ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Bacterial inoculums influence the growth and antioxidant enzyme activity in germinating *Vigna radiata.*

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Manuscript Info

Manuscript History:

Received: 12 June 2015
Final Accepted: 26 July 2015
Published Online: August 2015

Key words:

Vigna radiata; Bacillus; seed germination; SOD; Catalase

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Abstract

The present investigation was designed to study the regulation of antioxidant defense system of plant by *Bacillus* species. The study indicates that some bacteria of *Bacillus* sp are able to enhance the plant growth along with regulating the plant's antioxidant defence system. Experiment was conducted on germinating seeds of *Vigna radiata* inoculated with various strains of bacteria isolated from cave scrapings. Plant growth promoting effect of all 6 bacterial strains was confirmed by significant increase in tissue weight gain with increased protein content in comparison to control. 5 strains of bacteria were associated with a significant decrease in the super oxide dismutase (SOD) activity, whereas one strain was able to increase SOD activity in *Vigna radiata* significantly. All of the bacterial treated seeds except two, demonstrated unaltered catalase activity.

INTRODUCTION

An important response to stress by aerobic plant cells is the production of reactive oxygen species (ROS) like superoxide anion (O_2^{-}), hydroxyl radical (OH.), alkoxy radical, singlet oxygen and toxic hydrogen peroxide (H_2O_2) (Salin 1988; Luna 1994; Asada and Takahashi 1987 and Breusegen 2001). ROS, if not detoxified cause serious damage to proteins, lipids and nucleic acids (Elstner 1982; Alscher et al. 1997). When plants are exposed to a variety of adverse conditions including chilling, high light, drought and paraquat, oxidative stress ensures primarily due to the decrease in anti oxidant defense but also due to the increase in free radical production mediated by catalytic Fe (Becana et. al. 1998). Oxidative stress is essentially a regulated process, the equilibrium between the oxidative and anti-oxidative capacities determining the fate of the plant. Under non stressful conditions the antioxidant defense system provides adequate protection against active oxygen and free radicals (Asada and Takahasi 1987). Both natural and man- made stress situation provoke increased production of toxic oxygen derivatives. In response the capacity of antioxidative defense system is increased (Gressel and Salun 1994) but in most situations the response is moderate (Foyer et al. 1994). Plants possess very efficient scavenging systems for ROS that protect them from destructive oxidative reactions.

Alleviation of salt stress by antioxidant defense system in mung bean (*Vigna radiata*) has been reported by Saha *et al* 2010. Plants' capability of acclimation to various environmental stress shows their stress tolerance which can be developed by various factors. Chabot et al. (1993) demonstrated microbes being beneficial to plants such as production of phyto hormones, antibiotics, siderophores, vitamins, antifungal substances and hydrogen cyanide (Rodriguez and Fraga 1999). Increasing concern about conservation of cave animals has led us to delve deeper into the ecosystem of caves in which microorganisms play a pivotal role. Caves are being extensively used as a natural laboratory for ecology and evolutionary studies. Cave animals have also served as models for the study of adaptation because of their ability to survive in harsh environment.

The plant growth promoting bacteria can enter into a symbiotic relationship with plants or non-symbiotic free living bacteria can also promote plant growth (Glick, 1995). Beneficial bacteria are termed as either plant growth promoting rhizobacteria or plant health promoting rhizobacteria according to their mode of action (Sikora 1992). Reports on soil bacteria *Bradyrhizobium* sp. having high agronomic significance that establish a symbiotic nitrogen fixing association with peanut plant contributing to soil fertility (Fabra et al. 2010). Fravel (2005) reported that *Bacillus* sp being beneficial bacteria can be used as biopesticides to control plant diseases.

Regardless of the precise mechanism used by the bacterium to protect plants, the experiment with the germinating seeds reported by Burd et al. (1998) suggest that certain bacteria may eventually find a use in the development of phytoremediation strategies**.** Mung bean is an important cash crop in India, characterized by a relative high content of protein and is grown and harvested in summer season (February –May). As mung bean is a stress sensitive legume, the present investigation was undertaken to analyse whether bacterial inoculum produces or reduces oxidative stress during early stages of seedling development. SOD plays an important role for protection against superoxide derived oxidative stress in plant cells as reported by Asada and Kiso (1979). Little is known about the change of the SOD isozymes of plants in developmental course and in response to exogenous factors. Therefore this study was undertaken to assess the influence of bacterial inoculation on SOD and catalase activities of *Vigna radiata* germinating seeds.

Materials and methods

CHEMICALS

Most of the medium constituents for bacterial culture and biochemical characterisation such as tryptone, yeast extract, and agar were purchased from Difco Lab. Monobasic potassium phosphate (anhydrous) and dibasic potassium phosphate (anhydrous) from Merck were used to prepare buffer for performing all the tests. Potassium-sodium tartarate tertrahydrate, copper II sulphate pentahydrate , folin-ciocalteau's phenol, glacial acetic acid 99-100%, L-methionine, naphthyle ethylene diamine, sulphonylamide, riboflavin, hydroxylammoniumchloride crystal and ethylene diamino tertracetic acid disodium salt (dihydrate) were obtained from Merck. Glass wool, sodium carbonate (anhydrous) and bovine serum albumin were obtained from Himedia. Sodium hydroxide pellets were obtained from Sisco research laboratories.

BACTERIAL CULTURE IDENTIFICATION

Cave samples were obtained from four locations in a cave of Kutumsar. At each location 2 to 5 grams of surface material was scraped using sterile scalpel and placed inside zipper-closure plastic bags for transport to lab. After proper dilution the samples were spread on LB agar and potato dextrose agar plates to grow aerobic culturable microorganisms from the samples. Cultures were then isolated, purified and assayed for partial identification. Partial identification of bacteria was performed by Gram staining and Schaefer fulton spore staining method. All bacteria under investigation were of genus *Bacillus* which was confirmed from Bacillus specific biochemical tests. Most of the bacterial cultures were found to be of spore forming Bacilli species.

SEED GERMINATION

Seeds of *V.radiata* were sterilized by 0.1% $HgCl_2$ solution. Bacteria were grown in liquid L. B. medium. Culture of each isolate was centrifuged and resuspended in sterile water to 10^8cells per ml determined at 600nm. Seeds treated with bacterial suspension were allowed to germinate and grow under controlled light (16hr light/8hr dark) and temperature 28^0C for 3 days along with non treated seeds in separate petridishes. Initial and final weights of seeds were recorded. 10% homogenate of tissue was prepared in PBS and centrifuged for 10 min at 10,000 rpm at 4^0 C. The supernatant was used for various biochemical estimations.

ESTIMATION OF PROTEIN

Protein estimation of the samples was made according to the method of Lowry et.al. (1951). Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (bovine serum albumin) was taken as standard protein.

ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

SOD activity was determined according to the method of Das et al. (2000). 0.5ml of supernatant was passed through a 2ml column of sephadex G-50 and the elute was used for the estimation of SOD activity.

ESTIMATION OF CATALASE (CAT)

Catalase activity was estimated according to Beers and Sizer (1952).

STATISTICAL ANALYSIS

Each treatment was analysed and experiment was performed with 5 replications from petriplates. The results are presented as means and compared and analysed by one way ANOVA with standard deviation (±). Significance level adopted was 5% ($P \leq 0.05$).

Results and discussion

Bacterial identification

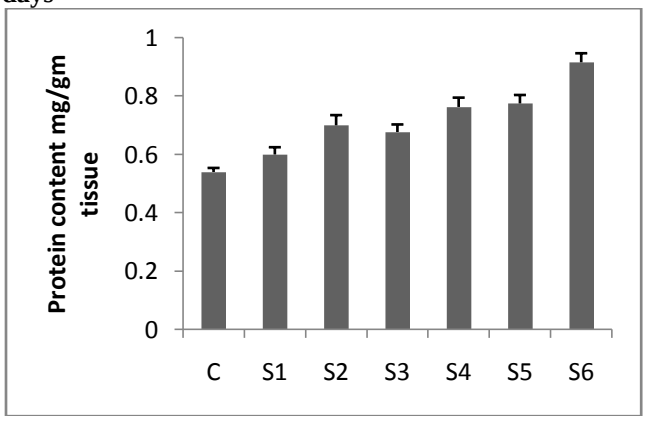
Six isolates from scrapings of cave wall were tested. Microscopic observation revealed that all were Gram positive aerobes. Cell free extracts of these six isolates were also evidenced for presence of catalase and superoxide dismutase. Biochemical tests were done for identification of all the isolates and were found to be of genus *Bacillus*. All six isolates were found to be spore forming rods.

Effect of bacterial inoculation on *Vigna radiata*

Protein Content

Protein accumulation in all types of bacterial inoculated seeds (Fig. 1) can be compared with the result obtained by Turan et al. (2007a) who studied protein accumulation in lentil plants under saline condition and suggested reason to provide storage form of nitrogen for future use and osmotic adjustment. However unaltered tissue weight along with protein gain in S2 and S6- treated seeds can be explained as result of decreased water retainment and degradation of some biomolecules other than protein

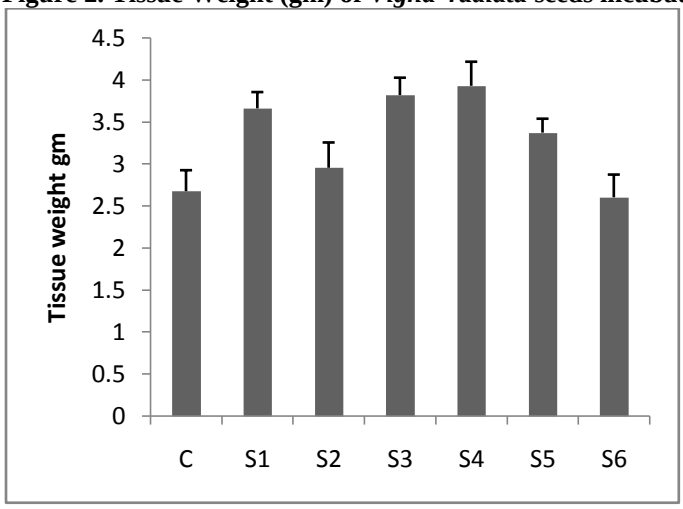
Figure 1. Protein content(mg/gm) of *Vigna radiata* seeds incubated with isolates(S1,S2,S3,S4,S5 and S6) for 3 days



Tissue weight gain

Significant increase in S1, S3, S4 and S5-treated tissue weight in comparison to control was observed (Fig.2). This result can be correlated to the results obtained by Ara et al. (2009) who proved that number of seeds pods and seed yield were influenced by bacterial biofertilizer.

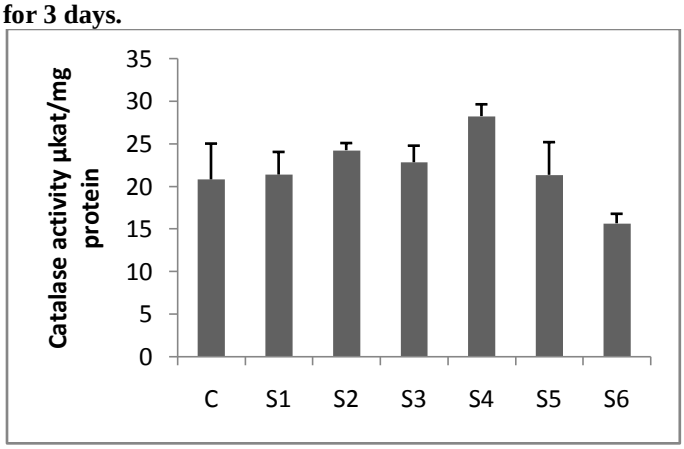
Figure 2. Tissue Weight (gm) of *Vigna radiata* seeds incubated with isolates(S1,S2,S3,S4,S5 and S6) for 3 days



Catalase activity

Treatment of S1, S3 and S5 show almost similar pattern of result without significant change (Fig. 3). S4 treatment increased the catalase activity which is supported by both tissue wt gain as well as increased protein content. S6 treatment resulted in increased protein content with unaltered tissue wt and reduced catalase activity. Unaltered tissue wt suggest increased susceptibility to H2O2 sensitivity which might have influenced catalase activity. Increased protein content can be presumed as result of two possibilities; disproportionate degradation of different cellular component due to bacterial stress, developing Increased resistant to stress by synthesis of new proteins. However before suggesting S6 isolate as plant growth promoter more parameters related to oxidative stress should be measured. Significant variations in catalase activity are clear in S4 and S6 treated samples as shown in graph (Fig. 3).

Figure 3. Catalase (µkat/mg) protein of *Vigna radiata* seeds incubated with isolates(S1,S2,S3,S4,S5 and S6) for 3 days.

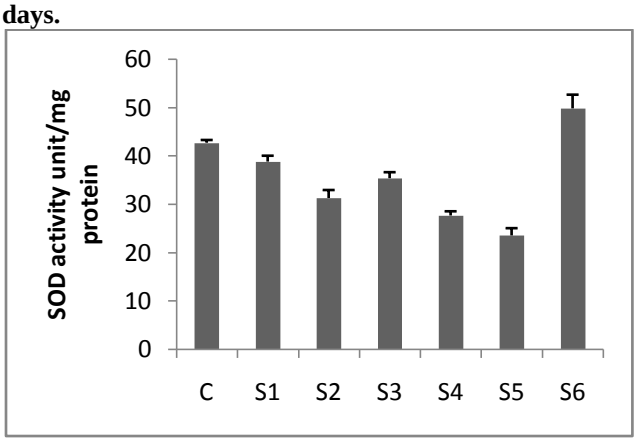


SOD Activity

Significant variations in SOD activity were found in S2, S4, S5 and S6 treated samples. S2, S4, S5 treated seeds demonstrated decreased level of SOD activity whereas S6 treated seeds indicated increase of SOD activity (Fig.4). Decreased level of SOD activity could be explained as the accumulating oxidative stress which should accompany with decreased tissue weight gain and protein content. As these two parameters (protein and tissue weight) did not show any decrease in our study, possibility of oxidative stress cannot be accepted. Decreased level of SOD may be compensated with other antioxidant system of *Vigna radiata* seeds which has to be

explored in future. Glick et al. (1998) reported that an enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) present in free living soil bacteria have unknown function in bacteria but have known to stimulate plant growth. The suggested mechanism may be due to modulation of ethylene level in developing plants. We sought in the work reported here to test whether some soil bacteria possessing such type of factors might lower the stress generated by ROS in plants. Increased activity of SOD in S6 treated seed in comparison to control can be suggested for antioxidant protection conferred by the bacteria directly to the plant. Thus S6 seems to have potential as a plant growth promoter. The identification of bacterial strain and the exact mechanism of their plant growth promoting activities need further investigation.

Figure 4. **SOD Unit/mg protein of *Vigna radiata* seeds incubated with isolates (S1,S2,S3,S4,S5 and S6) for 3 days.**



Conclusion

From the preliminary study it can be suggested that most of the bacteria of Bacillus species can serve as important defensive agents to overcome various biotic as well as abiotic stress for the plants and can be safely used as biofertilizers.

REFERENCES

Alscher, R.G., Donahue, J.L., Cramer, C.L. (1997): Reactive oxygen species and antioxidants:Relationships in green cells. Physiol.plant. 100: 224-223.

Ara, N., Zaman, M.R., Hasan, M.N., (2009): Effect of brady rhizobium and azotobacter on growth and yield of mungbean varieties. J.Bangladesh Agri. Univ. 7(1): 7-13

Asada, K., Takahashi, M., (1987): in *Photoinhibition: Topics in Photosynthesis* (eds Kyle, D.J., Osmond, C.B., Arnten, C.J., Elsevier, Amsterdam, vol. 9, pp. 227–287.

Becana, M., Moran, J.F., Iturbe- Ormaetxe, I., (1998): Iron dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. Plant and Soil, 201**:**137–147.

Beers Jr, R.F., Sizer, I.W., (1952): A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 195: 133–140.

Breusegem, F.V., James, E.V., Dat, F., Inze, D.,(2001): The role of active oxygen species in plant signal transduction. Plant Sci 161:423–431

Burd, G.I., Dixon, D.G., Glick, B.R**., (**1998): A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Applied and Environmental Microbiology. 64**:**3663–3668.

Chabot, R., Antoun, H., Cescas, M.P., (1993): Stimulation of maize growth and lettuce by inorganic phosphorus solubilizing microorganisms. Can J Microbiol 39:941–947

Clare, D.A., Duong, M.N., Darr, D., Archibald, F., Fridovich, I., (1984): Effects of molecular oxygen on detection of superoxide radical with nitroblue tetrazolium and on activity stains for catalase. Analytical Biochemistry.140**:**532–537.

Das, K., Samanta, L., Chainy, G.B.N., (2000): A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals, Indian Journal of biochemistry and Biophysics. 37:201-204.

Elstner, E.F., (1982): Oxygen activation and oxygen toxicity. Annu Rev Plant Physiol. 33:73-96
Fabra, A., Castro, S., Taurian, T., Angelini, J., Ibañez, F., Dardanelli, M., Tonelli, M., Bianucci, E., Valetti, L., (2010): Interaction among Arachis hypogaea L. (peanut) and beneficial soil microorganisms: how much is it known? Crc Rev Microbiol 36:179–194
Foyer, C.H., Descourvières, P., Kunert, K.J., (1994): Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell & Environment 17: 507–523.
Fravel, D.R., (2005): Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43: 337–359.
Glick, B.R., (1995): The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117
Gressel, J., Salun, E., (1994): in Causes of photooxidative stress and amelioration of defence systems in plants (eds Foyer, C.H. and Mullineaux, P.M.), CRC Press, Boca Raton FL,. pp. 237–274.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., (1951): Protein measurement with the Folin phenol reagent. J. biol. Chem, 193: 265.
Luna, C.M., González, C.A., Trippi, V.S., (1994):Oxidative damage caused by an excess of copper in oat leaves. Plant and Cell Physiology, 35(1):11-15.
Rodriguez, H., Fraga, R., (1999): Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
Saha P, Chatterjee P, Biswas, AK. (2010) NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mung bean (*Vigna radiata* L.Wilczek). Indian J Exp Biol, 48: 593-600.
Salin ML. (1988) Toxic oxygen species and protective systems of chloroplast. Physiol Plant. 72(3): 681-689.
Sikora RA. (1992) Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Annu Rev Phytopathol, 30:245–270
Turan MA, Turkmer N, and Taban N. (2007a) Effect of NaCl on stomatal resistance and proline chlorophyll, NaCl and K concentrations of lentil. Plants J Agron, 6: 378-381.