SYNOPSIS OF THE THESIS ON

# PLANT –MICROBE INTERACTIONS IN CEREAL- LEGUME INTERCROPPING SYSTEMS

Submitted To

The Maharaja Sayajirao University Of Baroda

For The Degree Of

Doctor of Philosophy in Microbiology

BY

**Siddhi. M. Vora**

**Supervisor: Prof.G.Archana**

Department Of Microbiology and Biotechnology Centre

Faculty of Science

The Maharaja Sayajirao University Of Baroda

Vadodara- 390002

Gujarat, India

# INTRODUCTION

## Intercropping

Intercropping is a form of sustainable agricultural system involving the cultivation of two or more crop species together in a same field. The most common advantage of intercropping is to produce a greater yield on a given piece of land by achieving more efficient use of the available growth resources that would otherwise not be utilized by each single crop grown alone. (Lithourgidis et al., 2011) Intercropping in practice is been undertaken by farmers practising high labour, low-yield farming on small lands, and is particularly common in countries with high amounts of subsistence agriculture and low amounts of agricultural mechanization. (Ngwira et al., 2012)

Legume-cereal intercropping systems have been widely studied in the context of diversity, ecosystem function and high yield, because Nitrogen ($N_2$) fixation by legumes increases ecosystem nitrogen supply. (Fujita et al., 1992) This facilitation is important to agriculture on a large scale, because dependence on chemical N fertilizer decreases over biological $N_2$ fixation by legumes. (Li et al., 2016) Legume-cereal intercropping not only allows crops to take up more nutrients than in mono-cultures but also reduces disease occurrence. (Gao X et al., 2014)

Pigeon pea (*Cajanus cajan*) as model legume plant enriches soil through symbiotic nitrogen fixation. It is primarily grown in tropical and subtropical areas of Asia, Africa and Latin America, and about 82% of all pigeon pea production takes place in India. (Kanoeka et al., 2015) Among cereal plants, maize (*Zea mays*) serves as model for intercropping studies as it is often intercropped with various legumes.

There are two pathways for $N_2$ transfer to non-nodulating plants: one is a direct transfer that N fixed by legumes is transferred to associated non-$N_2$ fixed plants via arbuscular mycorrhizal fungal (AMF) hyphae network and other way is an indirect transfer, wherein the residual and root exudates of legumes associated with symbiotic rhizobia release N to the soil system. (Sierra and Nygren, 2006) The former has been demonstrated in the soybean/maize intercropping system and improved the N fixation efficiency of soybean and promoted N transfer from soybean to maize. (Meng et al., 2015) Introduction of pigeon pea in maize based cropping systems resulted in a high proportion of N-derived-from-air (%Ndfa) (> 90%) in maize and thereby improved N budget. (Adu-Gyamfi et al., 2007)

## Plant growth-promoting rhizobacteria:

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. The beneficial effects of these rhizobacteria on plant growth can be direct or indirect. To exert their beneficial effects, bacteria usually must colonize the root surface efficiently. Examples of direct plant growth promotion include biofertilization, stimulation of root growth, rhizoremediation, plant stress control. Mechanisms of biological control by which rhizobacteria can promote plant growth indirectly are by reducing the level of disease,

include antibiosis, induction of systemic resistance, competition for nutrients and niche. (Lugtenberg and Kamilova 2009)

The ability of rhizobia to promote the growth of non-leguminous cereals plants such as maize, barley and rice is well known. (Hayat *et al*., 2010) Report suggested that the *nodD1* gene product of NGR234 responds to activation by phenolic compounds isolated from wheat extracts. (Le Strange et al., 1990) Other organisms like *Enterobacter* sp. strain FD17 showed both the highest growth-promoting activity under axenic conditions as well as colonization capacity. (Naveed et al., 2014)

## Role of root exudates in rhizosphere colonization:

Below ground plant roots release a broad variety of chemical compounds to attract and select microorganisms in the rhizosphere. Among plant root exudates, flavonoids act as chemo-attractant to draw rhizobia towards root. Rhizobia are known to nodulate via regulation of *nod* genes involved in nodulation on legume plants in response to flavonoids. (Huang et al., 2014) In mono-culture studies, legume–rhizobia symbiosis requires extensively a complex signal exchange between host plants and rhizobia. Adhesion of rhizobia to roots and root hair tips, the targets of infection, constitutes a very important stage in the initiation of symbiosis. This process involves both bacterial surface polysaccharides and secreted proteins. (Downie, 2010) Some rhizobial strains designated as "broad-host- range" have evolved to nodulate a larger variety of legume plants than others. Among those strains, *Sinorhizobium (Ensifer) fredii* NGR234 (here called NGR234) nodulates more than 120 genera of legumes and the non legume *Parasponia andersonii*.(Krysciak et al., 2014)

Efficient root colonization by plant-beneficial rhizobacteria is assumed to be essential for their role as biofertilisers as well as biocontrol of root pathogens. Root exudates are important carbon and energy sources for colonizing microorganisms. They are implicated as a key determinant of rhizosphere microbial community structure.

The ability to secrete a vast array of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots, with nearly 5% to 21% of all photosynthetically fixed carbon being transferred to the rhizosphere through root exudates. Root exudates have traditionally been grouped into low- and high-*M*r compounds. However, low-molecular weight exudates such as amino acids, organic acids, sugars, phenolics, and various other secondary metabolites are believed to comprise the majority of root exudates, whereas high-molecular weight exudates primarily include mucilage (high-*M*r polysaccharides) and proteins. **(**Oburger *et al*., 2013)

Root exudates are engaged in positive or negative interactions of plant-plant, plant microbe in the complex rhizosphere. Flavonoids found within the plant also constitute a large part of root exudates causes chemo-attraction of rhizobia towards the root, inhibit root pathogens. (Hassan *et al.*, 2011)

Quantities and quality of root exudates depends on plant age, plant species, environmental conditions, level of chemical, physical and biological stress and also microbial population.

Upon inoculation of AMF with *Fusarium,* root exudate analyses revealed an increase in sugars and organic acid (chlorogenic acid) in inoculated plants as compared with plants inoculated with AMF only. (Hage-Ahmed *et al*., 2013)

Plants can sense and respond to the presence of different plant neighbors and that the level of relatedness is perceived upon initial interaction. (Badri DV et al., 2012) This suggests that neighbouring plants could influence the quantity and quality of root exudates of the host plants. In the rhizosphere of intercropped plants, a greater diversity of microbial community and organic compounds could be expected if there are differences in root exudate composition between the intercropped species. In that case, the microbial community of intercropped species would not be a simple mixture of the communities of each of the two species. So far, effects of intercropping on microbial community structure are limited.

**Endophytes role in secondary metabolite production:**

Endophytic bacteria play a role for the metabolic potential of plants. Two indirect ways exists first, bacterial endophytes may strongly influence the performance, growth and stress tolerance of plants. Second, some metabolites are not only produced by a single organism, but might be produced by a plant in combination with associated bacteria**.** (Brader et al., 2014)

*H. seropedicae* is a known diazotrophic endophytic bacterium that colonizes plants of the family Poaceae (Baldani et al., 1986), as well as plants from other groups. The complete genome of *H. seropedicae* has been sequenced, allowed the identification of an operon containing 10 ORFs, whose proteins contain conserved domains with similarities to domains found in proteins involved in aromatic compound metabolism. This operon was named *fde* and is located upstream from a gene encoding a LysR-type transcriptional activator, which is divergently transcribed and it has been reported that this operon is involved in the degradation of naringenin and that its expression is regulated by flavonoids. (Marin et al., 2013)

Inoculation of *Azospirillium* resulted in early, strain-dependent modifications in the biosynthetic pathways of benzoxazine derivatives in maize in compatible interactions. This was the first study documenting a PGPR effect on plant secondary metabolite profiles, and suggests the establishment of complex interactions between *Azospirillum* PGPR and maize. (V.Walker *et al*., 2011)

## RATIONALE:

During mixed intercropping, it is reasonable to hypothesize that when roots of the plants come into closer contact below ground, the effect of root exudates of the different plants may not only have consequences on each other but also the microorganisms associated in the rhizosphere. The influence that intercropping has on the root exudate composition and on the subsequent colonization of rhizobacteria is not clearly understood. Among rhizobia, *E.fredii* NGR234 is a well-studied model organism because of its broad host range and mutants have been created considering strain roles in attachment, infection and nodulation on legume plants. However mechanism of rhizobia interaction with cereals in intercropping system is unknown. Hence, it would be interesting to know molecular interactions of rhizobia with non-legume in an intercropping system. Endophytes also have an influence on root exudates and it would be interesting to study the effect of endophyte colonization on rhizobacteria through modulation of root exudate composition.

## OBJECTIVES:

1. Influence of root exudates on chemotaxis and colonization of diverse plant growth promoting rhizobacteria in *Cajanus cajan – Zea mays* intercropping system
2. Identification of primary metabolites from the root exudates *Cajanus cajan – Zea mays* intercropping system and their influence on *Ensifer fredii* NGR234
3. Molecular effects of *C. cajan* & *Z. mays* root exudates on NGR234 and its interactions with monocropped and intercropped maize plants

## WORK DONE:

Present work deals with rhizobia and non rhizobia interaction in an intercropping system. Among rhizobia, *E.fredii* NGR234 was considered as it is a well-studied model organism and exhibits PGPR properties like broad host range in legumes for nodulation and nitrogen fixation also apart from it IAA production is also observed. (Theunis *et al*., 2004) Among non-rhizobia, *Enterobacter* sp. C1D was studied as it posseses PGPR properties like metal tolerance, ACC deaminase and phosphate solubilization. (Subrahmanyam *et al*., 2018)

To fulfil these objectives, work was divided into following three chapters:

### Chapter 1: Influence of root exudates on chemotaxis and colonization of diverse plant growth promoting rhizobacteria in *Cajanus cajan – Zea mays* intercropping system

Strain exchange between cereal –legume intercropping cross colonization was studied in the presence of barrier to prevent the root –root interaction and in the absence of barrier which allow the root-root interactions.

In cross colonization studies sterilized seedlings of *C.cajan* and *Z.mays* were taken. For studying cross colonisation in intercropping system in sann culture system two types of setups were designed. In first case, *C.cajan* seedlings were bacterized with log phase culture

and *Z.mays* seedlings were uninoculated. Both seedlings were grown at a distance of 5cm in pot containing sand system in the presence and after 30 days plants were harvested and CFU/g of root was calculated. It was observed that E.C1D and NGR234 cross colonized even in the presence of mesh barrier and colonized on *Z.mays* plant

In second case, *Z.mays* seedlings were bacterized with log phase culture and *C.cajan* seedlings were uninoculated. Like in first case, both plants were grown in pot containing sand and in the presence and absence of mesh barrier and after 30 days plants were harvested and CFU/g of root were calculated.

It was observed that like in previous case both organisms cross colonized in the presence of mesh barrier. By performing this experiment we observed that above both organisms cross colonized in the presence of barrier. So it might be the role of root exudates in cross colonization in an intercropping system.

To confirm the role of root exudates in cross colonization sodium orthovanadate, an ATPase inhibitor to block secretion of root exudates from ABC transporter (Sugiyama et al, 2007) was used. It was observed that at 30µM concentration the plants root-root interactions between the two plants was reduced, therefore our results confirmed that interspecific recognition was partly attributable to root exudates especially soluble substance.

Further to affirm cross colonization studies in 0.8 % Hoagland medium in presence of inhibitor, NGR234 strain was inoculated on one plant and cross colonization was observed at 10 DAS. Interestingly, we could observe cross colonization after elimination of root-root interaction which indicates root exudates playing a role in cross colonization.

To know the compositional difference in the root exudates of monocrops and intercrops, collection of monocrops- *C.cajan* and *Z.mays* and intercrops root exudates were collected from monocrops and intercropped plants at 30 DAS followed by extraction in methanol. Analysis of the metabolites was carried out by GC-MS, identification and quantification of metabolites was based on the measurements of reference compounds using characteristic fragment ions. Results indicated several differences in the root exudates of monocrops and intercrops plants with the release of new allelochemicals upon interspecific facilitation. Compounds like Aconitic acid and myoinositol were distinctly present in the root exudates of *Z.mays* while glycerol and 3-Mannobiose were unique in the intercrop *Z.mays* root exudates. In case of intercrop *C.cajan* root exudates fatty acids like arachidic acid, margaric acid and sugars like mannose were unique, however no unique metabolites were observed in monocrop *C.cajan* root exudates.

Capillary chemotaxis assay was performed to determine the response of NGR234 and E.C1D towards the root exudates of monocrops and intercrops. Organisms were grown up to late log phase in minimal media with 0.5% glucose (E.C1D) and 1% mannitol (NGR234). After 2 hrs of incubation, CFU/ml was counted. Negative control was taken as water and positive control was taken as 10mM glucose and 0.2µM Daidezin as a flavonoid. Differences in number of organisms migrated towards root exudates of monocrop and intercrop reflected difference in their preference for the compounds.

## Chapter 2: Identification of primary metabolites from the root exudates *Cajanus cajan* – *Zea mays* intercropping system and their influence on *Ensifer fredii* NGR234

Root exudates released from plant roots are known to be key mediators for their attachment. To decipher the molecular mechanisms involved in the interactions of NGR234 with the cereal plants, the relative gene expression studies of NGR234 through quantitative Real time polymerase chain reaction (qRT-PCR) was carried out. From strain NGR234, 13 genes were selected on the basis of their chemical signalling involved in attachment and nodulation with host plant legume. These included 3 housekeeping genes (*rpoD, gyrA, recA*) and other 10 genes (*nodA, cmrp, cheA, rhcN, virB, ngrI, flgB, exoI, cpaE, ndvB*) which are likely to be based on studies involved in the interactions with cereal plants. The differential response of NGR234 was observed by growing the strain in Rhizobium minimal glutamate (RMG) broth in absence and presence of root exudates at a concentration (0.5 mg/ml) of *C.cajan* and *Z.mays* for 6 h and 24 h.

Results demonstrated the chemotaxis gene (*cheA*) induction significantly at 6 hours both in *Z.mays* and *C.cajan* plants compared to flavonoid (Apigenin) used as a positive control. The critical finding from our qPCR results showed that root exudates of *Z.mays* stimulates nodulation gene (*nodA*) to a similar level compared to *C.cajan* (host) plant which indicates the presence and release of flavonoids from root exudates from *Z.mays* plants. In addition, to the induction of nod genes, it is known that the production of quorum sensing molecules by rhizobia was enhanced when they were cultured in the presence of flavonoids. (Pérez-Montaño, F. et al., 2011) Our data also suggest that autoinducer (AI) synthase gene (*ngrI)* for quorum sensing is significantly induced with the root exudates of *Z.mays* plant. Consequently it induces transcription of *exoI* gene responsible for the succinoglycan biosynthesis gene which is known to enhance the transcription of the *exo* cluster in the presence of AI molecules. (Krysciak et al., 2014) Root exudates of *Z.mays* induced genes involved in Type III secretion system (*rhcN*) and Type IV secretion system (*virB*) which suggests their role in the infection of *Z.mays* plants as observed similar in rice- *Bradyrhizobia* interactions. (Piromyou, P. et al., 2015) Hence, the results from qPCR studies signify that rhizobia interact with the root exudates of maize through mechanisms like chemotaxis, secretion systems, quorum sensing in response to flavonoids like genistein (Li et., 2017) found in the root exudates of *Z.mays* which are likely to present in the samples.

To support our findings, construct of promoter fusion (*nodA*) with green fluroscent protein *gfp* gene, and for gene deletion (*exoI*) were performed using standard techniques. Cloning for promoter fusions were carried out in pFAJ vector having tetracycline antibiotic selection and gene deletion was carried out in pJQSK200 suicide vector having gentamycin antibiotic as selection marker.(Kelly, S. J., 2013)

Further, NGR234 strain carrying P*nodA*:: *gfp* construct was inoculated on both the *C.cajan* and *Z.mays* plants at 5 day post inoculation and were visualised under confocal laser scanning microscopy (CLSM). NGR234 carrying P*nodA*:: *gfp* expressed on root hairs of both *C.cajan* and *Z.mays* plants indicating its expression in presence of metabolites released from roots of both plants.

It has been reported that flavanoids like genistein are been found in the root exudates of *Z.mays* had concentration of genistein in the solution grown with maize root exudates alone similar to that of faba bean exudates alone. However, the genistein concentration in root exudates collected from a mixture of maize and faba bean was 73% greater than that of the mean of the two crops grown alone. (Li et al., 2016) These results suggests us to check the phenotypic difference in the expression of genes (*nodA. ngrI, exoI, cheA,rhcN,virB*). Also, it will be interesting to check mechanism of colonization of NGR234 with *Z.mays* plants mediated through quorum sensing autoinducer (*ngrI*) mutant and succinoglycan (*exoI)* mutant.

**Chapter 3: Molecular effects of *C. cajan* & *Z. mays* root exudates on NGR234 and its interactions with monocropped and intercropped maize plants**

Endophytes induces host secondary metabolism through bilateral metabolite production and thereby endophytes modulates plant secondary metabolite synthesis. (Brader et al., 2014)

Depending on the type of endophyte, metabolites could also be reduced or remain unchanged. In our studies, *Herbaspirillum seropedicae* is used as model organism as it is a broad- host-range endophyte that colonizes sugarcane, rice, wheat, sorghum, and maize, and has been used as a biofertilizer. (Canellas.L. et al., 2013)

To study NGR234 interactions in presence of an endophyte in an intercropping system, flavanoids were extracted from the root extracts of *Z.mays* inoculated and uninoculated plants. Spectrophotometric studies were carried out to analyse the flavonoids at preliminary level, results demonstrated reduction in the flavonoid compared to the uninoculated plants probably it's due to presence of *fde* operon involved in degradation of flavonoids present in the *H. seropedicae* strain. (Marin et al., 2013)

Further, LC/MS/MS analysis will be carried out to know the qualitative and quantitative levels in the secondary metabolite production. For the relative gene expression studies with NGR234 in presence of the root extracts, genes selected are with respect to nodulation, chemotaxis, quorum sensing. With the root extracts of inoculated and un-inoculated plants, chemotaxis capillary assay and biofilm assay will be performed. Plant growth promotion by NGR234 in the presence of the endophyte in an intercropping system will be done.

## REFERENCES:


- Adu-Gyamfi, J. J., Myaka, F. A., Sakala, W. D., Odgaard, R., Vesterager, J. M., & Høgh-Jensen, H. “Biological nitrogen fixation and nitrogen and phosphorus budgets in farmer-managed intercrops of maize–pigeonpea in semi-arid southern and eastern Africa”. *Plant and soil*. (2007) *295*(1-2): 127-136.
- Badri, Dayakar V., et al. "Root secreted metabolites and proteins are involved in the early events of plant-plant recognition prior to competition." *PLoS One* 7.10 (2012): e46640.

- Baldani, J. I., et al. "Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium." *International Journal of Systematic and Evolutionary Microbiology* 36.1 (1986): 86-93.
- Brader, Günter, et al. "Metabolic potential of endophytic bacteria." *Current opinion in biotechnology* 27 (2014): 30-37.
- Brusamarello-Santos, Liziane Cristina, et al. "Metabolic profiling of two maize (*Zea mays L.*) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria *Herbaspirillum seropedicae* and *Azospirillum brasilense*." *PloS one* 12.3 (2017): e0174576
- Canellas. L. P. et al. "A combination of humic substances and *Herbaspirillum seropedicae* inoculation enhances the growth of maize (*Zea mays L.*)." *Plant and soil* 366.1-2 (2013): 119-132.
- Downie, J. A. "The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots". *FEMS microbiology* reviews. (2010) *34*(2): 150-170.
- Fujita, K., Ofosu-Budu, K. G., & Ogata, S. "Biological nitrogen fixation in mixed legume-cereal cropping systems". *Biological Nitrogen Fixation for Sustainable Agriculture*. Springer Netherlands. (1992):155-175.
- Gao X, Wu M, Xu R, Wang X, Pan R, Hye-Ji Kim, Hong Liao. "Root Interactions in a Maize/Soybean Intercropping System Control Soybean Soil-Borne Disease, Red Crown Rot". *PLoS ONE*.(2014) 9(5):1-9.
- Hage-Ahmed, Karin, et al. "Alterations in Root Exudation of Intercropped Tomato Mediated by the Arbuscular Mycorrhizal Fungus *Glomus mosseae* and the Soilborne Pathogen *Fusarium oxysporum f. sp. lycopersici*." *Journal of Phytopathology* 161.11-12 (2013): 763-773.
- Hayat, Rifat, et al. "Soil beneficial bacteria and their role in plant growth promotion: a review." *Annals of Microbiology* 60.4 (2010): 579-598.
- Huang, X. F., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., & Vivanco, J. M. "Rhizosphere interactions: root exudates, microbes, and microbial communities". *Botany.* (2014), *92*(4):267-275.
- Kaoneka, S. R., Saxena, R. K., Silim, S. N., Odeny, D. A., Rao, G., Rama, N. V. P., & Varshney, R. K. "Pigeonpea breeding in eastern and southern Africa: challenges and opportunities". *Plant Breeding*. (2016) 135:148–154.

- Kelly, S. J., Muszyński, A., Kawaharada, Y., Hubber, A. M., Sullivan, J. T., Sandal, N., & Ronson, C. W. "Conditional requirement for exopolysaccharide in the *Mesorhizobium–Lotus* symbiosis". *Molecular Plant-Microbe Interactions.* (2013), *26*(3), 319-329.
- Krysciak, D., Grote, J., Orbegoso, M. R., Utpatel, C., Förstner, K. U., Li, L., & Streit, W. R. RNA sequencing analysis of the broad-host-range strain *Sinorhizobium fredii* NGR234 identifies a large set of genes linked to quorum sensing-dependent regulation in the background of a *traI* and *ngrI* deletion mutant. *Applied and environmental microbiology*. (2014), *80*(18), 5655-5671.
- Le Strange, K. K., Bender, G. L., Djordjevic, M. A., Rolfe, B. G., & Redmond, J. W. "The *Rhizobium* strain NGR234 nodD1 gene product responds to activation by the simple phenolic compounds vanillin and isovanillin present in wheat seedling extracts". *Molecular Plant-Microbe Interactions*. (1990)*3*(4):214-220.
- Li, B., Li, Y. Y., Wu, H. M., Zhang, F. F., Li, C. J., Li, X. X. & Li, L. "Root exudates drive interspecific facilitation by enhancing nodulation and N2 fixation". *Proceedings of the National Academy of Sciences*. (2016):1-6.
- Lithourgidis, A. S., Dordas, C. A., Damalas, C. A., & Vlachostergios, D. "Annual intercrops: an alternative pathway for sustainable agriculture". *Australian journal of crop science*. (2011) *5*(4): 396-410.
- Lugtenberg BJJ, Kamilova F. "Plant-growth-promoting rhizobacteria". *Annu Rev Microbiol*. (2009) 63:541–556.
- Marin, A. M., et al. "Naringenin degradation by the endophytic diazotroph *Herbaspirillum seropedicae* SmR1." *Microbiology* 159.1 (2013): 167-175.
- Meng. L, Zhang.A ,Wang.F, Han.X, Wang.D and Li.S. "Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system." *Frontiers in plant science.* (2015) 6:1-10.
- Naveed, Muhammad, et al. "The endophyte *Enterobacter* sp. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics." *Biology and fertility of soils* 50.2 (2014): 249-262.
- Ngwira, A. R., Aune, J. B., & Mkwinda, S. "On-farm evaluation of yield and economic benefit of short term maize legume intercropping systems under conservation agriculture in Malawi". *Field crops research*. (2012)*132*: 149-157.

- Pérez-Montaño, F., Guasch-Vidal, B., González-Barroso, S., López-Baena, F. J., Cubo, T., Ollero, F. J., & Espuny, M. R. (2011). "Nodulation-gene-inducing flavonoids increase overall production of autoinducers and expression of N-acyl homoserine lactone synthesis genes in rhizobia". *Research in microbiology*, *162*(7), 715-723.
- Piromyou, P., Songwattana, P., Greetatorn, T., Okubo, T., Kakizaki, K. C., Prakamhang, J., & Minamisawa, K. (2015). "The type III secretion system (T3SS) is a determinant for rice-endophyte colonization by non-photosynthetic *Bradyrhizobium"*. *Microbes and environments*, *30*(4), 291-300.
- Saharan, B. S., and V. Nehra. "Plant growth promoting rhizobacteria: a critical review." *Life Sci Med Res* 21.1 (2011): 30.
- Sierra, Jorge, and Pekka Nygren. "Transfer of N fixed by a legume tree to the associated grass in a tropical silvopastoral system". *Soil Biology and Biochemistry* (2006): 1893-1903.
- Subrahmanyam, Gangavarapu, et al. "*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 (2018): 144-156.
- Sugiyama, Akifumi, Nobukazu Shitan, and Kazufumi Yazaki. "Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-*Rhizobium* symbiosis." *Plant Physiology* 144.4 (2007): 2000-2008.
- Theunis, Mart, et al. "Flavonoids, NodD1, NodD2, and nod-box NB15 modulate expression of the y4wEFG locus that is required for indole-3-acetic acid synthesis in *Rhizobium sp. strain* NGR234." *Molecular Plant-Microbe Interactions* 17.10 (2004): 1153-1161.
- Walker, Vincent, et al. "Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*." *New Phytologist* 189.2 (2011): 494-506.


| | |
|---|---|
| Siddhi Vora | Prof.G.Archana, |
| Candidate | Guide |