Harnessing the rhizobiome and pathobiome for mitigation of Fusarium wilt in *Cajanus cajan*

Conventional approaches to manage phytopathogens are associated with ecological drawbacks; chemical fertilizers pollute the environment, whereas bioinoculants often display inconsistent performance under field conditions. *Fusarium* is a notorious fungal pathogen causing a variety of diseases in different agricultural crops. Also, in soil ecosystems, environmental factors and pathogen pressure can disturb the rhizospheric microbiome, influencing plant fitness. A pathobiome comprises of microbial members that interact with both host plant and pathogen to promote disease establishment. In this study, the microbial diversity of rhizospheric soil from two varieties of *Cajanus cajan* (pigeonpea) grown in two agroclimatic zones were profiled under *Fusarium udum* infested and pathogen free control conditions. A *Fusarium*-induced disruption of the rhizosphere microbiome was identified, leading to the characterization of a distinct pathobiome. Correlation analysis revealed a negative association between *Bacillus* and *Fusarium*, prompting the generation of a Bacillaceae culture bank with strains exhibiting antagonism against *F. udum* both *in vitro* and *in planta*.

Building on this, a microbiome-assisted rhizosphere engineering approach was adopted to combat Fusarium wilt in *C. cajan*. Indigenous Bacillaceae strains from the culture bank with biocontrol and plant growth-promoting (PGP) traits were strategically assembled into synthetic microbial communities (SMCs). Compatibility testing, qualitative profiling for exhibition of several traits and then using a novel approach of iterative deconvolution to select strains exhibiting enhanced production of biocontrol traits when present in a community of other bacterial strains aided the selection of bacterial strains for generation of several SMCs that were tested through *in vitro* and *in planta* assays. Evaluation of growth attributes and stress markers identified one SMC with superior disease suppression potential, which was further validated under natural conditions. Persistence and colonization patterns of the five member strains of the shortlisted SMC were also confirmed in different root regions through scanning electron microscopy and viable cell counting methods. Further, whole genome sequencing of these five constituent strains revealed multiple biosynthetic gene clusters linked to biocontrol activity.

Mechanistic insights into wilt suppression were gained by investigating the bacterial volatilome. Volatile organic compounds (VOCs) released by biocontrol strains demonstrated both direct antifungal activity and indirect effects via plant immunity. Volatile-mediated interactions between *F. udum* and bacterial strains were characterized, revealing pathogen-induced upregulation of specific bacterial VOCs. The application of some synthetic volatiles effectively suppressed Fusarium wilt under controlled conditions *in planta*. Beyond volatiles, diffusible metabolites were also examined; cell-free supernatants (CFS) of biocontrol strains reduced disease severity in *C. cajan*. Subsequent metabolomic profiling identified several bioactive compounds, and synthetic analogs of these metabolites were confirmed to suppress Fusarium wilt in *C. cajan*.

Overall, this work provides proof-of-concept for the pathobiome in plants and demonstrates microbiome-assisted rhizosphere engineering as a viable strategy for sustainable disease management. By leveraging VOCs and diffusible metabolites from indigenous Bacillaceae strains, this study establishes an effective alternative to environmentally damaging chemical pesticides and unreliable conventional bioinoculants.