Synopsis of the thesis

on

Alleviation of Biotic and Abiotic stresses in *Cajanus cajan* using Bacterial consortium

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1. Introduction:

Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. Both of these factors can adversely affect plant growth and development and final yield performance of a crop. Drought, salinity, nutrient imbalances (including mineral toxicities and deficiencies) and extremes of temperature are among the major environmental constraints to crop productivity worldwide. Among these abiotic stresses, soil salinity is becoming one of the most serious problems in irrigated agriculture (Hassani et al., 2020).

1.1. Abiotic Stress factors:

Soil salinization describes the presence of elevated levels of different salts in the soil. Na^+ & Cl^- are the most prevalent soluble salt, but a range of other dissolved salts, such as Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3, can also contribute to salinity stress in soil and water (Essington, 2020; Kumar & Sharma, 2020). Sources of primary salinity may arise from weathering of rocks and minerals that releases soluble salts, precipitation that washes these salts downstream, wind-borne salts from oceans and sand dunes that are deposited inland, and influx of seawater followed by subsequent retreat (Singh & Nair, 2012), along with human activities. Furthermore, the salinized areas are increasing at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, irrigation with saline water, and poor cultural practices.

Soil salinization initially results in loss of soil productivity, but with increase in salinity, it leads to loss of all vegetation, causing barrenness of soil, loss of habitat and reduced microbial activity (Ramani et al., 2003). Soils are classified as saline when the electrical conductivity of a saturated paste soil extract (ECe) is 4dS/m or more. This is equivalent to approximately 40 mM (0.23%) NaCl, and severely affects yield of most of the crops (Zaman et al., 2018). It has been estimated that worldwide 20% of total cultivated and 33% of irrigated agricultural lands are afflicted by high salinity. It has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Etesami & Adl, 2020). It will then be a challenge to provide sufficient food for this population when environmental stresses affecting crop production are increasing.

Use of traditional breeding and plant genetic engineering with production of transgenic plants to improve crop yields is limiting. An alternative strategy to improve crop tolerance towards salinity could be to introduce salt-tolerant microbes that enhance crop growth. Soil microbes colonizing various tissues of the plants, are known to promote the growth in several crop species could also prove to be useful in developing strategies to facilitate plant growth in salt stress as well (Etesami & Maheshwari, 2018).

1.2. Biotic Stress factors:

The fungal pathogens affect a wide variety of hosts (Goswami et al., 2016). They generally develop symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. *Fusarium oxysporum, F. udum* are well known to cause the fusarium wilt disease in the pigeon pea plant which is a serious infection causing severe yield loses (up to 90%).

Rhizoctonia solani is well known to cause the web blight disease in pigeon pea along with a wide range of other crops. The disease is minor but is capable of causing serious losses under favorable conditions, especially during rains.

Thus, the fungal pathogens chosen for the present study were: ***F. udum*** (obtained from the Anand agriculture university, Vadodara, Gujarat) and ***R. solani*** (available in the department).

1.3. Plant Growth Promoting Rhizobacteria:

Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria, which when grown in association with a host plant, result in growth stimulation. These are known to confer beneficial effects on plant growth directly, through production of plant growth regulators like IAA, gibberellic acid, asymbiotic nitrogen fixation, or indirectly through enhancing bioavailability of bound minerals ions, antagonism against plant pathogens by producing antibiotics and cyanide etc., and the ACC deaminase activity, which reduces the stress responses exerted by ethylene on the plants (Jiao et al., 2021).

Several PGPR's under saline stressed conditions, have shown positive effects on plants, by improving plant-water relations, ion homeostasis and photosynthetic efficiency etc. thus, increasing the overall plant growth. Mechanisms of these PGPR traits are regulated by a complex network of signaling events occurring during the plant–microbe interaction and consequently result in stress alleviation (Ilangumaran & Smith, 2017).

1.4. Microbial Consortium:

Many different types of bacteria are found in the rhizosphere, and one bacterium in itself cannot be sufficient to fulfill all the requirements of a PGPR. Several studies have shown that co-inoculation of rhizobia with other plant-growth-promoting bacteria (PGPB) increases nodulation activity, biocontrol activity, nutrient acquisition, plant growth & survival against various other stress factors in a wide variety of crops (Fuentes et al., 2016; Samaddar et al., 2019). Thus, it is always beneficial to work with a consortium where we can get several benefits from different organisms which act in a synergistic manner to promote the plant growth and also alleviate stress under unfavorable conditions. Thus, in the present study, this concept of combined use of plant-growth-promoting rhizobacteria is an effort to shift microbiological equilibrium in favor of increased plant growth production, nutrient uptake, and protection.

1.5. *Cajanus cajan*:

Also known as the pigeon pea or the tropical green pea, it is a woody, short-lived perennial shrub that is cultivated widely in the tropics and subtropics, often as an annual. It is a drought-tolerant crop frequently grown in semiarid regions on poor soil (Nadeem et al., 2019). In some countries, it is also used as forage for animals or as a green manure or cover crop.

About 82% of the total world produce is cultivated in India being the 2nd most important (Narayanan et al., 2018). Though it grows well in areas with an annual rainfall of less than 650mm and abhors water-logging, legumes are highly salt sensitive, especially during the symbiotic stage (Garg & Bhandari, 2016; Kurdali et al., 2019). Pigeon pea is an important pulse crop in India and cultivated to a larger extent in Gujarat. Also, the rhizobia strains RST1 and RIC3109 are specific for the pigeon pea plant, while NGR234 is a highly nodulating strain with broad range specificity for a wide range of legume crops.

Fungal species known to affect *C. cajan* severely are *Fusarium udum* & *Fusarium oxysporum* (Fusarium wilt), *Rhizoctonia solani* (Web blight), *Aspergillus niger & Aspergillus flavus,* (seedling rot) etc.

2. Rationale:

A consortium provides the benefit of having multiple PGPR traits which can supplement the effect of each other and promote plant growth and development. The biocontrol properties of *Pseudomonas* spp., heavy metal tolerance along with mineral solubilization activity in *Enterobacter* sp. C1D and the bioinoculant property in *Rhizobium* spp. can be used to supplement each other under stressed conditions.

3. Objectives:

- Effect of salt stress on PGPR traits of PGPR strains individually and in consortium.
- Functioning and compatibility of the PGPR consortia in presence of multiple abiotic stresses using PCR based approach.
- Plant inoculation studies with PGPR consortia for salinity and biotic stress with *Cajanus cajan.*

4. Details of the Work Done:

Three different groups of PGPR's, each with specific type of PGPR properties, were chosen for the present study.

Members of Consortium:

- ***Enterobacter* sp. C1D (**EC1D)**:** It is a lab isolate (collected from a sediment sample, at an Industrial Waste Effluent settling lagoon situation near Sarod, Mahi river estuarine area, Gujarat, Western India) is known to alleviate heavy metal stress in plants as a PGPR. It is an efficient Phosphate solubilizer (Subrahmanyam et al., 2018)
- ***Pseudomonas* spp. :** Lab isolates *Pseudomonas* sp. G16, G19, G22, G38 (Chaubey et al., 2015; Patel & Archana, 2018) and 2 standard strains *P. protegens* CHA0 (Haas et al., 1991) & *P. fluorescence* Pf-5 (Howell & Stipanovic, 1979)*,* were used in this study. These are known to have good PGPR traits along with the biocontrol properties.
- ***Rhizobium* spp. :** ***Rhizobium*** sp. IC3109 (RIC3109) has been obtained from Indian Agricultural Research Institute, Delhi, India and is known to be a good nitrogen fixer strain, while ***Rhizobium*** sp. ST1 (RST1) is a lab isolate (Geetha et al., 2009).

  ***Sinorhizobium fredii* NGR234:** A unique alpha-proteobacterium (order Rhizobiales) that forms nitrogen fixing nodules with more legumes than any other microsymbiont (here called as NGR234). It known to be capable of forming nodules more than 120 genera of legumes and the nonlegume *Parasponia andersonii.* (Saldaña et al., 2003).

- **<u>Effect of salt stress on PGPR traits of PGPR strains individually and in consortium</u>**

The 10 bacterial strains as mentioned above were confirmed for their biochemical properties including gram nature, motility, carbohydrate utilization, citrate utilization, and Triple sugar iron test; and the PGPR traits which included phosphate solubilization, HCN production, ammonia production, IAA production, siderophore production and antifungal activity. The PGPR traits were then evaluated under saline stress at different concentrations ranging from 0 – 5% NaCl (w/v). EC1D and NGR234 were found to have the highest salinity stress tolerance in the growth medium (5%), followed by the *Pseudomonas spp.* (4%), *Rhizobium spp.* (3%) and NGR234 (~2%). 16S rDNA sequencing was done and the isolates G16, G19, G22, & G38 were confirmed to belong to the pseudomonads group and were identified as *P. putida* (G16, G19, G22) and *P. fluorescens* (G38).

It was observed that *EC1D* exhibits most of the PGPR traits, including phosphate release from rock phosphate, IAA production, biofilm production and siderophore production all of which are also the highest among all the strains under study. The pseudomonads also exhibited the PGPR traits such as phosphate solubilization, IAA production, biofilm production and siderophore production which are however less as compared to EC1D. The *Pseudomonas* spp. also exhibited ammonia production and HCN production and showed antifungal activity against the fungi *F. udum* and *R. solani*, ranging from highest in the order PG19 followed by PG22, PG38 and Pf-5. HCN production was observed only among some pseudomonads which was the highest to least in the order PCHA0, PG22, Pf-5 and PG38. In the *Rhizobium* spp., the PGPR traits such as IAA, biofilm and ammonia production were observed which are higher as compared to pseudomonads, however, no phosphate solubilization and low level of siderophore production was observed. *Rhizobium* spp. also had high exopolysaccharide (biofilm) production which was a characteristic of only this bacterial group among all the PGPR's under study. By using the thin layer chromatography method, the siderophores were characterized as hydroxamate type (pseudomonads) and catecholate type (EC1D, Rhizobia).

Antibiotic sensitivity was also checked for all the strains to establish a particular antibiotic resistance for each group of PGPR so as to evaluate survival of each strain in a consortium. However, selective screening on the basis of antibiotic sensitivity could not be established. No antagonistic effect among the PGPR's was observed during the compatibility study on solid medium.

<u>Effect of salt stress on the PGPR traits:</u> Salt in the range of 0 – 5% (w/v) NaCl, was applied to the growth medium for checking the effect of saline stress on the bacteria and their PGPR traits. IAA production was observed only till 3% salt stress, there was a decrease in phytohormone production with an increase in the saline stress. Highest siderophore production was found in EC1D which was consistent till 3% salinity, though the siderophore production decreased with increasing salt stress among the others with no siderophores at 3% salt stress although the isolates PG16, PG19 and PG38 showed a substantial level of siderophore production till 2% salt

stress. Biofilm production seemed to be elevated with increasing salt stress except EC1D. Although the maximum biofilm was observed at 1% salinity in all the strains, the maximum salt tolerance was found at 4% salinity. Phosphate solubilization, under salt stress, decreased among all the strains except EC1D, there was no zone of clearance but only slight growth was observed. EC1D is able to solubilize phosphate till 2% salinity and grows with zone of clearance till 4% salinity. Under saline stress, the HCN production decreased but was present till 3% salinity. Ammonia production seen in PCHA0, Pf-5, PG22, PG38, RST1 & RIC3109, decreased with increasing salt stress and was absent till 2% salt stress. Antifungal activity of the pseudomonads increased under increasing salt concentrations, which was maximum in PG19 and Pf-5 at high salt concentrations.

<u>PGPR traits of consortia in presence of salt stress</u>:

For consortia studies, combinations of 18 different consortia were prepared by mixing equal cell counts of three strains from each group and PGPR traits of consortia were studied under different salt concentrations. A few PGPR traits such as biofilm formation showed higher than 2-fold increase in production as compared to the individual strains, and there was an increase in concentration with increasing saline stress, maximum being at 4% salinity. IAA production among the consortia did not show any significant increase when compared to few individual strains such as EC1D and the *Rhizobium* spp., but the consortium containing Pf-5 showed the maximum IAA production which was at 2% salinity.

Under iron deficiency stress, it was seen that the consortia displayed an increased siderophore production under salt stress tolerance compared to individual strains. The consortia having strains PG19, PG22, and PG38 produced siderophores till 3% salinity. The siderophore cross utilization studies also revealed that most of the strains were able to utilize the siderophores from other strains, with the exception of RIC3109 and PG16 while NGR234 was able to utilize siderophores from Pf-5 and PG38 only. Siderophore cross utilization could be an important trait for establishing synergism among the consortial members during various stressed conditions.

- **<u>Functioning and compatibility of the PGPR consortia in presence of multiple abiotic stresses using PCR based approach</u>**

Based on the previous experiments, consortium consisting EC1D + 3 rhizobia (RST1*,* RIC3109 *&* NGR234*)* + 3 *Pseudomonas* spp. (Pf-5*,* PG22 *and* PG38) were narrowed down for further studies and a total of 9 consortia were formulated using the selected strains.

Phosphate starvation under saline stress: Using rock phosphate as the sole source of phosphorous in the medium in buffered conditions, the bacteria were tested to observe their ability to solubilize this bound form of phosphate so as to release the soluble inorganic form which can be easily taken up by the plants (Ames, 1966; Buch et al., 2008). It was observed the among all the strains, EC1D shows the fastest P-solubilization ie., within 12h, but PG22 and PG38 show the highest P-release followed by EC1D. But under saline stress, EC1D gives highest p-solubilization followed by, Pf-5, PG22 and PG38. At 4% salinity, P-solubilization was observed only in EC1D and some in Pf-5. The rhizobia did not grow beyond 2% salinity and there was no P-solubilization found in the *Rhizobium* spp., even though high turbidity was seen in the growth medium, which can be intimidated as the result of high EPS production due to stressed conditions. The overall phosphate production however, increased by more than 2 folds, under salt stress, in the consortia. No P-solubilization was observed at 4% salt stress in the consortia.

In consortia, the amount of P-released increased by 200% of that observed in individual strains. The consortia containing PG22 and PG38 showed a higher P-solubilization compared to Pf-5. Growth was observed at 4% salinity as well, and the delay in attaining log phase was also reduced in consortium. The 24h and 48h samples from each were collected for genomic DNA extraction for further experiments, to understand the factors responsible for an increased P-solubilization and if there is any synergistic effect on the growth of weaker constituents of the consortium members in order to survive the dual stressed conditions.

Semi-Quantitative PCR study: Genus specific primers were developed using the 16s rRNA sequence information specific for a particular group of the bacteria such as *Enterobacter* spp., *Pseudomonas* spp. *Rhizobium* spp. and *Sinorhizobium fredii* NGR234. The primers were verified for specificity to the respective strains and absence of cross specificity with those of other groups. Using these primers, 24h & 48h samples were collected, following DNA extraction, these were used as the template for PCR amplification. The assessment of the survival and population density experiments for individual strains in the consortium was done. It was observed that rhizobia, which had the least salt tolerance (2-3% salt) were present at higher saline concentrations (5% salt) as well, in consortia and under phosphate limited environment. RST1 was able to create a more stable consortium compared to RIC3109 as the mixed cultures containing RST1, showed the most intense bands and at almost all the salt concentrations. Among the pseudomonads, PG22 formed the most intense bands. Thus, by semi-quantitative PCR technique it was confirmed that the high P-release was a result of the combined effect of the EC1D and the pseudomonads, while the formation of consortium also enabled growth of the *Rhizobium* spp. at higher salt stressed conditions.

Quantitative studies by Real Time PCR method for estimating the growth and survival of individual members of a consortium under saline stress: Growth studies in Luria broth were conducted by applying saline stress ranging from 0 – 5 % NaCl (w/v), wherein individual strains as well as the consortia were subjected to growth till 24h time period in continuous shaking at 30ºC. As seen previously, only EC1D were able to grow at 5% salt stress, while the pseudomonads could grow only till 4% salt stress. The growth in individual cultures decreased with increasing salt concentrations, while in the consortium, growth equivalent to control conditions was observed at 5% salinity as well. Other key points observed include shorter log phase and the stationary phase was achieved early (24h).

For quantitative studies by Real time PCR method, the samples from each growth experiment were collected at 12h and 24h and genomic DNA extracted, which was then used as template for the amplification. The g-DNA collected at 12h was considered to be the growth phase samples, while those collected at 24h were taken for survival phase samples. Standard cycle threshold (Ct) value charts were prepared based on amplicon concentrations to be obtained using of each of the primes, so as to quantitate each of the member strains from a consortium. Among the consortia prepared using NGR234, it was observed that pseudomonads had grown in highest numbers and the growth of EC1D was higher at 4% salinity compared to the control. Only in the consortia C3N, the growth of both EC1D and PG38 was found to be most.

- **Plant inoculation studies with PGPR consortia for salinity and biotic stress with *Cajanus cajan***

Seed germination in *C. cajan* was reduced to 50% at 1% salt stress in water agar medium, however, plants grew only till 0.2% salt stress in the pot conditions.

Abiotic stress alleviation: Pot studies were conducted with *C. cajan,* under salt stress using the PGPR and their consortia as the bioinoculant, also confirmed the inability of pigeon pea to tolerate salinity beyond 0.2% NaCl, while treatment with most of the PGPR and their consortia alleviated the salt stress in plants but only till 0.3% NaCl concentration. Stress alleviation was evaluated based on the various physical (including shoot/root development, biomass content, salt tolerance index, biomass allocation, moisture content and soil electrical conductivity) as well as biochemical (including (total proteins, sugars, proline, carotenoids and chlorophyll content) growth parameters in the plants. The strains PG22, RST1 and consortia C2S, C2I showed maximum effect of salt stress alleviation in the plant growth parameters.

Biotic stress: Fungal spores were mixed with sterile soil and allowed to incubate for 10d for establishment of the infection. Germinated seedlings inoculated with pseudomonads and their respective consortia, which were then maintained in isolated conditions and watered regularly to maintain the moisture conditions. The growth and stress alleviation were evaluated based on the disease incidence and the infection symptoms observed on the plants and the number of plants survived. Under biotic stress in the form of Fusarium udum infection, PG22 along with consortia C3I, C1N showed the maximum stress alleviation of biotic stress on pigeon pea plants.

Thus, in the present study it was observed that the effectiveness of the consortium varied with the members of the consortia indicating strain specific interactions. The impact of population density led to the dominance in phenotype expression and consortial synergy enhanced the microbial sustenance. The consortium having EC1D, Pf-5, PG22, PG38, RST1, RIC3109 and NGR234 were most suitable for biotic and salt stress alleviation in pigeon pea.

5. References:

Ames, B. N. (1966). [10] Assay of inorganic phosphate, total phosphate and phosphatases. *Methods in Enzymology*, *8*(C), 115–118. https://doi.org/10.1016/0076-6879(66)08014-5

Buch, A., Archana, G., & Naresh Kumar, G. (2008). Metabolic channeling of glucose towards gluconate in phosphate-solubilizing *Pseudomonas aeruginosa* P4 under phosphorus deficiency. *Research in Microbiology*, *159*(9–10), 635–642. https://doi.org/10.1016/j.resmic.2008.09.012

Chaubey, S., Kotak, M., & G., A. (2015). New method for isolation of plant probiotic fluorescent pseudomonad and characterization for 2,4-diacetylphluoroglucinol production under different carbon sources and phosphate levels. *Journal of Plant Pathology & Microbiology*, *06*(02), 253. https://doi.org/10.4172/2157-7471.1000253

Essington, M. E. (2020). Soil Salinity and Sodicity. In *Soil and Water Chemistry* (Issue

September, pp. 586–609). https://doi.org/10.1201/b18385-15

Etesami, H., & Adl, S. M. (2020). Can interaction between silicon and non–rhizobial bacteria benefit in improving nodulation and nitrogen fixation in salinity–stressed legumes? A review. *Rhizosphere*, *15*(June), 100229. https://doi.org/10.1016/j.rhisph.2020.100229

Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and Environmental Safety*, *156*, 225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013

Fuentes, A., Almonacid, L., Ocampo, J. A., & Arriagada, C. (2016). Synergistic interactions between a saprophytic fungal consortium and *Rhizophagus irregularis* alleviate oxidative stress in plants grown in heavy metal contaminated soil. *Plant and Soil*, *407*(1–2), 355–366. https://doi.org/10.1007/s11104-016-2893-2

Garg, N., & Bhandari, P. (2016). Silicon nutrition and mycorrhizal inoculations improve growth, nutrient status, K+/Na+ ratio and yield of *Cicer arietinum* L. genotypes under salinity stress. *Plant Growth Regulation*, *78*(3), 371–387. https://doi.org/10.1007/s10725-015-0099-x

Geetha, R., Desai, A. J., & Archana, G. (2009). Effect of the expression of *Escherichia coli* fhuA gene in *Rhizobium* sp. IC3123 and ST1 in planta: Its role in increased nodule occupancy and function in pigeon pea. *Applied Soil Ecology*, *43*(2–3), 185–190. https://doi.org/10.1016/j.apsoil.2009.07.005

Goswami, D., Thakker, J. N., & Dhandhukia, P. C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food & Agriculture*, *2*(1), 1–19. https://doi.org/10.1080/23311932.2015.1127500

Haas, D., Keel, C., Laville, J., Maurhofer, M., OberhÄnsli, T., Schnider, U., Voisard, C., Wüthrich, B., & Defago, G. (1991). *Secondary metabolites of Pseudomonas Fluorescens strain CHA0 involved in the suppression of root diseases*. 450–456. https://doi.org/10.1007/978-94-015-7934-6_68

Hassani, A., Azapagic, A., & Shokri, N. (2020). Predicting long-term dynamics of soil salinity and sodicity on a global scale. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(52), 33017–33027.

https://doi.org/10.1073/PNAS.2013771117

Howell, C. R., & Stipanovic, R. D. (1979). Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium by the soil tube method described previously. *Phytopathology*, *69*(5), 480–482.

Ilangumaran, G., & Smith, D. L. (2017). Plant growth promoting rhizobacteria in amelioration of salinity stress: A systems biology perspective. *Frontiers in Plant Science*, *8*(October), 1–14. https://doi.org/10.3389/fpls.2017.01768

Jiao, X., Takishita, Y., Zhou, G., & Smith, D. L. (2021). Plant associated rhizobacteria for biocontrol and plant growth enhancement. *Frontiers in Plant Science*, *12*(March). https://doi.org/10.3389/fpls.2021.634796

Kumar, P., & Sharma, P. K. (2020). Soil salinity and food security in India. *Frontiers in Sustainable Food Systems*, *4*(October), 1–15. https://doi.org/10.3389/fsufs.2020.533781

Kurdali, F., Al-Chammaa, M., & Al-Ain, F. (2019). Growth and N_2 fixation in saline and/or water stressed *Sesbania aculeata* plants in response to silicon application. *Silicon*, *11*(2), 781–788. https://doi.org/10.1007/s12633-018-9884-2

Nadeem, M., Li, J., Yahya, M., Wang, M., Ali, A., Cheng, A., Wang, X., & Ma, C. (2019). Grain legumes and fear of salt stress: Focus on mechanisms and management strategies. *International Journal of Molecular Sciences*, *20*(4). https://doi.org/10.3390/ijms20040799

Narayanan, S. L., Manivannan, N., & Mahalingam, A. (2018). Correlation and path analyses of yield and its component traits in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *International Journal of Current Microbiology and Applied Sciences*, *7*(03), 614–618. https://doi.org/10.20546/ijcmas.2018.703.073

Patel, J. K., & Archana, G. (2018). Engineered production of 2,4-diacetylphloroglucinol in the diazotrophic endophytic bacterium *Pseudomonas* sp. WS5 and its beneficial effect in multiple plant-pathogen systems. *Applied Soil Ecology*, *124*(June), 34–44. https://doi.org/10.1016/j.apsoil.2017.10.008

Saldaña, G., Martinez-Alcántara, V., Vinardell, J. M., Bellogín, R., Ruíz-Sainz, J. E., & Balatti, P. A. (2003). Genetic diversity of fast-growing rhizobia that nodulate soybean

(*Glycine max* L. Merr). *Archives of Microbiology*, *180*(1), 45–52. https://doi.org/10.1007/s00203-003-0559-y

Samaddar, S., Chatterjee, P., Roy Choudhury, A., Ahmed, S., & Sa, T. (2019). Interactions between *Pseudomonas* spp. and their role in improving the red pepper plant growth under salinity stress. *Microbiological Research*, *219*, 66–73. https://doi.org/10.1016/j.micres.2018.11.005

Singh, P., & Nair, A. (2012). Environmental sustainability of cropping patterns in Gujarat. *Institute of Rural Management in Gujarat*, *November*. http://bit.ly/2UgKK2y

Subrahmanyam, G., Sharma, R. K., Kumar, G. N., & Archana, G. (2018). *Vigna radiata* var. GM4 plant growth enhancement and root colonization by a multi-metal-resistant plant growth-promoting bacterium *Enterobacter* sp. C1D in Cr(VI)-amended soils. *Pedosphere*, *28*(1), 144–156. https://doi.org/10.1016/S1002-0160(17)60448-X

Zaman, M., Shahid, S. A., & Heng, L. (2018). Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques. In *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques*. https://doi.org/10.1007/978-3-319-96190-3