GROWTH STIMULATION OF AKBAR VARIETY OF WHEAT IN SALINE SOIL THROUGH SALINITY TOLERANT RHIZOSPHERE BACTERIAS.

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خلاصہ

باٹنی ڈیپارٹمنٹ، نصرت جہاں کالج، ربوہ چناب نگر کی طرف سے کی جانے والی یہ تحقیق نمک روادار بیکٹیریا ہیلوموناس اور بیسیلس کے اثرات گندم کی ورائٹی اکبر پر کی گئی ہے۔ یہ ورائٹی ایوب ریسرچ سینٹر فیصل آباد سے لی گئی تھی جس کے بیجوں کو نمکیات والی مٹی میں اگایا گیا تھا۔ بیجوں کو مٹی میں دبانے سے پہلے چناب نگر سے نمکیات والی مٹی کو لیا گیا اور چار بیکٹیریل ٹریٹمنٹس میں تقسیم کیا گیا۔ ٹریٹمنٹ نمبر 1: ٹی0: 830 گرام مٹی لی گئی جس میں کوئی بیکٹیریا شامل نہیں کئے گئے، ٹریٹمننٹ نمبر 2:؛ٹی1: 830 گرام مٹی لی گئی جس میں ہر بیکٹیریا کی دو کالونیاں شامل کی گئیں، ٹریٹمنٹ نمبر 3: ٹی2: 830 گرام مٹی لی گئی جس میں چار کالونیاں شامل کی گئیں اور ٹریٹمنٹ نمبر 4 : ٹی3: 830 گرام مٹی جس میں چھ کالونیاں شامل کی گئیں۔ ہر ٹریٹمنٹس کے چار ریپلیکیٹس بنائے گئے۔ بیکٹیریل ٹریٹمنٹس کے بعد ہر ٹریٹمنٹ کے ہر ریپلیکیٹ میں گندم کے چار بیج بوئے گئے۔ سیڈلنگز کو بوائی کے اکتیسویں دن کاٹا گیا۔ کٹائی کے بعد تنے اور جڑوں کو علیحدہ علیحدہ پیسا گیا سینٹری فیوجڈ کیا گیا اور پھر پچاس ملی مولر پوٹاشیم فاسفیٹ بفر میں محفوظ کیا گیا۔ ان تمام محفوظ میٹیریلز کے مختلف سٹریس فزیالوجیکل ٹیسٹ کئے گئے۔ مزید یہ کہ مٹی کو بوائی سے پہلے اور کاشت کے بعد مائکروبائیولوجیکلی اور فزیالوجیکلی اینالائز کیا گیا۔

Abstract:

Botany Department, Nusrat Jahan College, Rabwah, Chenab Nagar conducted a research activity on examining the impact of salt tolerant rhizosphere bacteria: “Halomonas” and “Bacillus” (taken from National agriculture research center “NARC” Islamabad) on “AKBAR” wheat variety (taken from AYUB Research Center Faisalabad) grown in saline soil. Saline soil of Chenab Nagar was taken and was divided into four sets of bacterial treatments before seed sowing: T0=830g soil with no bacterial mixing, T1=2x10^{-2} CFU/ml of each bacteria mixed with 830g soil.T2=4x10^{-2} CFU/ml of each bacteria mixed with 830g soil and T3=6x10^{-2} CFU/ml of each bacteria mixed with 830g soil in containers. All treatments had 4 replicates each. After bacterial treatments, four seeds of wheat per container were sown in all treatments and their replicates. Wheat seedlings were harvested at 31st day of sowing. After harvest wheat shoots and roots were separately grinded, centrifuged and preserved in 50mM potassium phosphate buffer. The preserved material was used for various stress physiological tests. Soil was physiochemical and microbiologically analyzed before sowing and after harvest. Data of all types of tests was statistically analyzed by R studio 7.01.

Keywords: salinity, wheat, salt tolerant bacteria, physiochemical tests.

Introduction:

Increased environmental pollution including soil and water salinization, and depletion of resources of water (Shahbaz and Ashraf, 2013) as well as global climate change (Battisti and Naylor, 2009) are the major issues that marked the beginning of 21st century. According to an estimation the world current population is about 7 billion which will increased up to 8.9 billion approximately by 2050(Singh *et al*., 2011). Thus for feeding this growing population, sustainability of agriculture is an important matter. This sustainability is threatened by two things i.e. one is increase in population of humans and another is reduction in land for cultivation. (Shahbaz and Ashraf, 2013). This reduction in land area available for cultivation with reduction in both productivity and quality of crops is mainly due to soil salinization which is one of the most destructive a biotic environmental stess (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013).

According to Munns, 2005 and Jamil *et al*., 2011, a soil having 4 dSm-1 (which approximately equals to 40mM NaCl) the electrical conductivity (EC) of the saturation extract (ECe) in the root zone at 25°C with 15% exchangeable sodium is known as saline soil. ECe of soil may affect the crop yield and the yield may decrease at lower ECes. It is estimated that the salinized area would increase by the year 2050 by conversion of 20% of total the cultivated, 33% of irrigated agricultural and more than 50% of the arable land to salinized land worldwide. Moreover there are many abiotic factors which are responsible for annually 10% increase in area that has suffered from salinization. These abiotic factors are low precipitation, high surface evaporation, native rocks weathering, irrigation with saline water, and poor cultural practices (Jamil *et al*., 2011). Germination of seed, growth of plant either vegetative or reproductive and photosynthesis are negatively affected by soil salinity. Moreover toxicity of ions, osmotic as well as oxidative stress on plants, and deficiency of nutrients (N, CA, K, P, Fe, Zn) are some of the issues related to salinization and these are the factors that also limits uptake of water from soil (Akbarimoghaddam *et al*., 2011; Singh and Chatrath, 2001; Bano and Fatima, 2009; Netondo *et al*., 2004; Shrivasata, P., and Kumar, R., 2015).

For more than one-third of the world population including Asia, wheat is the major staple food crop (Shirazi *et al*., 2001; Godfray *et al*., 2010). In low- and middle-income countries like India, Nepal, Pakistan, and Bangladesh, etc. wheat ranks firstly as a source for calories and secondly for proteins (Joshi *et al*., 2007; Joshi *et al*., 2007; Joshi *et al*., 2011). It is estimated that in Pakistan 8.2 mha is the total production area for crops where the average yield is 2170 kg/hectare (Anonymous, 1999) and 2660 Kg//h is average wheat production (FAO, 2005).

Soil salinity may depress yield of wheat grains (Mehmet *et al*., 2006). While seed germination and its speeding growth is negatively affected by many factors like droughts (Passioura, J.B., 1988), salinity stresses (Hampson C.R., and Simpson, G.M., 1990) and soil sodicity (Singh, C. *et al*., 2008; Singh, R.P. *et al*., 2015). Whereas the reduction in germination is mainly due to salts high concentration and its toxicity that inhibits the uptake of water for seed (Mehmet *et al*., 2006, Khajeh-Hosseini *et al*., 2003).

Basically there are two kinds of strategies i.e. technological and biological that is used to overcome salinization stress (Ashraf and Foolad 2013). Thus coping salinity stress either by inculation of plant growth-promoting (PGP) (Bacilio *et al*. 2004; Shrivastava and Kumar 2015) or through salt tolerant bacteria, (Nanis G. Allam *et al*., 2018) both are appropriate biological strategy for growth of plants.

The main objective of this research paper is also to investigate the impact of salt-tolerant plant growth-promoting microbes i.e. Halomonas and Bacillus bacterias on the wheat crop at seedling stage.

Materials and methods

Saline soil of Chenab Nagar was taken and was divided into four sets of bacterial treatments before seed sowing: T0=830g soil with no bacterial mixing, T1=$2x10^{-2}$CFU/ml of each bacteria mixed with 830g soil,T2=$4x10^{-2}$CFU/ml of each bacteria mixed with 830g soil and T3=$6x10^{-2}$ CFU/ml of each bacteria mixed with 830g soil in containers. All treatments had 4 replicates each. After bacterial treatments, four seeds of wheat per container were sown in all treatments and their replicates. Wheat seedlings were harvested at 31^{st} day of sowing. After harvest wheat shoots and roots were separately grinded, centrifuged (at 14000rpm for fifteen minutes) and preserved in 50 mM potassium phosphate buffer. The preserved supernatants were used for various stress physiological tests. Soil before sowing and after harvest was analyzed microbiologically and physiochemically.

Fresh and dry weights of roots and shoots: The fresh weights of roots and shoots were measured. Also dry weights of roots and shoots were noted.

Root and shoot length: Roots and shoots lengths of all wheat seedlings were measured.

Malondialdehyde (MDA) test: For this test 2ml 0.6% thiobarbituric acid (TBA), 2ml TCA and 2ml of each supernatant were mixed and heated at 100°C for 20 minutes in water bath. After that the solution was allowed to cool for 4 to 5 min and the reading of each sample was checked through spectrophotometer at 532nm (Dhindsa etal. 1981).

Ascorbate peroxidase (APX) test: In APX test 0.8 ml supernatant, 1.6ml 0.5mM ascorbic acid and 0.6ml H2O2 were mixed and the activity of each supernatant was noted at 290nm through spectrophotometer (Asada and Takahashi 1987).

Catalase test: In catalase test 0.2ml supernatant and 0.8ml H2O2 were taken to record the catalase activity at 240nm by spectrophotometer (Chandlee and Scandalios, 1984).

Soil analysis: The values of pH, electrical conductivity (EC) and TDS of original soil sample as well as all soil samples of each replicate for each treatment after harvest were recorded via potable pH, EC and TDS meter.

Bacterial count: The drop of water from soil solution of each replicate was poured over MacConkey media in petri plates and left for 24 hours. After 24 hours bacterial growth was checked and number of colonies was counted.

Results and Discussion

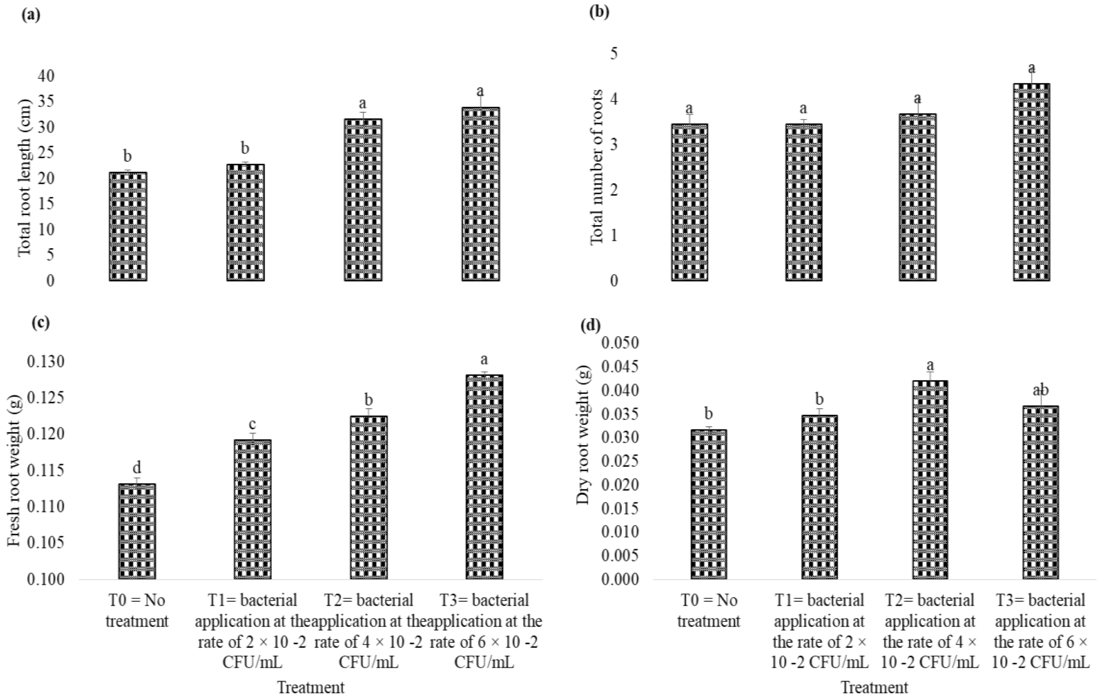

Fig.1. a-d: Root attributes of wheat seedlings under control conditions and bacterial treated saline conditions.

Total root length: Significantly increased in all three bacterial treatments as compared to control group, showing the reduction in the impact of Halomanas and Bacillus bacteria on soil salinity. In comparison to all three bacterial treatments, T3=6x10^{-2} CFU/ml showed maximum total root length.

Total number of roots: were also increased in all bacterial treatments and reduced number of roots were seen in control group, dipicting that due to absence of Halomonas and Bacillus bacteria roots have been harmed by salt stress while treatment groups have overcome harms of salt stress. Significantly highest number of roots was observed in T3 treatment 6× 10^{-2} CFU/ml.

Total fresh root weight: was also significantly enhanced in T1, T2 and T3 from T0, with largest fresh root weight observed in T3 treatment.

Total dry root weight: was also comparatively larger in T1, T2 and T3 treatments. Significantly highest increase in total dry weight of roots was obtained in T2 treatment.

Shoot length: Treatment T3=6× 10^{-2} CFU/ml showed maximum advancement in shoot length while T0 group showed least length of shoots.

Fresh shoot weight: Fresh shoot weight of all three bacterial treatments was observed to be increased as compared to control group. Largest increment in shoot fresh weight was recorded in T3 treatment.

Dry shoot weight: results of dry shoot weight reveal an increase in all bacterial treatments in comparison to control group with highest dry shoot weight recorded in third treatment.

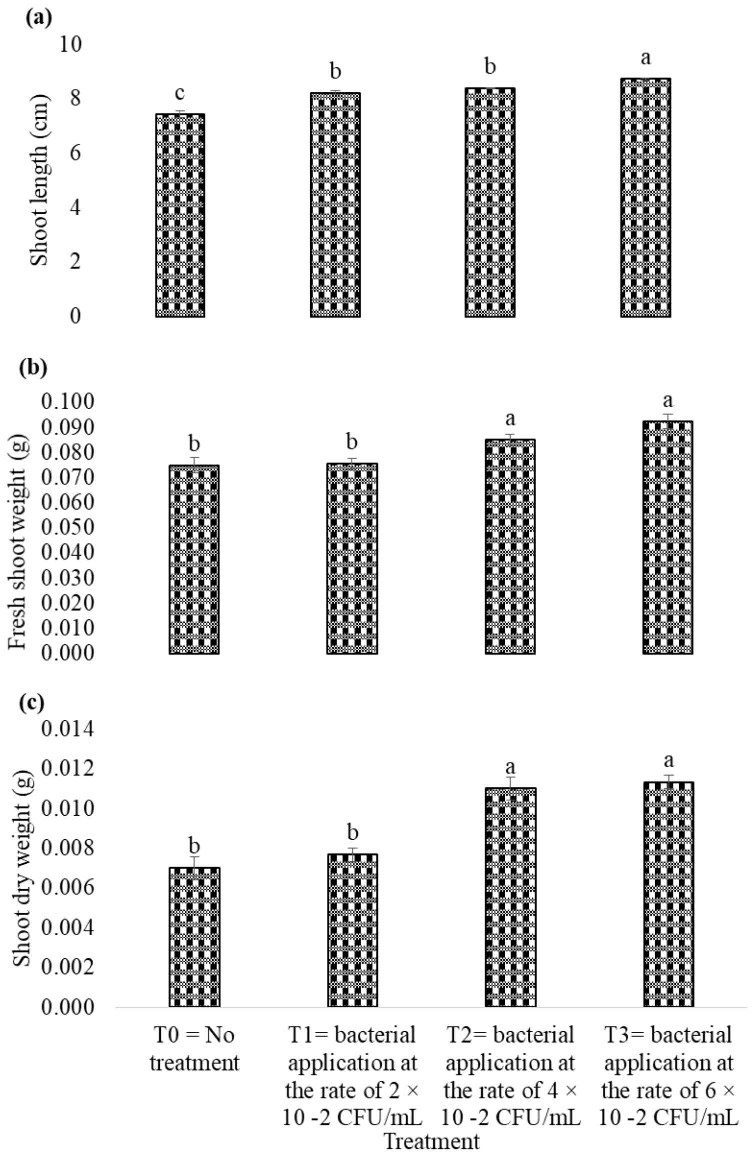

Fig.2. a-c: Shoot attibutes of wheat seedlings under control conditions and bacterial treated saline conditions.

MDA test: According to figure 3b malondialdehyde (MDA) content have been significantly reduced in both roots and shoots of all three bacterial treatments which increased in control group. This illustrates that treatment of Halomonas and Bacillus bacteria have protected wheat seedlings from adversity of soil salinity by reducing it.

APX activity: In accordance to results revealed from figure 3a, enhanced ascorbate peroxidase activity have been seen in roots and shoots of bacterial treatments T1, T2 and T3 and reduced APX activity in control group. Maximum increase was recorded in T2 treatment among all bacterial treatments.

Catalase activity: Figure 4 demonstrates increased catalase activity in bacterial treatments T1, T2 and T3 while in control group without any bacterial treatment reduced catalase activity have been seen. According to the results of this test it is evident that Halomonas and Bacillus bacteria have stimulated catalase enzyme to eradicate salinization effects by denaturing hydrogen peroxide (reactive oxygen species) generated during salt stress.

Soil analysis: According to results provided by Table 1, pH and EC values have been decreased in soils treated with bacterias as compared to control group. Number of bacterial colonies increased in bacterial treated soils.

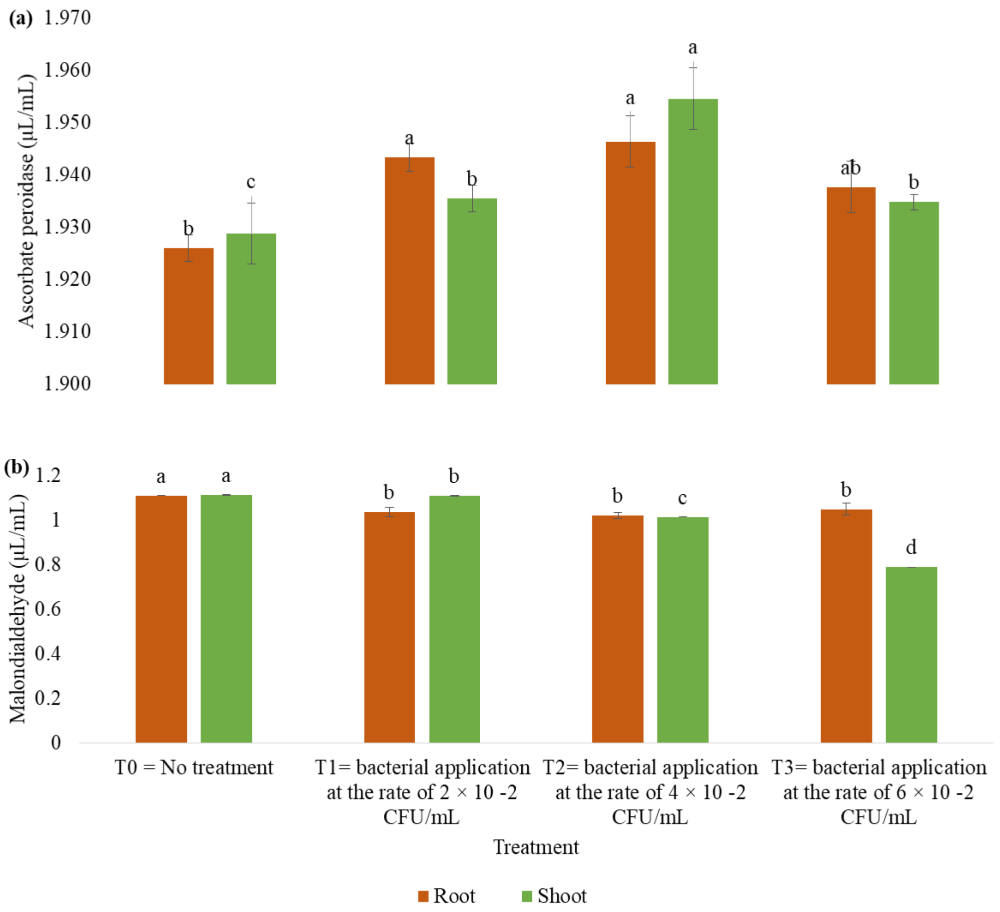

Fig.3. a: Ascorbate peroxidase (APX) activity in roots and shoots of control group and bacterial treatments in saline soil. 3b: Malondialdehyde (MDA) content in roots and shoots of control group and bacterial treatments in saline soil.

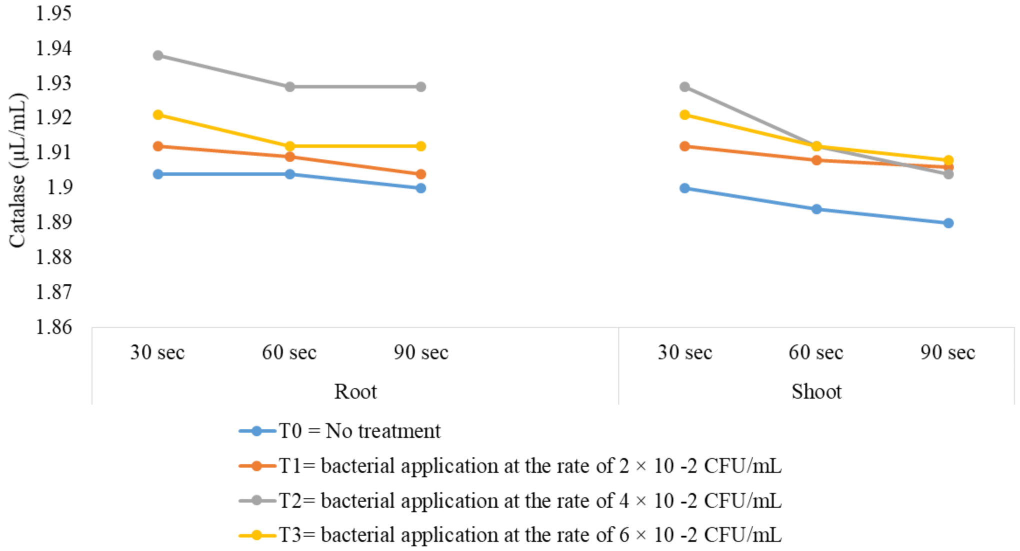

Fig.4. Catalase activity in roots and shoots of control group and bacterial treatments in saline soil.

Table 1. Results of soil analysis showing pH, EC values and bacterial colonies.

Treatment	pH	Electrical conductivity	Bacterial colonies CFU/mL
Before sowing	8.37 ± 0.03	657 ± 1.42	18 ± 0.88
After harvest			
T0 = No treatment	7.90 ± 0.06	642 ± 0.56	0 ± 0.00
T1= bacterial application at the rate of 2 × 10 $^{-2}$ CFU/mL	7.80 ± 0.06	535 ± 1.13	26 ± 0.33
T2= bacterial application at the rate of 4 × 10 $^{-2}$ CFU/mL	7.70 ± 0.06	525 ± 0.86	88 ± 0.88
T3= bacterial application at the rate of 6 × 10 $^{-2}$ CFU/mL	7.90 ± 0.06	536 ± 0.56	137 ± 0.56

According to previous studies, the improved growth in terms of biomass accumulation i.e. dry weight of plants (Bharti, N., *et al*., 2016; Singh UB, *et al*., 2021; Ansari, M. *et al*., 2019), root weight (Ansari, M. *et al*., 2019), root length (Bharti, N., *et al*., 2016; Ansari, M. *et al*., 2019), when inoculated with plant growth promoting bacteria in saline environment were reported.

Moreover reduction in MDA content, under inoculation of PGPB in saline environment in a correlation with lipid peroxidation to better stress tolerance were also reported (Bharti, *et al*., 2014; Singh UB, *et al*., 2021). Under abiotic stresses the reactive oxygen species (ROS) formation is stimulated by plants. The production of biomolecules like lipids, proteins and nucleic acid can be harmed by these ROS (Gill *et al*., 2010). Moreover ROS is majorly responsible for peroxidation of membrane lipid. To detect peroxidation of membrane lipid in plants, MDA is measured which is used as a marker of oxidative lipid injury due to environmental stress (Kong *et al*., 2016).

The increase in ascorbate peroxidase activity and catalase activity were also reported in previous studies (Bharti, N., *et al*., 2016; Singh UB, *et al*., 2021).

According to Singh UB, *et al*, 2021, a direct correlation is observed among the salt tolerance and related accumulation of biomolecules, peroxidation of lipids, antioxidant activity of enzymes, germination of seedlings, vigour indices, growth attributes and accumulation of biomass, while studying the plants under conditions of salt stress inoculated with PGPB.

The increased antioxidants production such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (AsA), glutathione (GSH), carotenoids, tocopherols and phenolics secures cell, membrane and biomolecules by reducing the damaging effects of ROS by microbial inoculants (Gouda *et al*., 2018; Arora *et al*., 2020). Results of this current research pertaining to plant biomass and antioxidant activites are in accordance to above mentioned previous findings.

Conclusion

Hence in view of all results of current research it is concluded that application of salt tolerant bacteria “Halomonas” and Bacillus” to saline soil is very effective in reducing salt stress and growing crops.

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