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Effect of PGPR on catalase activity of Indian mustard under saline stress

Meenakshi Nandal[1], Pragya mahajan[3], *Rajni hooda[2]

[1]*Assistant Professor Department of Environmental sciences M.D.U Rohtak India*

[2]*Research Scholar Department of Environmental sciences M.D.U Rohtak India*

[3]*Post graduate student Department of Environmental sciences M.D.U Rohtak India*

***Corresponding author: Meenakshi Nandal**

E mail: rachna_1310@yahoo.co.in

ABSTRACT

Plant Growth Promoting Rhizobacteria (PGPR) plays an important role in agricultural systems, especially as biofertilizer. Inoculation of plant material with PGPR can protect plants against salt stress through an increase in plant growth parameters and the regulation of ion concentration and antioxidant enzymes. Salinity produces oxidative stress by altering the general metabolic processes and enzymatic activities and enhanced occurrence of damaging toxic molecule i.e. reactive oxygen species (ROS). Plants can run away the damaging effects of reactive oxygen species by developing a physiologically powerful defence system together with antioxidant enzymes like CAT, POD and SOD. Catalase, which is involved in the degradation of H_2O_2 into water and oxygen, is the major H_2O_2 scavenging enzyme in all-aerobic organisms. Catalase is critical for maintaining the redox balance during oxidative stress and functions as a cellular sink for H_2O_2. Microbial inoculation to alleviate salinity stress could provide an innovative and cost-effective alternative to overcome salinity stress in soils. The objective of the present study was to evaluate the H_2O_2 scavenging effects of beneficial bacterial strains of *Bacillus* species under saline stress in three different varieties of Indian mustard.

Keywords: Catalase, Salt stress, Antioxidant enzymes, Reactive oxygen species (ROS), Plant Growth Promoting Rhizobacteria (PGPR).

INTRODUCTION

Salt stress has become one of the most damaging environmental hazards to crop productivity all over the world ([2]) by decreasing the photosynthetic capacity due to the osmotic stress and partial closure of stomata ([9]). Plants can suffer from membrane destabilization and general nutrient imbalance ([20] and [37]).Salinity alters general metabolic processes and enzymatic activities, causing increased production of reactive oxygen species ([29]).To counteract the toxicity of reactive oxygen species (ROS's), a highly efficient antioxidative defense system, including both nonenzymic and enzymic constituents, is present in plant cells. ROS's are partially reduced forms of atmospheric oxygen, which are produced in vital processes such as photorespiration, photosynthesis and respiration ([31] and [47]). The formation of ROS is

prevented by an antioxidant system: low molecular mass antioxidants (ascorbic acid, glutathione, tocopherols), regenerating the reduced forms of antioxidants and ROS-interacting enzymes such as SOD, peroxidases and catalases ([4]). Activation of the plant antioxidant system by H_2O_2 plays an important role in induced tolerance against salt stress ([15]). Both ROS and soluble antioxidants are involved in signaling processes in plants: the picture that is emerging suggests that relatively stable oxidants (H_2O_2) and antioxidants (ascorbate, glutathione) act as sensors of the 'oxidative load' on the cell ([33]). The antioxidant systems, including antioxidants and antioxidant enzymes, can ameliorate the deleterious effects of ROS in vivo and in vitro. Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) function, by catalyzing the decomposition of oxidants and free radicals. The balance between ROS generation and eradication determines the survival of the system ([25] and [16]). The activities of these antioxidant enzymes are reported to increase under various environmental stresses ([22] and [23]), and salt-tolerant cultivars generally show higher activity of these antioxidant enzymes as compared to salt-sensitive ones ([39] and [44]). Activities of antioxidant enzymes (SOD, CAT and POX) have been proved to be very effective in discriminating the oilseed rape cultivars to salt tolerance ([2]).Under salinity stress conditions, a constitutively high antioxidant capacity can prevent damages due to ROS formation ([19]).

Conventional farming practices that warrant high yield and quality require intensive use of chemical fertilizers, which are costly and have a high pollution effect ([36]). Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices ([11]). The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides and supplements. Some PGPR have been produced commercially as inoculants for agriculture to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity ([35] and [3]). Many environmental stresses affect crop productivity and impair electron transport system leading to the formation of activated oxygen, such as H_2O_2, O-2 and OH-, which may accumulate and damage the photosynthetic apparatus ([21]). In plants, the highly energetic reactions of photosynthesis and an abundant oxygen supply make the chloroplast a particularly rich source of reactive oxygen intermediates ([1]). To protect against oxidative stress, plant cells produce both antioxidant enzymes such as superoxide dismutase, peroxidase and catalase as well as non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol ([18]). Levels of antioxidant enzyme activity and antioxidant concentrations are frequently used as indicators of oxidative stress in plants. ([24]) found that inoculation of plant material with PGPR can protect paddy plants against salt stress through an increase in plant growth parameters and the regulation of ion concentration and antioxidant enzymes. Salinity produces oxidative stress by the enhanced occurrence of damaging toxic molecule i.e. reactive oxygen species (ROS) ([51]). Evidence suggests that membranes are the primary sites of salinity injury to cells and organelles ([6]) because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasmalemma or intracellular organelles ([6]). Plant cells produce antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), and nonenzymatic antioxidants such as ascorbate, glutathione, and α-tocopherol to protect the cells against oxidative stress ([7]). Activation of the plant antioxidant system by H2O2 plays an important role in induced tolerance against salt stress ([15]).

Catalase is unique among H2O2–degrading enzymes, as it not requires a reductant in order to catalyze H_2O_2 dismutation, the reaction of H_2O_2 removal being performed using a very energy-efficient mechanism ([41] and [30]). Although they have low substrate affinities at low substrate concentrations, catalases have a very fast turnover rate and a very high specificity for H_2O_2, characteristics that distinguish them from other peroxide-metabolizing enzyme ([36]). Catalase is critical for maintaining the redox balance during oxidative stress. Catalase functions as a cellular sink for H_2O_2 ([49]). Exposure to oxidative stress has been reported to cause a decrease in catalase activity in wheat as well as rice seedlings ([43]). ([38]) suggested the active involvement of at least catalase and peroxidases among the H_2O_2 scavenging enzymes in salt tolerant plants.

In the present research three mustard cultivars were selected (S_A, S_B, S_C), and were grown

hydroponically. Mustard seedlings were treated with Hoagland's solution along with salt tolerant isolated bacteria and NaCl treatments of 50, 100, 150 and 200 mM were applied, together with a control, for a period of 10 days. With the elapse of this period, the activity level of catalase was measured. The objective of present research was to study effect of PGPR treatments on enzymatic traits under salt stress condition.

MATERIALS AND METHODS

Bacterial isolation- Two salt tolerant bacterial strains were isolated from soil samples from saline areas of Rohtak district, Haryana. Bacterial Strains were cultured on nutrient agar plates by streaking and incubating at 37ºc for 24-48 hrs. amended with different NaCl (50, 100, 150 and 200 mM) concentrations.

Treatment of seeds-

Three different varieties of Mustard seeds (S_A, S_B, S_C) were surface sterilized with 0.1% $Hgcl_2$ before infection with selected bacterial strains. For infection with seeds, bacterial strains were grown on nutrient broth for 24-48 hrs in BOD shaker. The broth was centrifuged at 10,000 rpm for 1-2 min. The supernatant was discarded and pellet was suspended in MS media. Now the seeds were immersed in the above media for 24 hrs on shaker. Infected seeds (20) were placed on Petri dishes lined with two layers of filter paper supplied with different salt concentrations (50, 100, 150 and 200 mM) together with a control, for a period of 10 days.

Making of Enzyme Extract

Samples (0.5g) roots as well as shoots were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay ([10]).

CAT Assay

Total catalase activity was measured spectrophotometrically according to the method of ([27]), by monitoring the decline in absorbance at 240 nm due to H_2O_2 consumption. The 3 ml reaction mixture contained 66 mM sodium phosphate buffer (pH 7.0) and 30% H_2O_2 (the absorbance should be about 0.5 at 240 nm and with a 1 cm light path). The reaction was initiated by adding an appropriate dilution of the shoot or root crude extract to this solution. CAT activity was expressed as units per mg of protein, one unit being the amount of enzyme which liberates half the peroxide in 100 s at 25°C ([27]).

Extra cellular enzyme Catalase activity in bacterial strains-

Catalase test was performed by taking a 3- 4 drops of hydrogen peroxide (H2O2) was added to 48 hrs old bacterial colony which is grown on trypticase soya agar medium. The effervescence indicated catalase activity ([42]).

RESULT AND DISCUSSIONS

CAT is an important antioxidant enzyme that converts H_2O_2 to water in the peroxysomes ([28]). In this organelle, H_2O_2 is produced from β-oxidation of fatty acids and photorespiration ([32]). Plants with higher capacity for scavenging of H_2O_2 have higher resistance against oxidative stress .Higher activity of CAT and APX decrease H_2O_2 level in cell and increase the stability of membranes and CO_2 fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H_2O_2. A high level of H_2O_2 directly inhibits CO_2 fixation ([50]). The significant increase in catalase activity may be due to H_2O_2 which is one of the ROS produced in response to different environment stress including salt and ionic stress and there is significant reduction of shoot lengths caused by salts stress. These antioxidant enzymes show a role in imparting tolerance against salt stress and any type of environmental stress. ([45]).

Catalase in roots

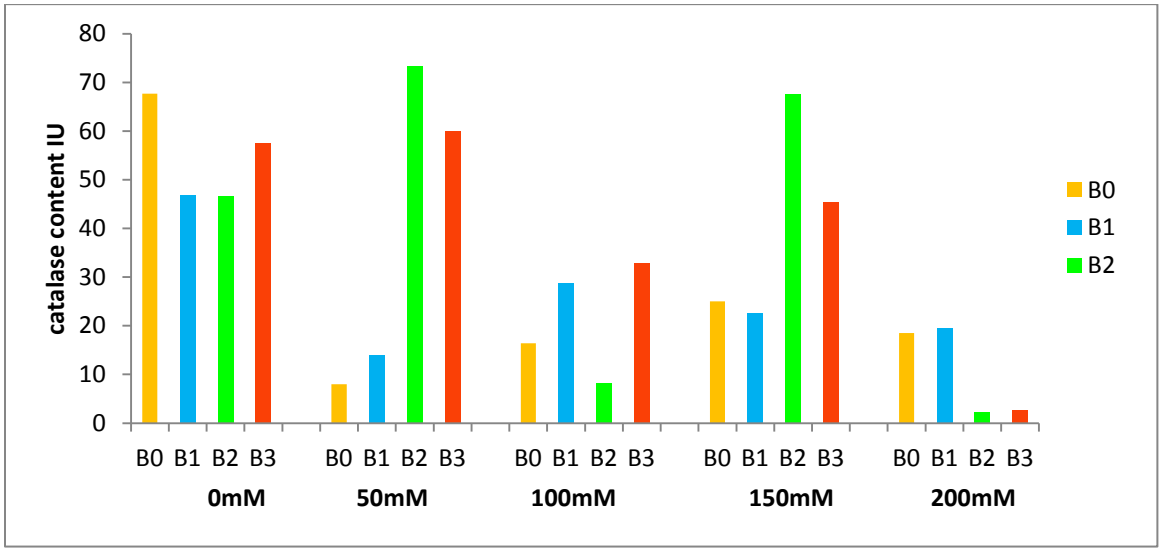

Fig.1: Seed S_A

B2 showed maximum catalase content at 50mM and 150mM salt concentrations. The decline in CAT activity is regarded as a general response to many stresses ([12, 17] and [26]). There are several reasons for the decreased activity of catalase. ([40]) showed that the reduction of catalase activity in pea plants was due to a decrease in the protein content. Also CAT is associated with peroxisomes and these organelles contain proteases, some of which are induced by senescence, catalase being a target of the peroxisomal protease activity ([8]). ([46]) have described the photo inactivation of catalase from rye leaves under different stress condition.

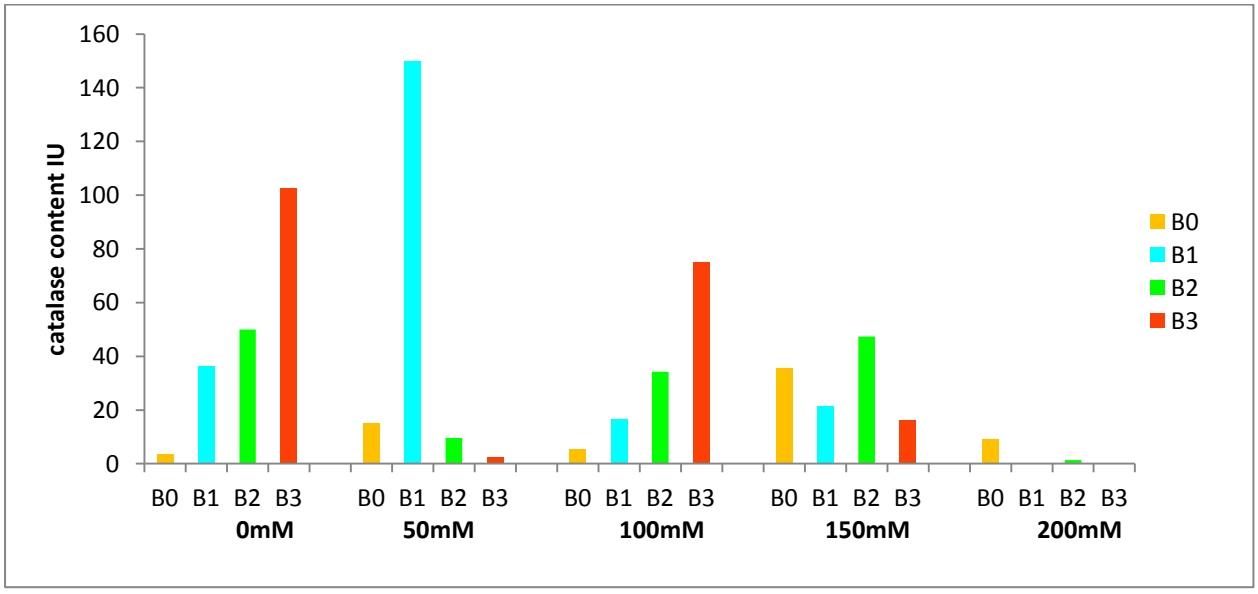

Fig.2: Seed S_B

In S_B the amount of catalase activity is shown maximum by B1 at 50mM salt concentration. There was no growth seen at 200mM salt concentration with B1, B2and B3.

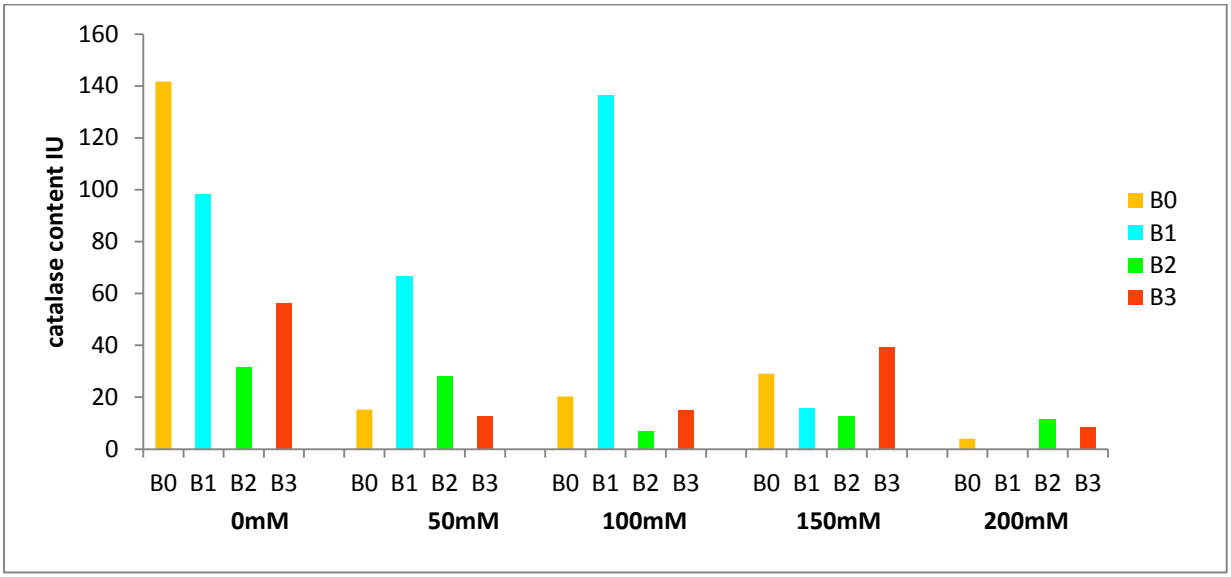

Fig.3: Seed S_C

Catalase activity was seen maximum at 100mM salt concentration when infested with B1. Catalase activity diminished with increased salt concentration. No growth was seen at 200mM concentration when infested with B1.

Catalase in Shoots

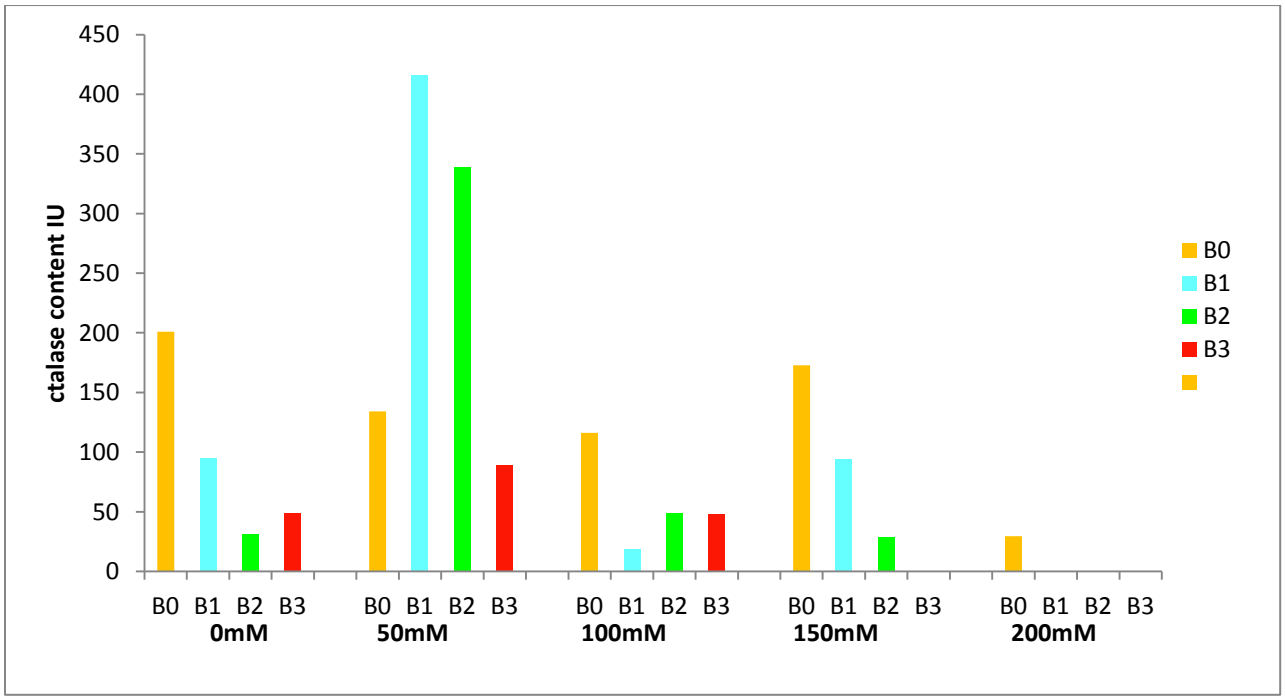

Fig.4: Seed S_A

B1 and B2 both showed high catalase content at 50mM salt concentration. Very less growth at 200mM salt concentration when infested with bacteria.

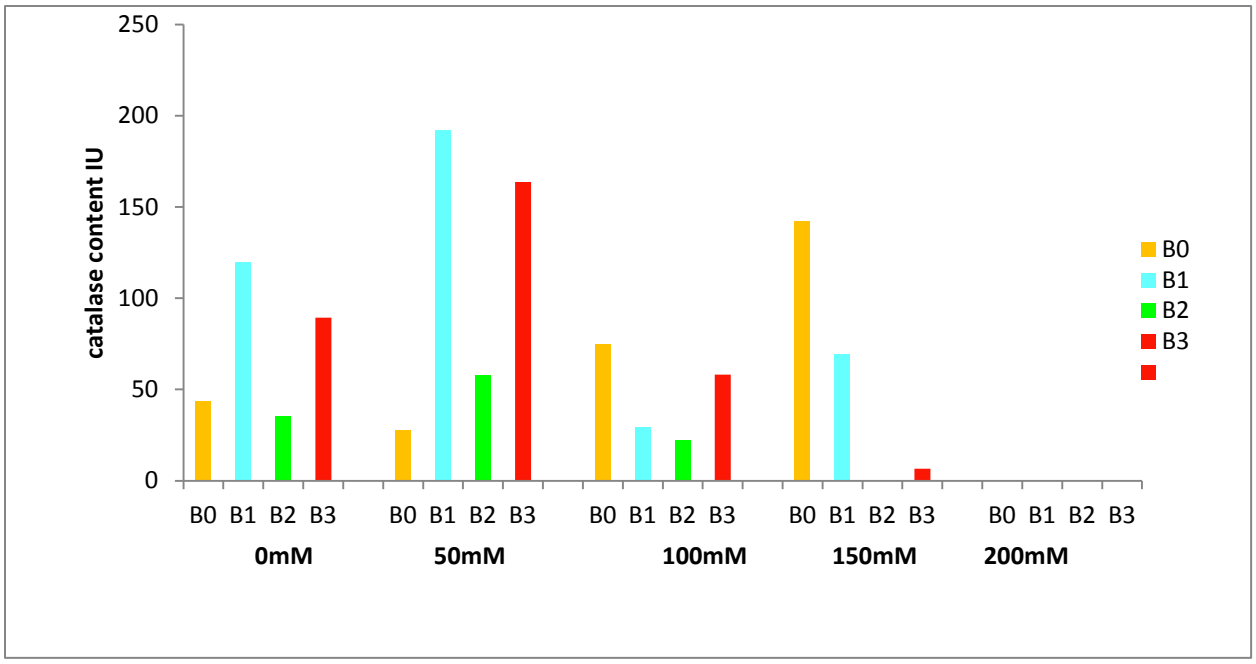

Fig.5: Seed S_B

Catalase activity was not observed at 200mM salt concentration with S_B. B1 and B2 showed increasing trend of catalase activity till 50mM salt concentration then decreased.

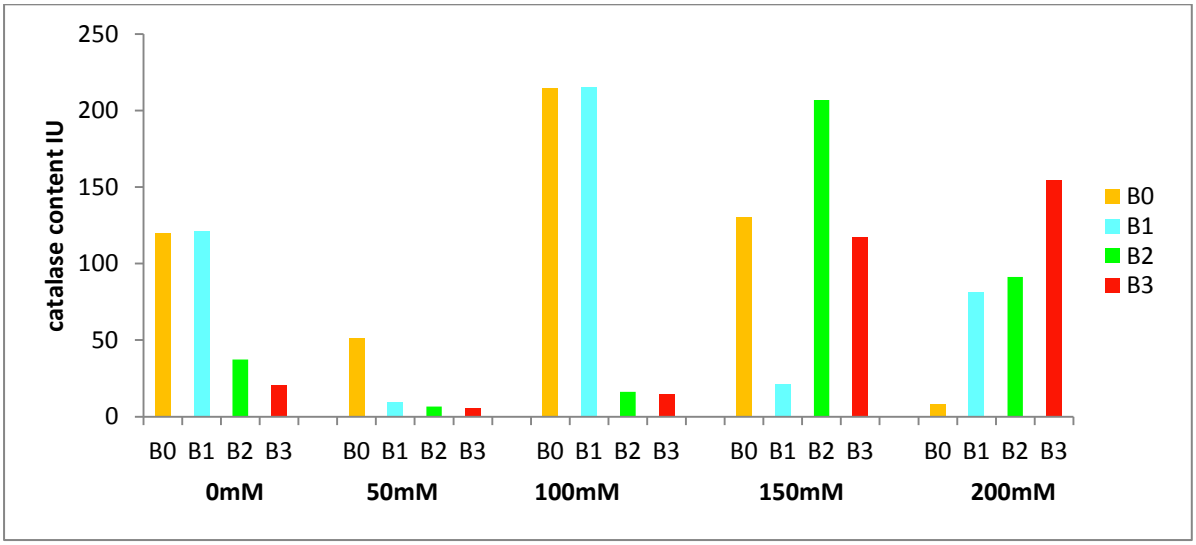

Fig.6 Seed S_C

In S_C, at different concentrations of salt stress different bacterial infestations showed maximum catalase activity. At 100mM B0 and B1 showed maximum content, at 150mM B2 showed maximum content and at 200mM consortia showed maximum catalase content. Our results were in conformity with study of ([48]) which showed increased CAT activity in root and leaf tissues of soybean under salinity stress. Reactive oxygen species include, for example, hydrogen peroxide, hydroxyl radicals and superoxide anions. ROS are usually generated by normal cellular activities such as photorespiration and beta-oxidation of fatty acids , but their levels increase when plants are exposed to biotic or abiotic stress conditions ([19]).In roots, this enzyme is located especially into glyoxysomes ([13]). The

significant drop verified in the CAT activity of *Pitiúba* cultivar roots might indicate a salt-sensitivity or, alternatively, that this cultivar could have a high constitutive level this enzyme in young root tissue able to scavenging H_2O_2 excess together other root peroxidases. The characterization of salt resistance just on the basis of enhanced antioxidative enzyme activities may be a simplistic approach taking into account the complexity of the oxidative metabolism, which is typically redundant in higher plants ([31]). Decline in CAT activity, a general response too many stresses, is supposedly due to inhibition of enzyme synthesis or change in assembly of enzyme subunits (Rae and Ferguson, 1985).

CONCLUSION

For successful scavenging of ROS by a scavenging system, some antioxidant enzymes must cooperate with each other. Exposure to salinity induced oxidative stress through the enhanced generation of ROS, which was accompanied by membrane damage, enhanced carotenoids levels, anthocyanins accumulation and by activation of antioxidant enzyme systems. Increased levels of scavenging enzymes indicate their induction as a secondary defense mechanism in response to salt stress. The PGPB used enhanced the optimization and acclimatization according to the prevailing soil conditions. The two isolates of Bacteria (*bacillus)* B1 and B2 showed potential as plant growth beneficial inoculants in Saline soil regions suggesting further studies on rhizo competence in commercial crops grown under salt stressed conditions. So that in future PGPR is expected to replace the chemical fertilizers, pesticides and artificial growth regulators which have numerous side-effects to sustainable agriculture. Our study suggests that the two strains of *bacillus* can also be applied for crop production in sustainable and ecological agricultural systems. Further research and understanding of mechanisms of PGPR mediated –phytostimulation would pave the way to find out more competent rhizobacterial strains which may work under diverse agro-ecological conditions. More studies are necessary to find out the molecular identity of isolates and ionic changes on plant cell against saline stress in presence and absence of bacteria. It has proved that the salinity will reduce the root colonization efficiency in PGPR probably due to variation in their quorum sensing signal disturbances need to be studied.

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