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Characterization of Plant Growth Promoting Attributes of *Cyamopsis Tetragonoloba* Rhizobacteria under Abiotic Stress for Sustainable Agriculture

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Abstract: ***Plant growth promoting rhizobacteria (PGPR) have potential to promote plant growth under extreme environmental conditions. In the present study, ten bacteria isolates having abiotic stress tolerance ability were selected from earlier study. These were isolated from rhizosphere region of cluster beans (Cyamopsis tetragonoloba), a drought - tolerant crop cultivated in arid and semi - arid regions of India. These isolates were tested for PGP attributes like phosphate solubilization, ACC deaminase activity, IAA and ammonia production under normal and stress (drought and salt) conditions. Isolates were further analyzed for exopolysaccharide (EPS) production, zinc solubilization, enzyme assay like protease, catalase and cellulase production. All 10 isolates were positive for phosphate solubilization and ammonia production, 6 isolates were showing IAA production while only 3 isolates were positive for ACC deaminase activity. All 10 isolates showed enzyme activity (protease and catalase) while only 3 isolates were positive for cellulase activity, EPS was produced by 7 isolates and only one isolate was positive for zinc solubilization. Principal Component Analysis (PCA) was also carried out to understand the variability in PGP activities shown by these 10 isolates and identify the most promising isolates. AK17 and KM6 were more prominent in PGP traits like ACC deaminase activity and IAA production while KM1 was better in phosphate solubilization.***

Keywords: Cluster beans (*Cyamopsis tetragonoloba*), Plant growth promoting rhizobacteria (PGPR), abiotic stress, Principal Component Analysis (PCA).

1. Introduction

The world's food crop productivity is greatly impacted by abiotic stresses such as drought and soil salinity. Both abiotic stresses lowers the soil water potential and plant water uptake capacity, which slows the pace of cell division in developing tissues, the stomatal conductance, and ultimately the rate of photosynthetic activity (Munns 2011) . Transgenic techniques, conventional breeding, and water - saving irrigation are all used as strategies to increase plant resistance to drought stress. However, they are extremely technological and labor - intensive techniques (Niu et al.2018) . The application of plant growth promoting rhizobacteria (PGPR) is an alternate strategy employed to mitigate abiotic stresses in plants. Sustainable agriculture is promoted by the use of stress - resistant PGPR since it is more environmentally friendly as compared to use of chemical - based fertilizers (Saikia et al.2018) . PGPR can either directly or indirectly promotes plant growth using various mechanism (Beneduzi et al.2012) . They mitigate stress condition in plant by production of phytohormones**,** controlling ethylene formation using the ACC (1 - aminocyclopropane - 1 - carboxylate) deaminase enzyme (Vurukonda et al.2016) fixing atmospheric nitrogen, and solubilizing mineral phosphates (Saleem et al.2018) . The ability of the PGPR to adequately protect plants under drought stress, especially in soils with low moisture content, depends on their ability to survive in the host plant's rhizospheric environment in sufficient numbers. This is because microorganisms that are not adapted to water - stressed soils may perish under these un - favorable circumstances (Van Meeteren et al.2008) . Therefore, PGPR that are abiotic stress tolerant may be preferable to others in terms of benefiting plants during stress conditions. So in the present work, we study the PGP traits of bacteria which were previously isolated from the rhizosphere of a drought tolerant crop guar or cluster beans [*Cyamopsis tetragonoloba* (L.) Taub]. *Cyamopsis* also known as guar is a drought and high temperature tolerant legume grown mostly in arid and semi - arid regions of the Indian subcontinent (Sharma et al.2012) . Guar is a versatile crop that is utilised in a variety of industries, including the food processing sector as an emulsifier and thickening. Additionally, it is utilised in textiles, printing, pharma, and cosmetics (Mudgil et al.2014) . Seeds of guar are the source of guar gum, which is used as thickening agent for hydraulic fracturing of shale in oil and gas industry (Abidi et al.2015) .

In the present study, selected abiotic stress tolerant rhizobacteria of *Cyamopsis* were assessed for PGP traits under drought and salinity stress. These rhizobacteria were also studied for exopolysaccharide production, zinc solubilization and enzymes assay. As a consequence of the research, it was concluded that the selected PGPR has enormous potential for use as bioinoculants in abiotic stressed agricultural systems.

2. Materials and Methods

2.1 Bacterial isolates

Rhizobacteria used for present study were isolated from rhizosphere soil of *Cyamopsis*, collected from different regions of Gujarat by us earlier (Jain and Saraf 2021) . Ten bacterial isolates (MN17, MN40, KM1, KM6, KM9, KM11, KM17, AK5, AK9 and AK17) were selected on the basis of their abiotic stress tolerance for present work.

2.2 Identification of Plant growth promoting (PGP) traits of bacterial isolates

Different PGP traits of bacterial isolates were studied under non stress and abiotic stress condition i. e drought and salinity (drought at - 0.15 MPa and - 0.73MPa; salinity at 5% and 10% NaCl concentration).

2.2.1 Phosphate solubilizing assay: Solubilization of phosphate was assessed using Pikovskayas agar medium (glucose: 10 gm, calcium phosphate: 5 gm, ammonium sulfate: 0.5 gm, potassium chloride: 0.2 gm, magnesium sulfate: 0.1 gm, yeast extract: 0.5 gm, agar: 15 gm, distilled water: 1000 ml). One loop full of active bacterial cultures was spot inoculated on plates and incubated at 28ºC for 6 days. The plates were observed for the appearance of a solubilization zone around the bacterial colony. Quantification of tri - calcium phosphate solubilization was done under normal and abiotic stress conditions in Pikovskayas liquid medium containing PEG - 6000 using ammonium molybdate reagent (Fiske and Subbarow 1925) .5ml of Pikovskayas liquid medium in 30 ml test tubes were inoculated with 50µl of active bacterial cultures. Test tubes were incubated at 28ºC in an incubator shaker at 180 rpm for 7 days. The supernatant was analyzed for phosphate solubilization by ammonium molybdate test and concentration was calculated by the calibration curve prepared using KH_2PO_4.

2.2.2 Production of indole acetic acid (IAA): The bacterial cultures were inoculated in sterile tryptone yeast extract broth (tryptone: 10 gm, yeast extract: 3 gm, NaCl: 5 gm, L - tryptophan: 0.204 gm, distilled water: 1 L, pH: 7) to test the production of IAA under normal and abiotic stress conditions. Inoculated flasks were covered with paper to create dark and kept in a shaker for 72h at 28ºC. One ml of the culture's supernatant was added to an equal volume of Salkowski reagent (50ml, 35% hydrogen peroxide, 1ml 0.5M $FeCl_3$ solution). This mixture was incubated in dark for 1h at room temperature. The development of pink color indicated the production of IAA by bacterial cultures. Quantification of IAA was done by measuring the absorbance at 535nm using a spectrophotometer and concentration was determined by standard curve of IAA (Sarwar and Kremer 1995) .

2.2.3 Production of ammonia: The bacterial cultures were grown in peptone water broth for 72h at 28ºC under static conditions. After incubation, the cell - free supernatant was mixed with 1ml Nessler's reagent. The development of yellow to brown color showed the presence of ammonia (NH_3). Quantification was done by measuring the absorbance at 450 nm using a spectrophotometer and the concentration of ammonia was calculated by the standard curve of ammonium sulfate (Dye 1962) .

2.2.4 Determination of ACC deaminase activity: The ability of bacteria to use 1 - aminocyclopropane - 1 - carboxylate (ACC) as a sole nitrogen source determined the presence of ACC deaminase activity in them. The bacterial cultures were inoculated in NB medium and incubated for 24 hrs at 28ºC at 120 rpm. The cells were extracted, washed 2 - 3 times with sterile 0.1M Tris - HCl (pH 7.5) and spot inoculated on Petri plates containing DF salt minimal medium (Dworkin and Foster 1958) (glucose: 2.0 gm; gluconic acid: 2.0 gm; citric acid: 2.0 gm; KH_2PO_4: 4.0 gm; Na_2HPO_4: 6.0 gm; $MgSO_4.7H_2O$: 0.2 gm), micronutrient solution ($CaCl_2$: 200 mg; $FeSO_4.7H_2O$: 200 mg; H_3BO_3: 15 mg; $ZnSO_4.7H_2O$: 20 mg; Na_2MoO_4: 10 mg; KI: 10 mg; NaBr: 10 mg; $MnCl_2$: 10 mg; $COCl_2$: 5 mg; $CuCl_2$: 5 mg; $AlCl_3$: 2 mg; $NiSO_4$: 2 mg; distill water: 1000 ml) supplemented with 3mM ACC instead of $(NH_4)_2SO_4$ as a nitrogen source. The plates were incubated for 72 hat 28ºC and checked for growth after every 24h. Quantification of ACC deaminase activity under non - stress and stress conditions was done by measuring the production of α - Ketobutyrate produced by the cleavage of ACC by ACC deaminase (Penrose and Glick 2003) .

2.2.4 Other traits: To test the production of EPS, bacterial isolates were inoculated on nutrient agar plate supplement with 5% sucrose and incubated for 3days at 28ºC. Colonies showing thick mucoid like appearance on plates were producing EPS. The zinc solubilization assay of the bacterial isolates was analyzed on the modified Pikovskaya medium having 1g insoluble zinc oxide (ZnO). The cultures were streaked on medium plates and incubated at 28ºC for 4 - 5 days. The formation of clear zone around colonies confirmed the sobubilization of zinc by bacterial isolates (Singh et al.2020).

2.2.5 Enzymes assay: Cellulase activity of bacterial isolates was examined by using method of Cattelan (Cattelan et al.1999) . Pure bacterial cultures were spot inoculated with nutrient agar supplement with 1% carboxymethylcellulose (CMC) and incubated for 48 - 72 h at 30ºC. Cellulase activity was observed by pour 0.1% congo red solution in plates for 15 min and then destained with 1M NaCl solution for 15 min. Then the plates were examined for positive cellulase production. Protease activity of bacterial isolates was observed according to Smibert (Smibert and Krieg 1994) . The medium used for this activity was skim milk agar with 0.04 % bromo cresol green as a pH indicator. Bacterial isolates was spot inoculated on medium plates and incubated for 48 - 72 h at 30ºC. A halo zone around colonies indicates positive protease activity. Catalase test was examined by spotting freshly grown bacterial isolates on sterile slide with the help of sterile wire loop and then add a drop of 3% hydrogen peroxide on it. The formation of bubbles showed positive catalase activity of bacterial isolates.

2.3 Statistical Analysis

Analysis of Variance (ANOVA) was carried out to investigate the effect of stress conditions on PGP traits of the

selected isolates. Following ANOVA, Tukey's HSD post hoc test was carried out for comparison of means (at $p<0.05$). Significantly different means were indicated with different letters. All statistical computations were carried out using SPSS 24.0. Data from PGP traits was used to generate the biplot ordination diagram of principal component analysis with Python 3.7.15 using PCA package.

3. Results and Discussion

3.1 Plant growth promoting traits of bacterial isolates:

PGP traits of the selected isolates were observed under non - stress and stress conditions.

Phosphate solubilization: All ten isolates were able to solubilise phosphate. Among the ten selected isolate, the best phosphate solubilization was observed for isolate AK17 (59.7±2.3 µg/ml) under non - stress conditions. Three isolates (KM1, KM6 and AK17) showed an increase in phosphate solubilization up to osmotic potential of - 0.79MPa, whereas in isolates MN17, KM17 and AK9 an increase in phosphate solubilization was observed only upto - 0.15MPa osmotic potential and beyond that either a decrease or no phosphate solubilization was observed. In other four isolates (MN40, KM9, KM11 and AK5) phosphate solubilization decreased with increase in drought stress. The highest solubilization was observed in AK17 isolate at osmotic potential of - 0.73MPa (85.37±1.5µg/ml) followed by KM1 and KM6 (Fig 1a). There wasa42.8 % increases in phosphate solubilization under osmotic potential of - 0.73MPaas compared to non - stress in the AK17 isolate. Similarly at salt stress highest solubilization was observed for isolate AK17 (88.76±0.34µg/ml) at 10% NaCl. The MN40 and KM6 isolates showed decrease in the phosphate solubilization with increase in salt stress while isolate KM1 showed highest solubilization at 5% salt concentration (Fig 1b). Isolate AK17 showed the highest solubilization of phosphate under both the stresses. Similarly, *Pseudomonas libanensis* EU - LWNA - 33 strain isolated by Kour showed higher solubilization of phosphate under drought condition as compare to normal (Kour et al.2019). Upadhyay found that their bacterial isolates were able to solubilise phosphate at 8% NaCl concentration (Upadhyay et al.2009). Previous study have reported solubilization of phosphate by PGPR under drought and salt stress condition (Toribio - Jimenez et al.2017).

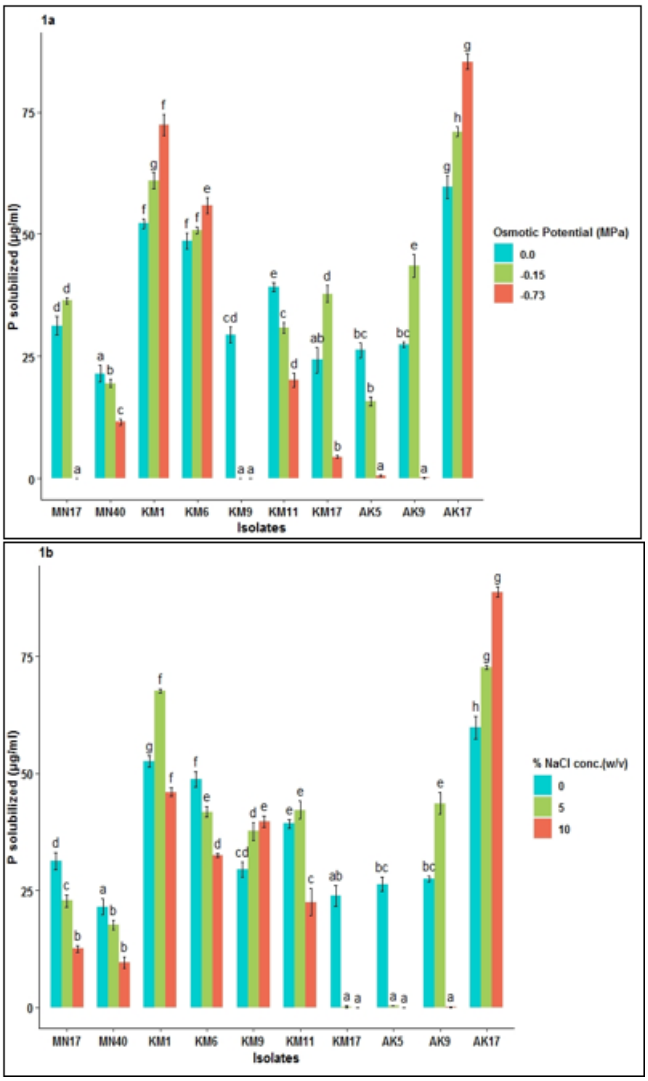


Figure 1: Phosphate solubilization by bacterial isolates under **1a** drought stress **1b**salt stress

3.1.2 IAA production: Out of ten bacterial isolates six (KM1, KM6, KM9, KM11, KM17 and AK17) were positive for IAA production. Under non - stress conditions, the IAA production was highest in bacterial isolate AK17i. e133.07±0.51 µg/ml but the production decreased with an increase in osmotic potential. While in the case of KM6isolate IAA production increased with osmotic potential as compared to non stress condition with the best activity obtained at - 0.15 MPa osmotic potential (136.09±1.64µg/ml). There was a33.6% increase in the production of IAA under stress conditions (- 0.15 MPa) compared to control in theKM6 isolate. At - 0.73MPa osmotic potential, the production of IAA decreased in all three strains (Fig 2a). Under salt stress, the highest IAA production was observed in AK17 isolate (135.4±0.95 µg/ml) at 5% NaCl followed by KM6 and production was decrease at 10% salt concentration (Fig 2b). It has been reported by many researchers (Dimkpa et al.2009; Saleem et al.2018) that IAA producing bacteria are helpful to mitigate stress by enhancing the root growth, formation of the lateral root, root hair thereby increasing the uptake of water and nutrients by the plant. Dimkpa found that inoculation of plants with IAA producing *Azospirillum* enhanced tolerance to abiotic stress (Dimkpa et al.2009). Similarly, German reported that inoculation of *Phaseolus vulgaris* with *A.*

brasilense Cd - an IAA producing PGPR resulted in a significant change in the root morphology of the plant under drought stress (German et al.2000).

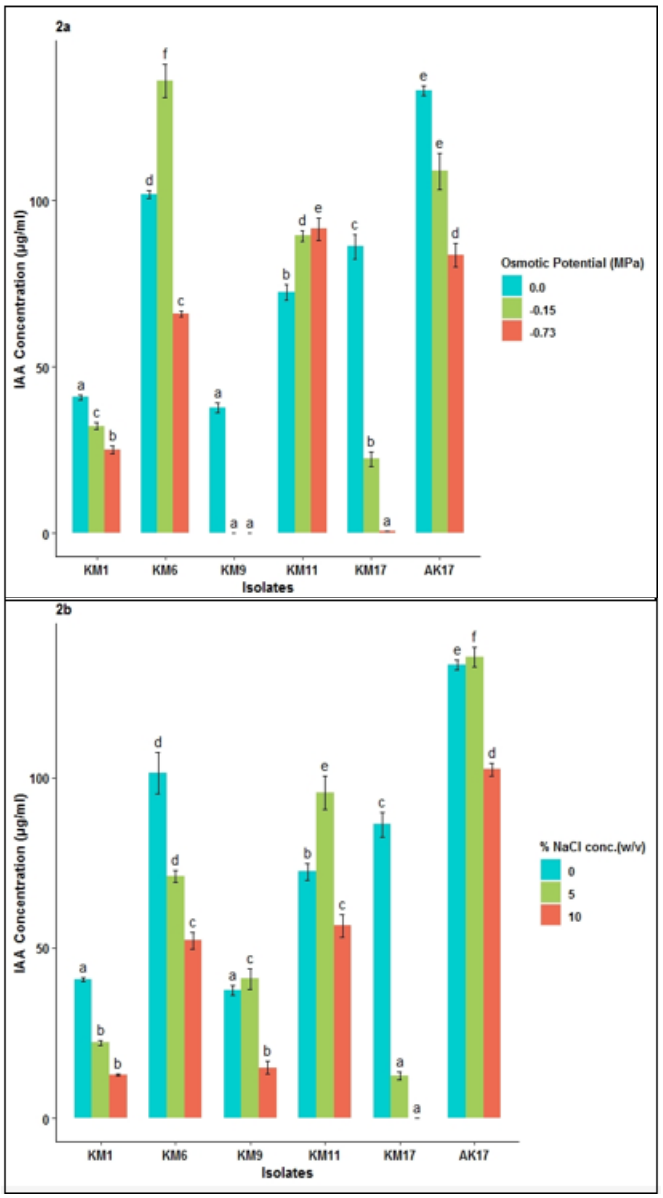


Figure 2: IAA production by bacterial isolates under **2a** drought stress **2b** salt stress

3.1.3 Ammonia production: Ammonia production help in cell signaling and improves plant growth during stress conditions (Anwar et al.2016). All ten isolates were positive for ammonia production, out of which the highest production was observed for KM6 (28.5±0.40µg/ml) followed by AK17 (27.49±0.5 µg/ml) under non stress condition. But under stress condition (drought and salt) decrease in the ammonia production was observed for all isolates expect AK17 which showed highest production (40.83±0.51µg/ml) at - 0.73 MPa osmotic potential (Fig 3a, b).

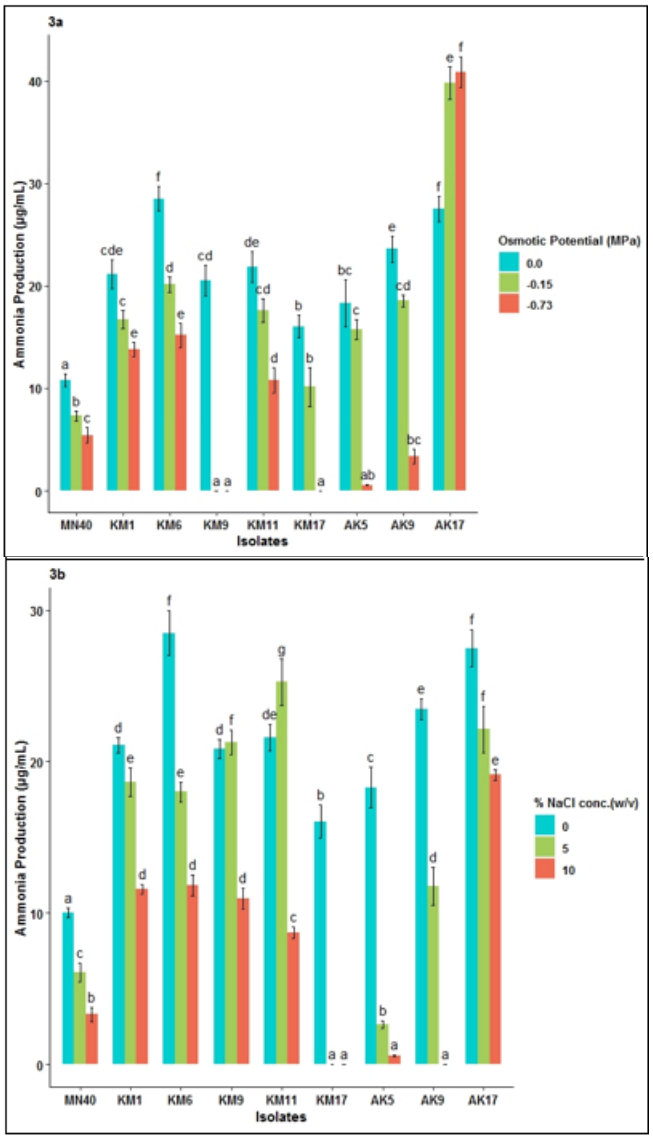


Figure 3: Ammonia production by bacterial isolates under **3a** drought stress **3b** salt stress

3.1.4 ACC deaminase activity: Among ten isolates, three isolates i. e KM1, KM6 and AK17 were positive for ACC deaminase activity and grew well on Petri plates having DF salt minimal medium with ACC as a sole nitrogen source. The assay for ACC deaminase enzyme activity under non - stress and stress conditions was done by quantifying the amount of α - Ketobutyrate produced when ACC deaminase cleaves ACC. Under non stress condition, isolate KM6 showed a greater amount of ACC deaminase activity (43.88±1.4µm α - Ketobutyrate/mg/h) followed by AK17 (35.9±0.7µm α - Ketobutyrate/mg/h). Under drought stress isolates KM6 and AK17 showed an increase in ACC deaminase activity by 36% and 40% at - 0.73 MPa osmotic potential compared to control. While a decrease in activity was noticed for isolate KM1 with increase in drought stress compared to non stresscondition (Fig 4a). At 10% salt stress, an increase in ACC deaminase activity was observed for only KM6 isolate (15%) compare to control (Fig 4b). According to previous report, bacteria with ACC deaminase activity reduce the amount of "stress ethylene" and hence increase resilience to several stress (Nukui et al.2000).

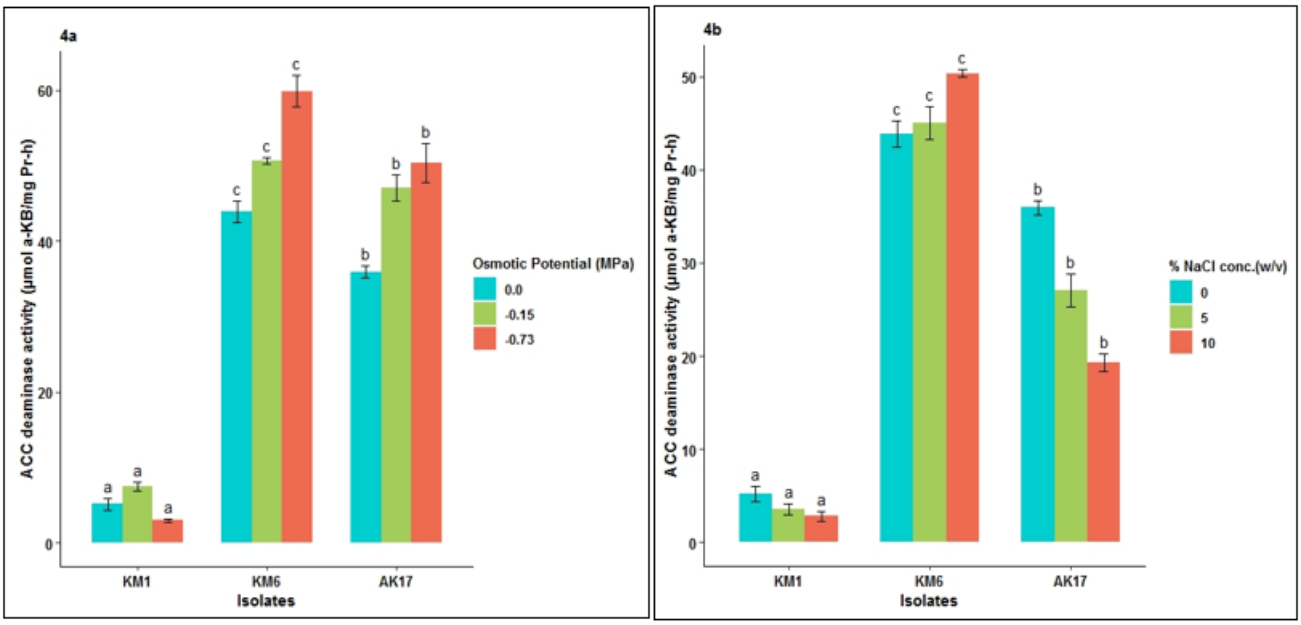
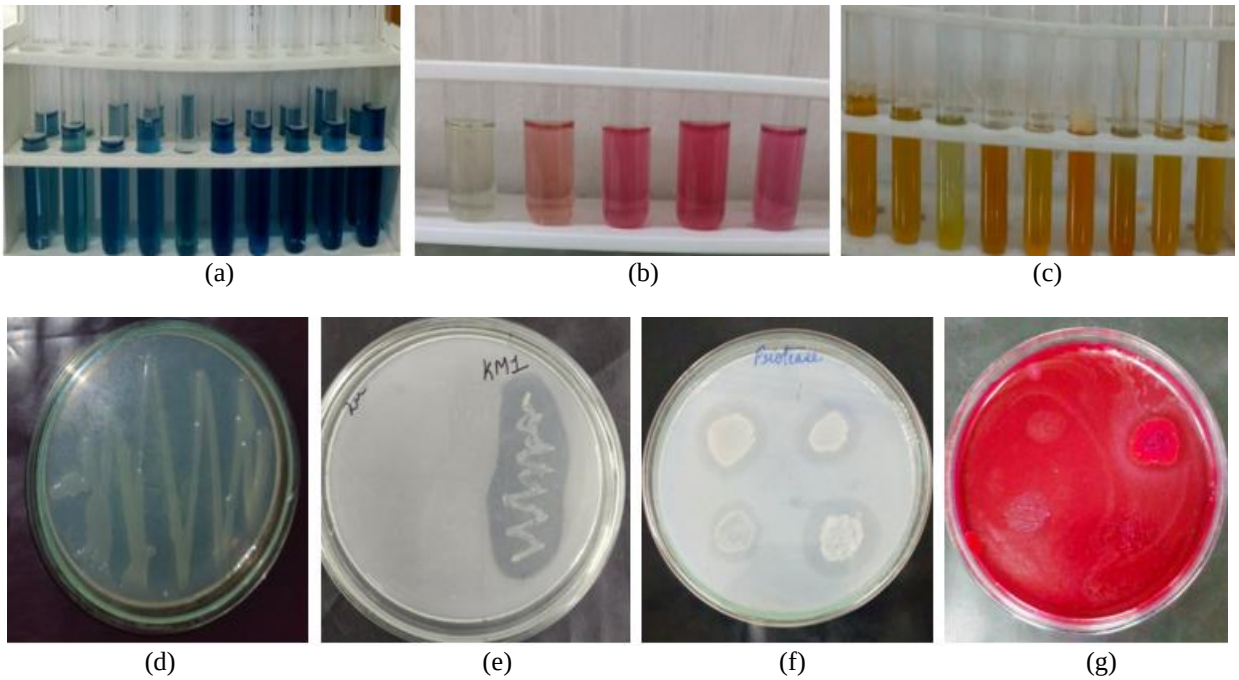


Figure 4: ACC deaminase activity of bacterial isolates under **4a** drought stress **4b** salt stress

3.1.5 Other traits: Out of 10 isolates, 7 showed the mucoid like appearance on plate and were positive for EPS production. Zinc solubilization assay was showed by only one isolate i. e KM1. Isolates showing hydrolytic enzymes activity such as cellulase and protease, help in decomposition of organic matters and nutrient mineralization, as well as the entry of microbes into host tissues (Lima et al.1998) . Cellulase activity was showed by only three isolates (KM1, KM6 and AK17) while all ten isolates were positive for protease and catalase activity. The different PGP traits of isolates are shown in fig 5.



(a) (b) (c)

(d) (e) (f) (g)

Figure 5: In vitro screening of PGP traits (a) Phosphate solubilization (b) IAA production (c) Ammonia production (d) EPS production (e) Zn solubilization (f) Protease activity (g) Cellulase activity

Table 1: Plant growth promoting traits of bacteria isolates

Isolates	EPS production	Zn solubilization	Protease activity	Cellulase activity	Catalase activity
MN17	-	-	+	-	+
MN40	+	-	+	-	+
KM1	+	+	+	+	+
KM6	+	-	+	+	+
KM9	+	-	+	-	+
KM11	+	-	+	-	+
KM17	+	-	+	-	+
AK5	-	-	+	-	+
AK9	-	-	+	-	+
AK17	+	-	+	+	+

(+) Present, (-) Absent;

Principal Component Analysis was carried out to better visualize the PGP trait variability of the 10 isolates. The first principal component PC1 explained 74.5% of variance while second principal component PC2 explained 9.68% variance. The biplot diagram of the principal component analysis describes the plant growth promoting traits of 10 isolates under normal and various drought and salinity conditions. PCA revealed that isolates AK17 has the most prominent PGP activities amongst all isolates. Further isolate KM6 gave the best ACC deaminase activity under normal as well as stress conditions. Isolates KM1 and KM11 showed similar PGP traits but they were distinguished by their higher ammonia and phosphate solubilization activity under drought and salinity stress conditions. PCA analysis helped us in identifying the most promising isolates based on the PGP activity. KM6 and AK 17 were the obvious choices while amongst KM1 and KM11, KM1 was selected for further activities as it had marginally higher PGP activity compared to KM11 (Fig 6).

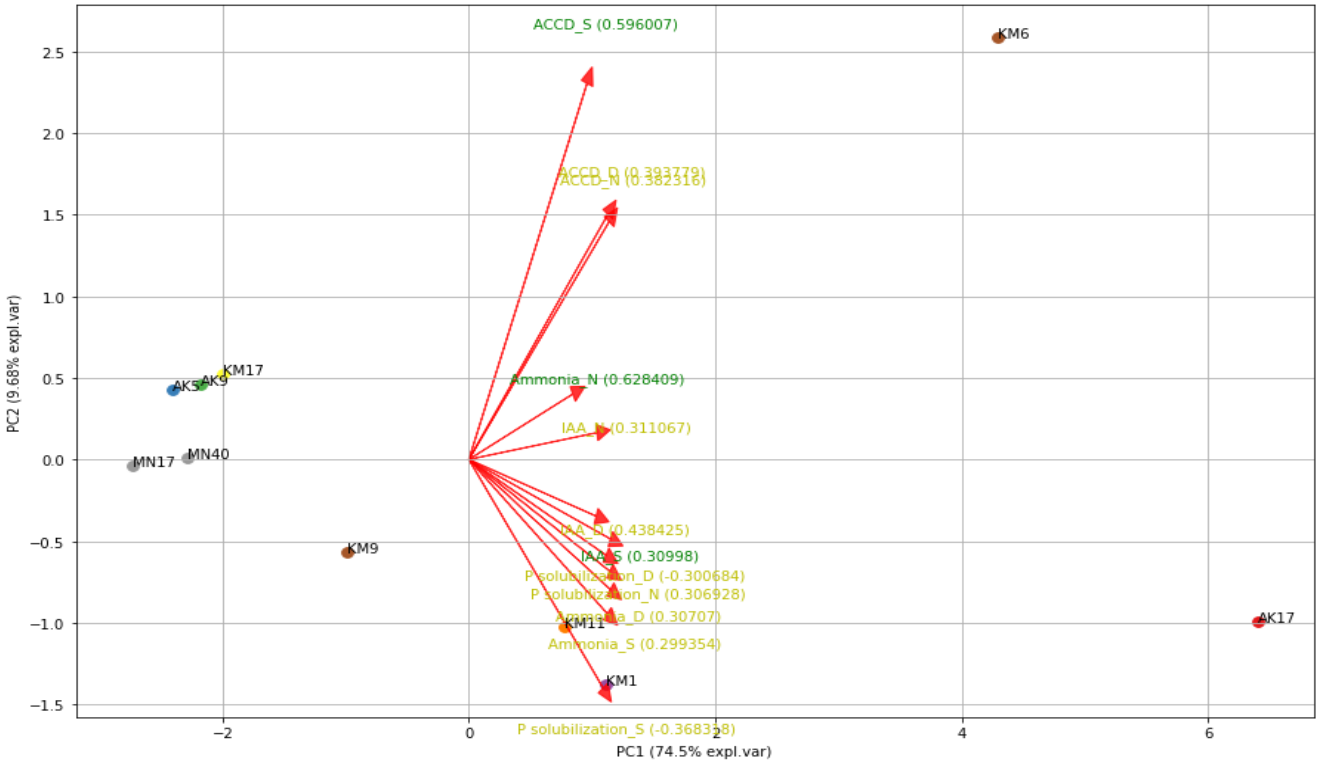


Figure 6: Biplot ordination diagram of principal component analysis describing plant growth promoting traits of 10 bacterial isolates under normal (N), drought (D) and salt (S) stress conditions

4. Conclusion

This study revealed that the abiotic stress tolerant rhizobacteria associated with drought tolerant cluster beans display PGP traits such as phosphate solubilization, IAA production and ammonia production activity even under drought and salinity stress conditions. The ACC deaminase activity shown by selected isolates plays crucial role to withstand under abiotic stress conditions. Other activities such as EPS and enzymes production enhance their survival rate under drought and salt stress. Therefore, the roots of cluster beans act as a good source of stress tolerant rhizobacteria competent in guarding the plant against stress. The application of such stress tolerant PGPR as bioinoculants for stress sensitive crops may be a promising approach towards sustainable agriculture.

References

[1] Abidi, N., Liyanage, S., Auld, D., Norman, L., Grover, K., Augadi, S., Singla, S., Trostle, C., 2015. Challenges and opportunities for increasing guar production in the United States to support unconventional oil and gas production. Chapter 12: Challenges and Opportunities for Increasing Guar Production in the United States to Support Unconventional Oil and Gas Production 207–225.

[2] Anwar, S., Ali, B., Sajid, I., 2016. Screening of rhizosphericactinomycetes for various in - vitro and in - vivo plant growth promoting (PGP) traits and for agroactive compounds. Frontiers in microbiology 7, 1334. https: //doi. org/10.3389/fmicb.2016.01334

[3] Beneduzi, A., Ambrosini, A., Passaglia, L. M., 2012. Plant growth - promoting rhizobacteria (PGPR): their

potential as antagonists and biocontrol agents. Genetics and molecular biology 35, 1044–1051. https: //doi. org/10.1590/S1415 - 47572012000600020

[4] Cattelan, A., Hartel, P., Fuhrmann, J., 1999. Screening for plant growth–promoting rhizobacteria to promote early soybean growth. Soil Science Society of America Journal 63, 1670–1680. https: //doi. org/10.2136/sssaj1999.6361670x

[5] Dimkpa, C., Weinand, T., Asch, F., 2009. Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant, cell & environment 32, 1682–1694. https: //doi. org/10.1111/j.1365 - 3040.2009.02028. x

[6] Dworkin, M., Foster, J., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. Journal of bacteriology 75, 592–603.

[7] Dye, D., 1962. The inadequacy of the usual determinative tests for the identification of Xanthomonas spp. New Zealand Journal of Science 5.

[8] Fiske, C. H., Subbarow, Y., 1925. The colorimetric determination of phosphorus. J. biol. Chem 66, 375–400.

[9] German, M. A., Burdman, S., Okon, Y., Kigel, J., 2000. Effects of Azospirillumbrasilense on root morphology of common bean (Phaseolus vulgaris L.) under different water regimes. Biology and fertility of soils 32, 259–264. https: //doi. org/10.1007/s003740000245

[10] Jain, R., Saraf, M., 2021. EXPLORING THE ABIOTIC AND BIOTIC STRESS TOLERANCE POTENTIAL OF RHIZOBACTERA ISOLATED FROM CYAMOPSIS. Journal of Advanced Scientific Research 12, 190–194. https: //doi. org/10.55218/JASR.202112327

[11] Jha, B., Gontia, I., Hartmann, A., 2012. The roots of the halophyte Salicorniabrachiata are a source of new halotolerantdiazotrophic bacteria with plant growth - promoting potential. Plant and Soil 356, 265–277. https: //doi. org/10.1007/s11104 - 011 - 0877 - 9

[12] Kour, D., Rana, K. L., Sheikh, I., Kumar, V., Yadav, A. N., Dhaliwal, H. S., Saxena, A. K., 2019. Alleviation of drought stress and plant growth promotion by Pseudomonas libanensis EU - LWNA - 33, a drought - adaptive phosphorus - solubilizing bacterium. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 1–11. https: //doi. org/10.1007/s40011 - 019 - 01151 - 4

[13] Lima, L., Marco, J. de, Felix, J., 1998. Enzimashidroliticasenvolvidas no controlebiologicopormiciparasitisma. Controlebiologico 11, 263–304.

[14] Mudgil, D., Barak, S., Khatkar, B. S., 2014. Guar gum: processing, properties and food applications—a review. Journal of food science and technology 51, 409–418. https: //doi. org/10.1007/s13197 - 011 - 0522 - x

[15] Munns, R., 2011. Plant adaptations to salt and water stress: differences and commonalities. Advances in botanical research 57, 1–32. https: //doi. org/10.1016/B978 - 0 - 12 - 387692 - 8.00001 - 1

[16] Niu, X., Song, L., Xiao, Y., Ge, W., 2018. Drought - tolerant plant growth - promoting rhizobacteria associated with foxtail millet in a semi - arid agroecosystem and their potential in alleviating drought stress. Frontiers in microbiology 8, 2580. https: //doi. org/10.3389/fmicb.2017.02580

[17] Nukui, N., Ezura, H., Yuhashi, K. - I., Yasuta, T., Minamisawa, K., 2000. Effects of ethylene precursor and inhibitors for ethylene biosynthesis and perception on nodulation in Lotus japonicus and Macroptiliumatropurpureum. Plant and Cell Physiology 41, 893–897. https: //doi. org/10.1093/pcp/pcd011

[18] Penrose, D. M., Glick, B. R., 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiologiaplantarum 118, 10–15. https: //doi. org/10.1034/j.1399 - 3054.2003.00086. x

[19] Qin, S., Zhang, Y. - J., Yuan, B., Xu, P. - Y., Xing, K., Wang, J., Jiang, J. - H., 2014. Isolation of ACC deaminase - producing habitat - adapted symbiotic bacteria associated with halophyte Limoniumsinense (Girard) Kuntze and evaluating their plant growth - promoting activity under salt stress. Plant and soil 374, 753–766. https: //doi. org/10.1007/s11104 - 013 - 1918 - 3

[20] Saikia, J., Sarma, R. K., Dhandia, R., Yadav, A., Bharali, R., Gupta, V. K., Saikia, R., 2018. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Scientific reports 8, 1–16. https: //doi. org/10.1038/s41598 - 018 - 21921 - w

[21] Saleem, A. R., Brunetti, C., Khalid, A., Della Rocca, G., Raio, A., Emiliani, G., De Carlo, A., Mahmood, T., Centritto, M., 2018. Drought response of Mucunapruriens (L.) DC. inoculated with ACC deaminase and IAA producing rhizobacteria. PLoS One 13, e0191218. https: //doi. org/10.1371/journal. pone.0191218

[22] Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold spring harbor laboratory press, New York

[23] Sarwar, M., Kremer, R., 1995. Determination of bacterially derived auxins using a microplate method. Letters in applied microbiology 20, 282–285. https: //doi. org/10.1111/j.1472 - 765X.1995. tb00446. x

[24] Sharma, P., Gummagolmath, K., 2012. Reforming guar industry in India: Issues and strategies §. Agricultural Economics Research Review 25, 37–4818.

[25] Singh, T. B., Sahai, V., Goyal, D., Prasad, M., Yadav, A., Shrivastav, P., Ali, A. and Dantu, P. K., 2020. Identification, characterization and evaluation of multifaceted traits of plant growth promoting rhizobacteria from soil for sustainable approach to agriculture. Current Microbiology, 77, pp.3633 - 3642. https: //doi. org/10.1007/s00284 - 020 - 02165 - 2

[26] Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society of Microbiology, Washington DC, pp 607–654

[27] Toribio - Jiménez, J., Rodríguez - Barrera, M. Á., Hernández - Flores, G., Ruvacaba - Ledezma, J. C., Castellanos - Escamilla, M., Romero - Ramírez, Y., 2017. Isolation and screening of bacteria from Zea mays plant growth promoters. RevistaInternacional de

ContaminaciónAmbiental 33, 143–150. https: //doi. org/10.20937/RICA.2017.33. esp01.13

[28] Upadhyay, S. K., Singh, D. P., Saikia, R., 2009. Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Current microbiology 59, 489–496. https: //doi. org/10.1007/s00284 - 009 - 9464 - 1

[29] Van Meeteren, M., Tietema, A., Van Loon, E., Verstraten, J., 2008. Microbial dynamics and litter decomposition under a changed climate in a Dutch heathland. Applied Soil Ecology 38, 119–127. https: //doi. org/10.1016/j. apsoil.2007.09.006

[30] Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., SkZ, A., 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiological research 184, 13–24. https: //doi. org/10.1016/j. micres.2015.12.003