean (*Phaseolus vulgaris* L.) is the most important cash crop in Cameroon with great nutritional value in human diet consisting of micronutrients such as phenolic compounds. (Myers et al., 2019). It also provides iron, copper, zinc, phosphorus, magnesium and calcium, high protein content and abundant fiber, complex carbohydrates, and other daily food needs such as vitamins and minerals (Imran et al., 2014). Understanding the modifications and possible mechanisms involved in the mitigation of salt stress

by the AMF contributes to improve salt tolerance in plants for research and breeding programs. Therefore the main objective of this work was to evaluate the influence of arbuscular mycorrhizal biofertilizer (*Scutellospora gregaria*) on some metabolites, mineral distribution, chlorophyll content, growth and agronomic parameters of Black bean (*Phaseolus vulgaris* L.) under saline conditions.

MATERIALS AND METHODS

Study area and plant material

The study was performed in a greenhouse and field conditions of Faculty of Science, The University of Bamenda located in Bambili-Cameroon from January 2019 to November 2021. The plant material, black bean (*Phaseolus vulgaris* L.) is one of the most important legume crops grown in all continents of the world, because of its high protein, fibre, and complex carbohydrate content (Imran et al., 2014). The variety Dor 701 and AMF biofertilizer (***Scutellospora gregaria*)** used were provided by the Institute of Agronomic Research and Development (IRAD Yaoundé) breeding program of Cameroon.

Plant growth conditions and salt treatments

After viability test, the seed of black bean variety "Dor 701" were sterilized with 3% of sodium hypochlorite during 10 minutes, washed five times with demineralized water and transplanted into 2 L polythene bags filled with 2 kg of sterilized sand. The plants were arranged in a complete randomized block design, with one plant each and five replications per treatment. They were enriched on daily basis with a modified nutrient solution. (in g/L) made of 150 g $Ca(NO3)_2$, 70 g KNO_3, 15 g Fe–EDTA, 0.14 g $KH2PO_4$, 1.60 g $K2SO_4$, 11 g $MgSO_4$, 2.5 g $CaSO_4$, 1.18 g $MnSO_4$, 0.16 g $ZnSO_4$, 3.10 g $H3BO_4$, 0.17 g $CuSO_4$ and 0.08 g MOO_3 (Hoagland & Arnon 1950). The pH of the nutrient solution was adjusted to 7.0 by adding HNO_3 0.1 mM. Black bean plants were subjected to different salt concentrations (0, 50, 100 and 200 mM NaCl) with (0 as control) in culture medium for a period of four weeks for the determination of physiological and biochemical responses of cultivars to salt stress. The average day and night temperatures in the greenhouse were between 20 and 26 °C respectively during the growth period with average relative air humidity of 69.5%. Parameters were evaluated under greenhouse condition: stem height, leaf area, dry biomass of roots and shoots, chlorophyll (a+b) content, metabolites (proline, soluble carbohydrates, total phenol and flavonoids content) and mineral (Na, K, Ca and Mg contents of roots and shoots).

Growth parameters

The leaf area, stem height and dry weight (DW) were recorded after four weeks. The stem height was determined by measuring with a ruler (Hand et al., 2017). Roots and shoots were dried separately at 60 °C for 72 Hours and their dry biomasses were determined (Nouck et al., 2016). Leaf area was calculated using the Taillièz and Ballo (1992) formula, surface area (cm^2) = 1/3 (Length × Width).

Mineral distribution

The sodium, potassium, calcium and magnesium in the shoots and roots were determined . 2 g of dried organs were separately reduced to ashes by heating at 550 °C for 4 hours and thoroughly mixed with 250 mL of deionized water. The filtrate was analysed with an atomic absorption spectrophotometer (Rayleigh WFX-100) (Pauwels et al., 1992) method.

Chlorophyll content

The chlorophyll content was determined using the Arnon (1949) method. 0.80 g sample of fresh leaves were crushed and extracted with 80% of alkaline acetone (v/v). The filtrate was analyzed using a spectrophotometer (Pharmaspec model UV-1700) at 645 and 663 nm. **Osmolytes**

Proline content

Proline content (PRO) was estimated using Bates et al. (1973) method. 0.5 g of fresh leaves were weighed and put inside a flask. 10 mL of 3% aqueous sulphosalicylic acid was poured in the same flask. The mixture was homogenized, and then filtered with a Whatman N° 1 filter paper. 2 mL of filtered solution was poured into a test tube, and then 2 mL of glacial acetic acid and ninhydrin acid were respectively added into the same tube. The test tube was heated in a warm bath for 1 h. The reaction was stopped by placing the test tube in an ice bath. 4 mL of toluene was added to the test tube and stirred. The toluene layer was separated at room temperature, the mixture purple color and the absorbance of the purple mixture was read at 520 nm by spectrophotometer UV (Pharmaspec model UV-1700). At 520 nm, the absorbance was recorded and the concentration of PRO was determined using a standard curve as µg/g FW.

Soluble carbohydrate content

Soluble carbohydrate (CH) content was obtained using phenol-sulphuric acid (Dubois et al., 1956). The fresh leaves (1 g) were grounded in 5 mL of 80% ethanol and filtered with the Whatman N° 1 filter paper. The collected extracts were diluted by deionized water to 50 mL. 1 mL of each sample was poured in test tube, then 1 mL of phenol solution and 5 mL of sulphuric acid were added. The mixture was then swirled. The absorbance was read at 490 nm using a spectrophotometer (Pharmaspec UV-1700 model). The quantity of CH was deduced from the glucose standard curve.

Flavonoids content

Flavonoids content (FLA) of crude extract was determined by the aluminum chloride colorimetric method (Chang et al., 2002). 50 µL of crude extract (1 mg/mL ethanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 minutes of incubation, and the mixture was allowed to stand for 6 minutes. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 mins, and absorbance was recorded on spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a grutin calibration curve, and the result was expressed as grutin equivalent per g dry weight.

Total phenolic content

Total phenolic content (TP) was determined using the folin-ciocalteu method (Marigo 1973). 1 g of fresh leaves was ground at 4 °C for 20 minutes in 3 mL of 0.1 NHCl, the homogenate was centrifuged at 6000 g during 40 minutes. The pellet re-suspended in 3 mL of 0.1 NHCl and centrifuges previously. The two supernatant are mixed and constitute the crude extract of soluble phenol. The reaction mixture containing 15 µL of extract 100 µL folin-ciocalteu reagents, 0.5 mL of 20% Na_2CO_3 was incubated at 40 °C for 20 minutes and absorbance read at 720 nm wavelength with a spectrophotometer (Pharmaspec UV-1700 model). The TP (mg/g FW) content was determined through a standard curve established by using chlorogenic acid.

Yield components

The field experiment was performed at the University of Bamenda agricultural research farm located in Bambili-Cameroon. Bambili (5° 60' 33" North and longitude 10° 15' 21" East, Elevation 1444 m) is found in Mezam division of the North West region of Cameroon. The work was carried out from January 2019 to November 2021, Average rain fall and temperatures are 854 mm/year and 30 °C and relative humidity is nearest to 84%. Prevailing winds carry the tropical monsoon. Table 1 shows the physico-chemical properties of the soil taken from 0-20 cm depth of the experiemental site in Bambili. The plots were arranged in a randomized complete block design within a split plot layout with two main treatments (NaCl only and NaCl + Bio at 0 and 50 mM NaCl) with three replications and 0 as a control. Plots were 4×1 m surface and intra spacing was 1.5 m and inside the plots the cultivars were 0.50 m. Yield datas were collected from eighteen plants per repetition for each variant of the experiment.

The agronomic parameters assessed were the flowering time, number of flowers per plant, number of pods/plant, yield and harvest index. The number of flowers were determined by counting flowers every week for each treatment until the emergence of the first pods. The number of pods per plant was determined every week for each treatment until harvesting time. The flowering time was gotten by noting the date of first appearance of flower for each treatment. The yield was obtained Yield (t/h) = Total production (tonne)/surface (hectare). The harvest index (HI) was calculated. HI = (WP/(WP+Biomass (shoot and root)) x 100. (Bijalwan & Manmohan, 2014). Where; HI = Harvest Index, WP = Weight of pods.

Statistical analysis

The experiment was performed using the complete randomized design. All data were presented in terms of mean (± standard deviation), statistically analysed using Graph pad Prism version 5.01 and subjected to analysis of Variance (ANOVA). Statistical differences between treatment means were established using the Fisher Least Significant Difference (LSD) at $P < 0.05$. Probability level using Duncan's Multiple Range Test (DMRT).

RESULTS

Plant growth

The growth parameters stem height (SH) and leaf area (LA) were generally influenced by intake doses of NaCl and decreased significantly in the culture medium during four weeks from 100 to 200 mM of NaCl for both treatments (Figure 1). The treatment with AMF significantly increased ($p < 0.001$) leaf area and stem height in all concentrations compared to the treatment with NaCl only in general.

Dry biomass

The dry weight partioning (roots dry weight (RDW) and shoots dry weight (SDW)) was generally influenced by intake doses of NaCl and decreased significantly in the culture medium during four weeks from 100 to 200 mM of NaCl for both treatments (Table 2). The treatment with AMF significantly increased ($p <0.001$) the dry biomass in plant organs in all concentrations compared to the treatment with NaCl only.

Mineral distribution

The Na^+ significantly increased ($p < 0.001$) with increased doses of NaCl in plant partitioning from 100 mM NaCl (Table 3). The others minerals (K^+, Ca^{2+} and Mg^{2+}) showed significant decrease ($p < 0.001$) respectively from 100 mM NaCl. These minerals are generally higher in the shoots compared to the roots. The treatment with AMF significantly increased ($p < 0.001$) the various minerals in plant organs in all concentrations compared to the treatment with NaCl only (Table 3).

Chlorophyll (a+b) content

The chlorophyll (a+b) content was generally affected by the increased doses of NaCl in the culture medium. They significantly sloped down (P

< 0.001) from 100 mM NaCl with increased salinity in the culture medium for both treatments (NaCl only and NaCl + Bio) (Figure 2). The treatment with AMF significantly increases ($p<0.001$) the Chlorophyll (a+b) concentrations in all concentrations compared to the treatment with NaCl only (Figure 2). **Osmolytes**

The proline content, soluble carbohydrates and total phenol content significantly increased ($P < 0.001$) with intake doses of NaCl from 50 mM NaCl compared to the control in all treatments (Figure 3). The NaCl in the culture medium generally influenced the production of flavonoid and significantly decreased ($P <0.001$) with increased salinity compared to the control (Figure 3). The treatment with AMF significantly increases ($p<0.001$) the osmolytes in general in all concentrations compared to the treatment with NaCl only (Figure 3).

Yield components

The agronomic parameters; flowering time, number of flowers per plant, number of pods per plant, yield and Harvest index were influenced significantly with the NaCl in the field (Table 4). The flowering time significantly decreased ($P <0.05$) under 50 mM NaCl in both treatments (NaCl only and NaCl + Bio). The treatment with AMF significantly increases ($p< 0.001$) the flowering time in general compared to the treatment with NaCl only (Table 4). The number of flowers significantly decreased ($P < 0.01$) at 50 mM NaCl in both treatments (NaCl only and NaCl + Bio). The treatment with AMF significantly increases ($P < 0.05$) the number of flowers per plant compared to the treatment with NaCl only. The same results were obtained with the number of pods per plant, yield and Harvest index which significantly decreased ($P < 0.05$); ($P < 0.01$) and ($p<0.001$) respectively at 50 mM NaCl, with favourable results obtained in plants growing with AMF (Table 4).

DISCUSSION

The stem height and leaf area decreased from 100 mM of NaCl due to the negative effects of NaCl which inhibit plant growth and development, negatively affecting photosynthesis and availability of water. According to Kaymakanova & Stoeva (2008), Kosovà et al. (2013); Nouck et al. (2021) salinity induces an imbalance in water potential, cellular dehydration, inhibition of intracellular enzyme activities, cell division and expansion which negatively affects the growth and productivity. In this study, the treatment with AMF increased leaf area and stem height in all concentrations compared to the treatment with NaCl only. These findings corroborate Kaya et al. (2009), Beltrano et al. (2013), Chun et al. (2018). They stated that the association of plants with arbuscular mycorrhizae enabled plants to absorb more water and mineral nutrients and enhanced ability of the plants to resist osmotic imbalances such as physiological drought and toxicity imposed by salinity.

The dry weight partioning decreased in the culture medium from 100 mM of NaCl for both treatments. According to Meguekam et al. (2014), Nouck et al. (2021) is due to limited hydrolysis of food reserved from storage tissue to the developing embryo and a reduction of water uptake. Previous studies show the positive effects of mycorrhiza on plant growth (Beltrano et al., 2013, Elhindi et al., 2017). In our study AMF positively affected the dry biomass in plant organs in all concentrations compared to the treatment with NaCl only and improved the dry weight of shoots and roots. According to Elhindi et al. (2017), Chun et al. (2018) the improved water uptake, mineral nutrition and photosynthetic activities of the plants were directly responsible for increased biomass enhanced by the presence of arbuscular mycorrhizal fungi (AMF).

The results showed that Na^+ increased with increased salinity in both shoots and roots while others minerals (K^+, Ca^{2+} and Mg^{2+}) decreased. According to Nouck et al. (2021), Na^+ in plants increased with increased salinity due to imbalance in the K^+/Na^+ transporters. The ratio is distorted by the high salt concentration in the soil, which enhanced the uptake of Na^+. These results correspond to those of Hand et al. (2017) and Ndouma et al. (2020). They showed that, the competition of Na^+ and K^+ for aerial plants resulted in greater accumulation of Na^+ in the shoots than in the roots, additional it can be caused by the loss of osmotic potential of root medium and the lack of nutritional ions. The quantity of Na^+ continued to increase in the culture medium for biofertilizer with increasing salt concentrations, while K^+, Ca^{2+} and Mg^{2+} decreased with increased salinity and increased significantly with time. Previous researchers Beltrano et al. (2013) and Hashem et al. (2018) explained that the uptake of Na^+ actually reduced comparatively and that due to the increased absorption, it existed in the plants in a diluted state. They also explained that the Na^+ were stored in old leaves which eventually fell off. AMF improved root development and some Na^+ stored in their hyphae. According to Abd_Allah et al. (2015), Hashem et al. (2018), AMF would alleviate the detrimental effects of salinity and stimulated the absorption of K^+, Ca^{2+} and Mg^{2+} so as to reinstate ionic balance. This is because K^+ and Ca^{2+} are involved in energy metabolism and Mg^{2+} is a central component of chlorophyll pigments.

The treatment with biofertilizer has a great effect on Chlorophyll (a+b) content compared to treatment

with NaCl only. These results agree with many previous findings. Abd_Allah et al. (2015); Elhindi et al. (2017) reported that the high chlorophyll contents is due to AMF, which improves water absorption, resulting in greater photosynthetic efficiency, maintenance of nutrient balance, enzyme activity, and more importantly upregulation of the antioxidant defense system.

Accumulation of soluble carbohydrates and proline with intake doses of NaCl enhance plant salt tolerance. These results corroborate Khalil et al. (2016); Nouck et al. (2021). They showed that the production of certain osmolytes under salinity stress is a result of osmotic adjustment, stabilizing and protecting membrane integrity. Previous workers maintained that the accumulation of TP and FLA are physiological responses to plant stress. Their accumulation is a cellular adaptive mechanism for scavenging oxygen free radicals, while maintaining chlorophyll levels and cell turgor to protect photosynthetic activities. Inoculation of AMF in crops has been reported by Yang et al. (2008) to modulate the biosynthesis of important osmoprotectants such as proline and soluble carbohydrates, thereby protecting photosynthesis by improving chloroplastic membrane integrity. in the same line, Ahanger et al. (2014), Hashem et al. (2016) showed that the greater accumulation of osmolytes under salinity conditions due to inoculation with AMF makes AMF to enhance the salt stress tolerance of sensitive plant species. AMF inoculation improves the activity of key antioxidant components (Total phenolic and flavonoids content), developing a strong protective mechanism against the harmful effects of ROS (Beltrano et al., 2013).

Flowering time significantly decreases in the presence of NaCl for both treatments. These results are in agreement with those of Pushpavalli et al. (2014) on chickpea (*Cicer arietinum* L.). According to him, salinity stress not only impairs plant growth but also delays flowering in many species. Shekoofeh et al. (2012) explained that mycorrhizal biofertilizer colonization in salt stressed plants suggests the beneficial role of AMF in enhancing the stress tolerance by contributing to maintenance of cellular water content (Eveline et al., 2012). The number of flowers per plant decreases and Nouck et al. (2016) showed that salinity might have reduced the number of flowers per plant and production of crop by overturning water and nutritional balance of plant and loss of photosynthetic capacity. Under salinity the treatment with mycorrhizal biofertilizer colonization in salt stressed enhance the stress tolerance by contributing to maintenance of cellular water content (Eveline et al., 2012). The number of pods per plant was varied. These results are in agreement with those of Pushpavalli et al. (2014) on chickpea (*Cicer arietinum* L.). According to him; salinity stress not only impairs plant growth but also reduces the number of pods in many species. The application of biofertilizer significantly increases the number of pods per plant Shekoofeh et al. (2012); (Eveline et al., 2012) explained that mycorrhizal biofertilizer in salt stressed enhance the stress tolerance. According to Nouck et al. (2016) the yield could not be affected by low levels of salinity even though the leaf area and the plants biomass are reduced. These results agree with findings of Beltrano et al. (2013); they showed that AMF enhance the stress tolerance by contributing to maintenance of cellular water content, improving productivity. The harvest index results are in consonance with those of Afifi et al. (2003) this promoting effect of biofertilizer could be attributed to the biologically active substance produced by these biofertilizers such as auxins, gibberellins, cytokinins, amino acids and vitamins.

CONCLUSION

The results revealed that *Phaseolus vulgaris* was negatively affected by NaCl stress in the culture medium. The growth parameters, the dry biomass, the mineral uptake, Chlorophyll (a+b) decreased with salinity doses of NaCl from 100 to 200 mM NaCl while Na^+ and metabolites increased from 50 mM NaCl. AMF enhanced the accumulation of all the study parameters than non-AMF plants under salinity. Agronomic parameters were improved from 50 mM NaCl in the treatment with AMF than treatment with NaCl only in the field. The AMF appeared to alleviate the impacts of salinity on the black bean resulting to significant improvements. The high accumulation of metabolites with intake doses of NaCl could be added as indicators of early identification and osmotic adjustment ability for salt-tolerant plants in salt stress conditions. The black bean could be cultivated in the soil with moderate salinity. Using AMF as an alternative way of decreasing the NaCl stress in plants will be more beneficial as it maintains the soil fertility and the yield. There is a need to experiment on several types of biofertilizers and black bean varieties so as to obtain the one that will give maximum yield in salty zones.

REFERENCES

Abd_Allah E F, Hashem A, Alqarawi A A, Alwathnani H A (2015). Alleviation of adverse impact of cadmium stress in sunflower (*Helianthus annuus* L.) by arbuscular mycorrhizal fungi. *Pakistan Journal of Botany*, 47(2):785–795.

Afifi M H, Manal F M and Gomaa A M (2003). Efficiency of applying biofertilizers to maize crop under different levels of mineral

fertilizers. *Annals of Agricultural Sciences Moshtohor*, 41 (4): 1411 – 1420.

Ahanger M A, Hashem A, Ab_Adllah E F and Ahmad P (2014). Arbuscular mycorrhiza in crop improvement under environmental stress. In: Ahmad P, Rasool S (eds) *Emerging technologies and management of crop stress tolerance*, vol 2. Elsevier, New York, pp 69–95.

Arnon D I (1949). Copper enzymes in isolated chloroplasts. Polyphenylodase in *Beta vulgaris*. *Plant Physiology*, 24 (1): 1-15.

Bates L, Waldren R P, Teare I D (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39 (2): 205-207.

Beltrano J, Ruscitti M, Arango M C and Ronco M (2013). Effects of arbuscular mycorrhizal inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. *Journal of Soil Science and Plant Nutrition*, 13(1), 123-141.

Bijalwan A and Manmohan J R D (2014). Productivity of Wheat (*Triticum Aestivum*) as Intercropin *Grewia Optiva* Based Traditional Agroforestry System along Altitudinal Gradient and Aspectin MidHills of Garhwal Himalaya, India."*American Journal of Environmental Protection*, 5(2): 89-94.

Chang C C, Yang M H, Wen H M and Chern J C (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food Drug Analysis*, 10(3): 178-182.

Chun S C, Paramasivan M and Chandrasekaran M (2018). Proline Accumulation Influenced by Osmotic Stress in Arbuscular Mycorrhizal Symbiotic Plants. *Frontiers in Microbial*, 9:2525.

Colla G, Rouphael Y, Cardarelli M, Tullio M, Rivera CM, Rea E (2008). Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biology and Fertility Soils*, 44, 501-509.

Di Ferdinando M, Brunetti C, Fini A, Tattini M. 2012. *Flavonoids as Antioxidants in Plants under Abiotic Stresses*. In: Ahmad P, Prasad MNV. (Eds.), *Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability*. Springer: New York, pp159–179.

Dubois M, Gilles K A, Hamil J K, Rebers P A, Smith F (1956). Colorimetric method for determination of sugars and related substances. *Anal Chemistry*, 28(3): 350-356.

Elhindi K M, El-Din A S and Elgorban A M (2017). The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). *Saudian Journal of Biological Sciences*, 24(1):170–179.

Evelin H, Giri B and Kapoor R (2012). Contribution of Glomus intraradices inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonellafoenum-graecum*. *Mycorrhiza*, 22:203–217.

Ghassemi F, Jakeman AJ, and Nix HA. (1995). Salinisation of Land and Water Resources. CAB International, Wallingford, 526 p.

Grigore M N, Boscaiu M and Vicente O (2011). Assesment of the relevance of osmolytes biosynthesis for salt tolerance of halophytes under natural conditions. *European Journal of plant Sciences and Biotechnology*, 5, 12:19.

Imran H, Asif A, Tariq M, Anwaar A and Shaukat B (2014). Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.). *Critical reviews in food science and nutrition*, 54, 580-592.

Hand M J, Taffouo V D, Nouck AE, Nyemene K P J, Tonfack L B, Meguekam T L and Youmbi E (2017). Effects of Salt Stress on Plant Growth, Nutrient Partitioning, Chlorophyll Content, Leaf Relative Water Content, Accumulation of Osmolytes and Antioxidant Compounds in Pepper (*Capsicum annuum* L.) Cultivars. *Notulae Botanicae Horticulture Cluj-Napoca Agrobotanici*, 45(2): 481-490.

Hashem A, Abd_Allah E F, Alqarawi A A and Egamberdieva D (2016). Bioremediation of adverse impact of cadmium toxicity on Cassia italica Mill by arbuscular mycorrhizal fungi. *Saudian Journal of Biological Sciences*, 23:39–47.

Hashem A, Alqarawi A A, Radhakrishnan R, Al-Arjani A F, Aldehaish H A, Egamberdieva D and Allah E F A (2018). Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. *Saudi Journal of Biological Sciences*? 25(6): 1102–1114.

Hoagland DR, Arnon DI. 1950. *The water-culture method for growing plants without soil*. University of California, College of Agriculture, Berkley, 34p.

Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna A L, Cullu M A (2009). The infuence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. *Science Horticultura*, 121, 1- 6.

Kaymakanova M. and Steova N. (2008). Physiological reaction of bean plants (*Phaseolus vulgaris* L.) to salt stress. *General Applied Plant Physiology*, 34 (3-4) 177-188.

Khalil C, Abdelmajid H, Mohammed HE, Houssein BB (2016). Growth and proline content in NaCl stressed plants of annual medic species. *International Journal of Advanced Research in Biological Sci*ences, 3(9): 82-90.

Khosravinejad F, Heydari R and Farboodnia T (2009). Effect of salinity on organic solute content in Barley. *Pakistan Journal of Biological Sciences*, 12, 158-162.

Kosová K, Prášil I T and Vítámvás P (2013). Protein Contribution to Plant Salinity Response and Tolerance Acquisition. *International Journal of Molecular Sciences*, 14(4): 6757-6789.

Marigo G (1973). On a fractionation method and estimation of the phenolic compounds in Plant. *Analysis*, 2(2): 106-110.

Meguekam T L, Taffouo V D, Grigore M N, Zamfirache M M, Youmbi E, Amougou A (2014). Differential responses of growth, chlorophyll content, lipid perodxidation and accumulation of compatible solutes to salt stress in peanut (*Arachis hypogaea* L.) cultivars. *African Journal of Biotechnology*, 13(50): 4577-4587.

Myers J R, Wallace T L, Mafi SM, Kleintop A E, Echeverria D, Thompson H J, Brick M A, Lee R and McClean P E (2019). Improving the health benefit of snap bean. Genome-wide association studies of total phenolic content. *Nutrients*, 11, 2509.

Nasri N, Saidi I, Kaddour R and Lachaal M (2015). Effect of salinity on Germination, seedling growth and acid phosphatase activity in lettuce. *American Journal of Plant Sciences*, 6(01):57-63.

Ndouma M C, Nouck A E, Titah M A, Ndjouondo G P, Ekwel S S, Fotso, Taffouo V D (2020). Growth parameters, mineral distribution, chlorophyll content, biochemical constituents and non-enzymatic antioxidant compounds of white yam (*Dioscorea rotundata* (L) var. gana) grown under salinity stress. *GSC Biological and Pharmaceutical Sciences*, 12(03): 139-149.

Nouck A E, Taffouo V D, Tsoata E, Dibong S D, Nguemezi S T, Gouado I and Youmbi E (2016). Growth, Biochemical Constituents, Micronutrient Uptake and Yield Response of Six Tomato (*Lycopersicum esculentum* L.). *Journal of Agronomy*, 15 (2): 58-67.

Nouck A E, Hand M J, Numfor E N, Ekwel S S, Ndouma M C, Wiraghan S E and Taffouo V D (2021). Growth, mineral uptake, chlorophyll content, biochemical constituents and non-enzymatic antioxidant compounds of white pepper (*Piper nigrum* L.) grown under saline conditions. *International Journal of Biological and Chemical Sciences*, 15(4): 1457-1468.

Pauwels J M, Van Ranst E, Verloo M, Mvondo Z A (1992). *Analysis Methods of MajorPlants Elements. Pedology Laboratory Manual: Methods of plants and Soil Analysis. Stock Management Equipment of Worms and Chemical Equipment*. AGCD, *Agriculture Publications* : Brussels, 28p.

Pushpavalli R, Zaman A M, Turner C N, Baddam R, Rao M V and Vadez V (2014). Higher flower and seed number leads to higher yield under water stress conditions imposed during reproduction in chickpea (*Cicer arietinum* L.).*Functional Plant Biology.* 42(2) 162-174.

Shekoofeh E, Sepideh H and Roya R. (2012). Role of mycorrhizal fungi and salicylic acid in salinity tolerance of *Ocimum basilicum* resistance to salinity. *African Journal of Biotechnology*, 11: 2223-2234.

Taïbi K, Taïbi F, Abderrahim L A, Ennajah A, Belkhodja M, Mulet J M (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defense systems in *Phaseolus vulgaris* L. *South Africa Journal of Botany*, 105, 306–312.

Taillièz and Ballo Koffi (1992). Une méthode de mesure de la surface foliaire du palmier á huile. *Oléagineux*, 47(8-9): 537-553.

Yang X, Liang Z, Wen X and Lu C (2008). Genetic engineering of the biosynthesis of glycine betaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. *Plant Molecular Biology*, 66,73–86.

Table 1 : Physico-chemical properties of the soil taken from 0-20 cm depth of the experiemental site in Bambili, Cameroon

Properties	**Values**

Fine sand (%)	17.66±1.52
Coarse sand (%)	16.33±0.57
Fine silt (%)	15.16±0.76
Coarse silt (%)	15.66±1.15
Clay (%)	36.66±1.52
Moisture content (%)	14.37±2.37
porosity (%)	38.33±1.52
pH water	5.45±0.2
pH kcl	4.93±0.05
Organic carbon (%)	5.04±0.05
Organic mater (%)	8.69±0.20
Nitrogen (%)	1.88±0.07
Ratio C/N	2.68±0.03
Exchangeable cations (cmol + kg^{-1})	0.2±0.04
Cation Exchange capacity (cmol + kg^{-1})	11.23±0.25
Phosphoros (ppm)	63.73±0.25
Potassium (g kg^{-1})	0.01±0.01
Calcium (g kg^{-1})	2.22±0.07
Magnesium (g kg^{-1})	1.35±0.14
Sodium (g kg^{-1})	0.04±0.01
Sulfur (g kg^{-1})	3.56±0.03
Iron (g kg^{-1})	117.97±6.90
Conductivity (mS/cm)	0.08±0.01

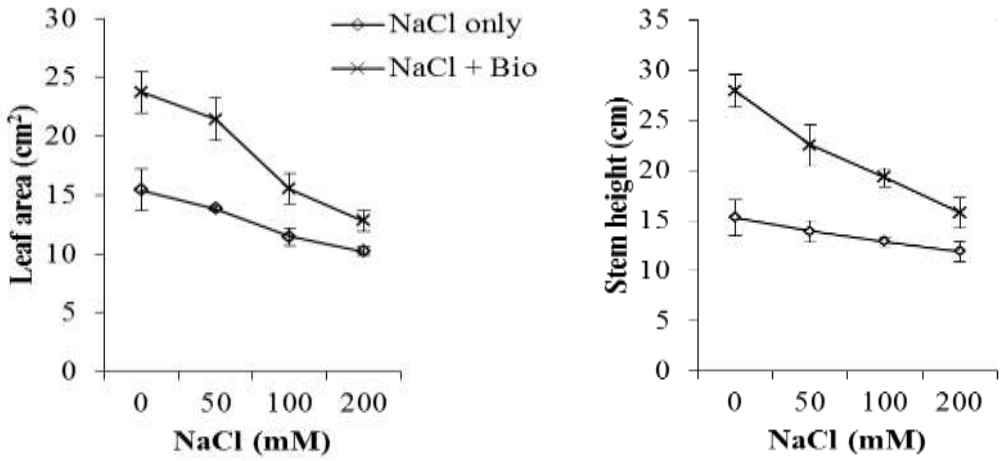

Figure 1: Effects of arbuscular mycorrhizal biofertilizer application on growth parameters in black bean after four weeks of treatment at different salt concentrations.

Table 2: Effects of arbuscular mycorrhizal biofertilizer application on dry biomass (g) in black bean after four weeks of treatment at different salt concentrations.

		Concentration	Dry weight (g)		
Cultivar	Treatment	mM NaCl	RDW	SDW	Total DW
		0	1.62±0.04a	2.46±0.05a	4.08±0.08a
	NaCl only	50	1.36±0.07b	2.16±0.09b	3.52±0.09b
		100	0.92±0.05c	1.65±0.05c	2.58±0.4c
Dor 701		200	0.46±0.04d	1.09±0.07d	1.55±0.06d

		0	2.10±0.05e	2.98±0.07e	4.88±0.46e
	NaCl +Bio	50	1.86±0.04f	2.62±0.05f	4.47±0.06f
		100	1.46±0.06b	2.14±0.05b	3.59±0.06b
		200	0.95±0.53c	1.61±0.02c	2.58±0.08c
Two way ANOVA Result					
NaCl			*	*	*
Biofertilizer			*	**	***
NaCl×Biofertilizerer			*	*	**

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different ($P < 0.05$). (* = $P < 0.05$; ** = $P < 0.01$ and *** = $P < 0.001$).

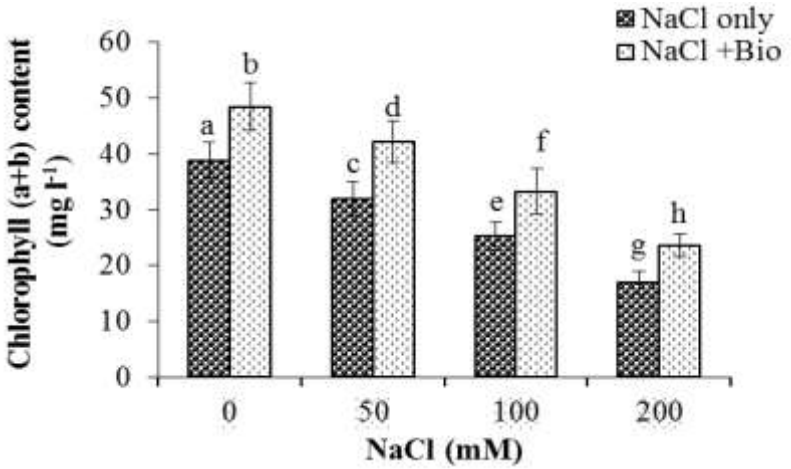

Figure 2: Effects of arbuscular mycorrhizal biofertilizer application on growth parameters in black bean after four weeks of treatment at different salt concentrations. Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different ($P < 0.05$).

Table 3: Effects of arbuscular mycorrhizal biofertilizer application on mineral uptake (µg g^{-1} MS) after four weeks at different salt concentrate. Mean results of five

Cultivar	Treatment		Concentration	Mineral uptake (µg g^{-1} MS)				
			mM NaCl	Na^+	K^+	Ca^{2+}	Mg^{2+}	K^+/Na^+
		Roots	0	1.21±0.03a	8.46±0.79a	14.74±2.09a	46.23±4.41a	4.02a
			50	2.11±0.06b	5.25±0.04b	10.64±0.97b	37.59±3.90b	2.5b
	NaCl only		100	3.79±0.06c	2.34±0.04c	4.17±1.10c	27.58±2.51c	0.61c
			200	5.2±0.05d	1.21±0.04d	2.68±0.35d	20.61±2.63d	0.23d
		Shoots	0	2.15±0.03b	24.53±1.30e	37.03±2.11e	17.55±2.24e	11.68e
			50	4.24±0.04f	20.83±1.84f	31.46±1.84f	13.61±1.55f	4.92f
Dor 701			100	7.95±0.55g	16.55±1.44g	23.72±2.31g	8.28±0.80g	2.08g
			200	15.36±1.05h	11.12±0.19h	17.85±2.20h	4.59±0.67h	0.72h
		Roots	0	1.56±0.07i	11.79±0.87h	17.98±1.88h	20.50±2.31d	7.55i
			50	2.79±0.07j	6.42±0.08j	13.62±0.73a	7.87±0.91g	2.3b
	NaCl +Bio		100	4.57±0.08k	3.8±0.07k	8.99±0.52k	12.67±0.82f	0.83h
			200	6.44±0.09l	1.84±0.05l	5.53±0.46c	8.2±0.68g	0.28d
		Shoots	0	1.88±0.04m	29.62±1.29m	46.4±5.66m	55.54±3.4m	15.83m
			50	5.31±0.07d	24.29±1.49e	38.18±2.36e	46.24±3.81a	4.59n
			100	11.73±0.67o	19.63±1.67f	28.53±2.04o	34.9±3.0o	1.67o
			200	20.09±1.69p	16.01±1.05g	22.69±1.97g	27.70±1.20c	0.79h
Two way ANOVA Result								
NaCl				**	*	*	*	*
Biofertilizer				**	*	*	*	*
NaCl×Biofertilizerer				*	*	*	*	*

replications ± SD; within each column, mean followed by the same letter are not significantly different ($P < 0.05$). (* = $P < 0.05$ and ** = $P < 0.01$

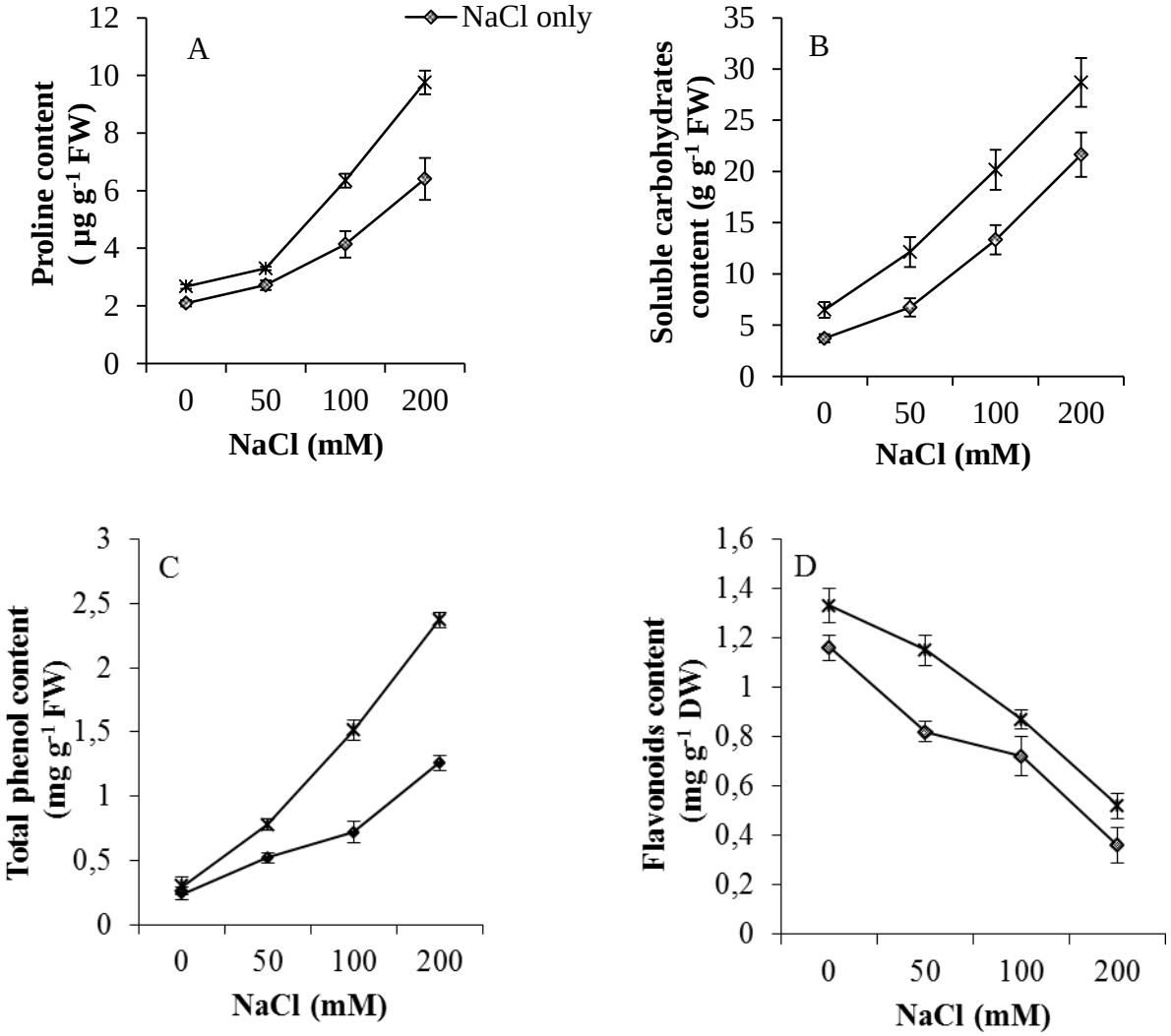

Figure 3: Effects of arbuscular mycorrhizal biofertilizer application on metabolites in black bean after four weeks of treatment at different salt concentrations. A: Proline content; B: Total soluble carbohydrates; C: Total phenol content and Flavonoids content. Bars are means (n= 5) ± SD.

Table 4: Changes in yield components measured as flowering time; number of flowers per plant; number of pods per plant; yield and Harvest index after addition of NaCl at 0 (control) or 50 mM and arbuscular mycorrhizal biofertilizer application.

			Yield components				
Cultivar	**Treatments**	**Concentrations (mM NaCl)**	**Flowering time (week)**	**Number flowers per plant**	**Number of pods/plant**	**Yield (t/hectar)**	**Harvest index (%)**
Dor 701	NaCl only	0	8.33±0.40a	28.33±1.75a	24.83±2.13a	2.24±0.03a	0.69±0.01a
		50	7.1±0.40b	21.33±1.21b	18.33±1.75b	1.36±0.09b	0.57±0.03b
	NaCl + Bio	0	10.83±0.14c	39.5±1.83c	30.16±3.18c	3.21±0.18c	0.76±0.05c
		50	9.5±0.54d	33.16 ±1.87d	36.33±2.73d	2.66±0.06a	0.72±0.06a
Two way ANOVA Result							
NaCl			*	*	*	*	*
Biofertilizer			*	*	*	*	*
NaCl×Biofertilizerer			*	*	*	*	*

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different ($P < 0.05$). (* = $P < 0.05$ and ** = $P < 0.01$.