ABSTRACT

Cereal- Legume intercropping systems, in the context of diversity, ecological function, and better yield have been widely studied. Such systems enhance nutrient phytoavailability by balancing root-rhizosphere interactions. Plant beneficial microbes or plant growth-promoting rhizobacteria (PGPR) resides on/ around the root surface and promote plant growth directly or indirectly. These are a diverse group of bacteria found as symbiotic and non-symbiotic that are used as bio inoculants. Root exudates play a major role in the rhizospheric interactions of plant-plant and/or plant-microbiome interaction. However, the influence of the primary metabolites of root exudates on plant-microbe interactions, in a cereal-legume intercrop system, is not known.

To investigate the influence of rhizosphere colonization by beneficial bacteria in an intercropping system, a model *Zea mays- Cajanus cajan* intercropped plants was determined to know colonization of four different PGPR strains including *Enterobacter* sp. C1D, *Pseudomonas* sp. G22, *Rhizobium* sp. IC3109 and the well-studied broad host range legume nodulating *Ensifer fredii* NGR234. The present study highlights microbial interactions of different genera of non-symbiotic (*Pseudomonas* sp. G22, *Enterobacter* sp. C1D) and symbiotic *(Rhizobium* sp. IC3109, *E. fredii* NGR234) beneficial bacteria with plants grown in the *Z. mays – C. cajan* intercropping system. Cross colonization experiments demonstrated that bacterial movement from one plant species to another can occur in the presence/absence of a mesh barrier that prevented root-root interactions, implying the role of intercrop root exudates in promoting microbial migration from one plant to another. Plant inoculation studies and in vitro assays displayed that non-symbiotic bacteria like *Enterobacter* sp. C1D had a preference for monocropped plants and *Pseudomonas* sp. G22 is evenly colonized in both conditions. On the other hand, symbiotic bacteria like *Rhizobium* sp. IC3109 colonized better on intercropped plants and *E. fredii* NGR234 on both monocropped and intercropped plants. The bacteria under study exhibited differential physiological responses in terms of chemotaxis and biofilm formation towards root exudates of monocropped and intercropped plants. Further primary metabolites identified by both targeted LC/MS/MS (MRM) and a non-targeted metabolomics-based approach revealed a clear separation between intercropping and monocropping root exudates of the two plants. Intercropped *C. cajan*

showed an increase in the myo-inositol, and proline, while intercropped *Z. mays* showed enhanced galactose, D-glucopyranoside, and arginine in the root exudates.

As NGR234 is a broad-host-range legume symbiont and responds well to cereal plants like maize and wheat therefore to understand its adaptation to the intercropping system at the physiological and molecular level, further studies were carried out with this strain. The physiological assays of NGR234 with the root 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. Further, genes related to nodulation, quorum sensing, type III and type IV secretions system were found to be significantly upregulated by the *Z. mays* root exudates NGR234. Promoter fusion *nodA* tagged with GFP was created and confirmed on the roots of *Z. mays*. To investigate in detail the differential protein expression in the presence of intercropped and monocropping *Z. mays* plants, a label-free proteomics approach was used and we identified a total of 2570 proteins of NGR234 covering 50% annotated protein sequences upon exposure to monocropped and intercropped *Z. mays* root exudates. Furthermore, intercropped *Z. mays* root exudates upregulated bacterioferritin migratory protein (BCP), putative nitroreductase, IlvD, LeuC, D (branched-chain amino acid proteins), and chaperonin proteins GroEL2. The current study offered new insights into the metabolome of the cereal-legume intercrop and proteome patterns of NGR234 – exposed to *Z. mays* root exudates that underline the new molecular candidates likely to be involved in the fitness of rhizobium in the intercropping system.

Overall, this study provides different lines of evidence that co-cultivation of cereal and legume plants leads to the variation in root exudates which in turn alters the response of plant beneficial bacteria. Thus, these plant-microbe interactions provide a better understanding of the mechanisms relevant to the application of the PGPR for agricultural production in cereal-legume intercropping systems.