Summary & Conclusion

"*Success means having the courage, the determination, and the will to become the person you believe you were meant to be.*"

-George Sheehan

Summary

Based on the literature the rhizospheric microbes and root exudates were found to play an important role in intercropping systems. Therefore, to understand their potential a model of *Cajanus cajan- Zea mays* intercropping system and four plant growth-promoting rhizobacteria (*Enterobacter* sp. C1D, *Pseudomonas* sp. G22, *Rhizobium* sp. IC3109, and *Ensifer fredii* NGR234) were used for this study.

- Through cross colonization experiment, this study highlights that all PGPR strains cross colonized from *C. cajan – Z. mays* plants and vice versa in the absence/presence of barrier placed in the middle of 10 cm distance. However, there was a definite chemotactic response exhibited by this PGPR towards the root exudates of both plants under mono as well as co-cultivation conditions.
- Plant inoculation studies and in vitro assays revealed that non-symbiotic bacteria such as C1D had a preference for both monocropped plants and G22 is evenly colonized in both conditions. On the other hand, symbiotic bacteria such as IC3109 colonized better on intercropped plants and NGR234 on both monocropped and intercropped plants.
- Detection of organic acids from the root exudates of monocropped and intercropped plants was carried out through LC/MS/MS (MRM) mode to which relative differences in fumarate, malate, and succinate were observed in monocropped plants.
- Chemotaxis studies with strain C1D and strain NGR234 revealed that chemo-attraction towards all 4 organic acids was similar, G22 strain migrated towards succinate, fumarate, and citrate with a 3 fold increase in response and IC3109 strain manifested 3 fold increased migration towards fumarate and to succinate with 2 fold increase compared to control.
- Biofilm formation in the presence of organic acids revealed that IC3109 strain had a strong potential to form biofilm formation in presence of fumarate with a 1.2 fold increase while C1D strain, NGR234 strain, and G22 strain demonstrated comparatively less biofilm ability in presence of all four organic acids.

To explore the physiological role and the molecular signaling for the attachment of rhizobia with host legume (*C. cajan)* and non-host cereal plants (*Z. mays)*, further studies were carried out with model organism NGR234.

- By carrying out untargeted metabolomic analysis of root exudates of monocropped and intercropped plants, a clear and distinct difference between monocrop and intercrop root exudates metabolome for each plant. In total 62 metabolites were identified putatively across four different samples.
- When monocrop and intercrop conditions were compared among the plant, it was observed that intercrop *C. cajan* released more of myo-inositol, mannose, and L-proline compared to monocrop *C. cajan*. In the case of intercrop *Z. mays* root exudates, metabolites like D-galactose, D-glucopyranoside, and L-arginine were increased.
- Chemotaxis of NGR234 was found to be significant towards myo-inositol, proline, and glycerol while towards galactose and mannose no significant difference was found in its behavior.
- The growth and biofilm studies with NGR234 showed that exudates of intercropped *C. cajan* exhibited a significant enhancement in biofilm formation, while intercropped *Z. mays* root exudates accelerated the bacterial growth in the late log phase.
- NGR234 tagged with *dsReD* was found to be colonized along with the root hairs of *C. cajan* and *Z. mays* plants visualized through confocal laser scanning microscopy (CLSM) at 23DAS & root hairs of *Z. mays* roots at 15 DAS respectively.
- Relative gene expression studies by qRT-PCR suggested that both *C. cajan* and *Z. mays* root exudates significantly induces the expression of the key genes involved in nodulation (*nodA*), chemotaxis (*cheA2*), secretion systems(*rhcN* & *virB*), quorum sensing (*ngrI* & *traI*)systems.
- The expression of P*nodA*:: *gfp* construct was found to be along with the root hairs of *C. cajan* plants after 5 days of inoculation. While on *Z. mays* plants, its expression was found after 7 days of inoculation along the elongation region of the roots.
- To know the molecular signaling of NGR234 with monocropped and intercropped Z. mays plants, total proteins of NGR234 were isolated upon treatment with root exudates at 48 h and analyzed by a label-free quantitative proteomic approach.

- A total of 2570 proteins were identified across all three samples (untreated cells (control), intercrop *Z. mays*, and monocrop *Z. mays* root exudates treated NGR234 cells).
- However, notable variance in proteins are related to chaperonin proteins (GroES2 and GroEL2), and efficient symbiosis/interaction (BCP, IlvD, LeuC, LeuD) are expressed significantly by NGR234 in the presence of intercropped *Z. mays* root exudates.
- The upregulation of proteins linked to the biosynthesis of branched-chain amino acid in NGR234 by the root exudates of intercrop *Z. mays* further provides an additional basis for understanding the rhizobial –*Z. mays* interaction in intercropped plants.

Conclusion

The behavior of the rhizobacterial species (*Enterobacter* sp. C1D, *Pseudomonas* sp. G22, *Rhizobium* sp. IC3109, and *Ensifer fredii* NGR234) studied here indicates that during the *C. cajan* – *Z. mays* intercropping, the microbes of the two co-cultivated plants might readily colonize across the roots of both plant types. Further, the present study suggests that the behavior of root-associated bacteria is influenced by intercropping, which may be a consequence of the changes in root exudates composition of primary/secondary metabolites. It also highlights new lines of evidence on the contributions of the root exudates in the cereal-legume intercropping system and novel insight into the understanding of rhizobia- cereal interactions.