HARNESSING THE RHIZOBIOME AND PATHOBIOME FOR MITIGATION OF FUSARIUM WILT IN *CAJANUS CAJAN*

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by

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Submitted

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DEDICATED TO
THE DIVINE
CREATOR, MY
GRANDPARENTS,
PARENTS, AND
SISTER

CERTIFICATE

This is to certify that the thesis entitled "**Harnessing the rhizobiome and pathobiome for mitigation of Fusarium wilt in *Cajanus cajan***" submitted by **Ms. Rashi Tyagi** has been prepared under my guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

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Rashi Tyagi

ABSTRACT

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. The core bacterial genera identified included *Steroidobacter*, *Pseudomonas*, and *Acidibacter*, while *Mortierella*, *Actinomortierella*, and *Naganishia* dominated the fungal core. A *Fusarium*-induced disruption of the rhizosphere microbiome was identified, leading to the characterization of a distinct pathobiome. While certain genera of pathobiome such as *Acidibacter* and *Actinomortierella* overlapped with the core microbiome, others including *Brevundimonas*, *Calenema*, *Delftia* and fungal taxa like *Aplosporella*, *Cristinia*, and *Kwoniella* were unique to *Fusarium*-infested soil samples. 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 potential biosynthetic gene clusters (BGCs) that might be 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.

सार

फाइटोपैथोजेन्स (Phytopathogens) के प्रबंधन के पारंपरिक तरीके पारिस्थितिक कमियों से जुड़े हैं; रासायनिक उर्वरक पर्यावरण को प्रदूषित करते हैं, जबकि बायोइनोक्यूलेंट्स (bioinoculants) अक्सर क्षेत्र की स्थितियों के तहत असंगत प्रदर्शन प्रदर्शित करते हैं। फ्यूजेरियम (*Fusarium*) एक कुख्यात फंगल (fungal) रोगाणु है जो विभिन्न कृषि फसलों में कई तरह की बीमारियों का कारण बनता है। इसके अलावा, मिट्टी के पारिस्थितिक तंत्रों में, पर्यावरणीय कारक और रोगाणु दबाव राइजोस्फेरिक माइक्रोबायोम (rhizospheric microbiome) पर प्रतिकूल प्रभाव डाल सकते हैं, जिससे पौधे की फिटनेस (fitness) प्रभावित होती है। एक पैथोबायोम (pathobiome) में सूक्ष्मजीवी (microbial) सदस्य शामिल होते हैं जो रोग की स्थापना को बढ़ावा देने के लिए पौधे और रोगाणु दोनों के साथ वार्तालाप करते हैं। इस अध्ययन में, दो कृषि-जलवायु क्षेत्रों में उगाए गए अरहर (*Cajanus cajan*) की दो किस्मों से राइजोस्फेरिक (rhizospheric) मिट्टी की सूक्ष्मजीवी (microbial) विविधता को फ्यूजेरियम उदुम (*Fusarium udum*) संक्रमित और रोगाणु मुक्त स्थितियों के तहत प्रोफाइल (profile) किया गया था। फ्यूजेरियम (*Fusarium*)-प्रेरित राइजोस्फीयर माइक्रोबायोम (rhizosphere microbiome) में व्यवधान की पहचान की गई, जिससे एक विशिष्ट पैथोबायोम (pathobiome) का निर्धारण हुआ। पैथोबायोम की कुछ प्रजातियाँ, जैसे एसिडीबैक्टर (*Acidibacter*) और एक्टिनोमॉर्टिएरेला (*Actinomortierella*), कोर माइक्रोबायोम (core microbiome) के साथ अतिव्याप्त थीं, जबकि ब्रेवुंडिमोनस, कैलेनेमा, डेल्फ़्टिया (*Brevundimonas*, *Calenema*, *Delftia)* और एप्लोस्पोरेला, क्रिस्टिनिया और क्वोनिएला (*Aplosporella*, *Cristinia*, and *Kwoniella*) जैसे कवक वर्ग, फ्यूजेरियम (Fusarium)-संक्रमित मृदा नमूनों के लिए विशिष्ट थे। सहसंबंध विश्लेषण से बैसिलस (*Bacillus*) और फ्यूजेरियम (*Fusarium*) के बीच एक नकारात्मक संबंध का पता चला, जिससे एक बैसिलेसी (*Bacillaceae*) कल्चर बैंक (culture bank) का निर्माण हुआ, जिसमें ऐसे उपभेद थे जो इन विट्रो और इन प्लांटा (in plants) दोनों में एफ. उदुम (*F. udum*) के प्रति विरोध प्रदर्शित करते थे।

इसके आधार पर, अरहर में फ्यूजेरियम विल्ट (Fusarium wilt) से निपटने के लिए एक माइक्रोबायोम (microbiome)-सहायता प्राप्त राइजोस्फीयर इंजीनियरिंग (rhizosphere engineering) दृष्टिकोण अपनाया गया। कल्चर बैंक (culture bank) से जैव-नियंत्रण और पादप वृद्धि-प्रवर्तक (पीजीपी) गुणों वाले स्वदेशी बैसिलेसी (*Bacillaceae*) उपभेदों को रणनीतिक रूप से सिंथेटिक माइक्रोबियल (synthetic microbial) समुदायों (एसएमसी, SMC) में एकत्रित किया गया। संगतता परीक्षण, कई लक्षणों के प्रदर्शन के लिए गुणात्मक रूपरेखा तैयार करना और फिर अन्य जीवाणु उपभेदों के समुदाय में मौजूद होने पर जैवनियंत्रण लक्षणों के उन्नत उत्पादन को प्रदर्शित करने वाले उपभेदों का चयन करने के लिए पुनरावृत्त विसंवर्तन के एक नए दृष्टिकोण का उपयोग करने से कई एसएमसी की पीढ़ी के लिए जीवाणु उपभेदों के चयन में सहायता मिली, जिनका परीक्षण इन विट्रो (*in vitro*) और इन प्लांटा (*in planta*) परख के माध्यम से किया गया। विकास विशेषताओं और तनाव मार्करों के मूल्यांकन ने बेहतर रोग दमन क्षमता वाले एक एसएमसी की पहचान की, जिसे प्राकृतिक परिस्थितियों में आगे मान्य किया गया। स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (Scanning Electron Microscopy) और व्यवहार्य कोशिका गणना विधियों के माध्यम से विभिन्न जड़ क्षेत्रों में चयन किए गए एसएमसी (SMC) के पांच सदस्य उपभेदों की दृढ़ता और उपनिवेशण पैटर्न की भी पुष्टि की गई। इसके अतिरिक्त, इन पाँच घटक उपभेदों के संपूर्ण जीनोम अनुक्रमण से कई संभावित जैवसंश्लेषी जीन क्लस्टर सामने आए, जो जैव-नियंत्रण गतिविधि से जुड़े हो सकते हैं।

एफ. उदुम (*F. udum*) और जीवाणु उपभेदों के बीच वाष्पशील (Volatile organic compounds)-मध्यस्थ अंतःक्रियाओं का वर्णन किया गया, जिससे विशिष्ट जीवाणु के रोगजनक-प्रेरित अपरेगुलेशन का पता चला। कुछ सिंथेटिक वाष्पशील पदार्थों के अनुप्रयोग ने पौधों में नियंत्रित परिस्थितियों में फ्यूजेरियम विल्ट को प्रभावी ढंग से दबा दिया। वाष्पशील पदार्थों के अलावा, विसरित मेटाबोलाइट्स (metabolites) की भी जाँच की गई; जैवनियंत्रण उपभेदों के कोशिका-रहित सुपरनैटेंट्स ने अरहर में रोग की गंभीरता को कम किया। बाद में मेटाबोलोमिक प्रोफाइलिंग (metabolomic profiling) ने कई जैवसक्रिय

यौगिकों की पहचान की, और इन मेटाबोलाइट्स (metabolites) के सिंथेटिक एनालॉग्स (synthetic analogues) की पुष्टि अरहर में फ्यूजेरियम विल्ट (Fusarium wilt) को दबाने के लिए की गई।

कुल मिलाकर, यह कार्य पौधों में पैथोबायोम (pathobiome) के लिए अवधारणा का प्रमाण प्रदान करता है और स्थायी रोग प्रबंधन के लिए एक व्यवहार्य रणनीति के रूप में माइक्रोबायोम-सहायता प्राप्त राइजोस्फीयर इंजीनियरिंग को प्रदर्शित करता है। स्वदेशी बैसिलेसी उपभेदों से VOCs और विसरित मेटाबोलाइट्स (metabolites) का लाभ उठाकर, यह अध्ययन पर्यावरण को नुकसान पहुँचाने वाले रासायनिक कीटनाशकों और अविश्वसनीय पारंपरिक बायोइनोक्युलेंट्स (bioinoculants) का एक प्रभावी विकल्प स्थापित करता है।

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ABBREVIATIONS

ADAP	Automated data analysis pipeline
AI	Artificial intelligence
BGCs	Biosynthetic gene clusters
Bioc	Biocompost
CFS	Cell free supernatant
CFU	Colony forming unit
CLSA	Closed-loop stripping analysis
DAS	Days after sowing
EPS	Exopolysaccharides
FACS	Fluorescence-activated cell sorting
FYM	Farmyard manure
FW	Fresh weight
GBDP	Genome BLAST distance phylogeny
GC-QTOF	Gas chromatography/quadrupole time of flight
HR-LCMS-QTOF	High resolution liquid chromatography mass spectrometry / quadrupole time of flight
ITS	Internal transcribed spacer
ISR	Induced systemic resistance
LPX	Lipid peroxidation
MDA	Malondialdehyde
ML	Machine learning
NRPS	Non-ribosomal peptide synthetase
OD	Optical density
OTU	Operational taxonomic unit
PBS	Phosphate buffer saline
PCoA	Principal coordinates analysis
PDA	Potato dextrose agar
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
PTFE	Polytetrafluoroethylene
RI	Retention index
RiPP	Ribosomally synthesized and post-translationally modified peptide
SEM	Scanning electron microscopy
SMC/SynCom	Synthetic microbial community
SPME	Solid phase microextraction
TYGS	Type strain genome server
VOC	Volatile organic compound

Elements

Carbon	C
Nitrogen	N
Phosphorus	P
Potassium	K

Units

Cm	Centimetre
ºC	Degrees Celsius
G	Gram
H	Hours
$m\ s^{-1}$	Meter per second
µL	Microliter
µm	Micrometre
µM	Micromolar
Mg	Milligram
mL	Milliliter
mM	Millimolar
Min	Minutes
M	Molar
m/z	Mass/charge
Nm	Nanometre
%	Percent
rpm	Revolution per minute
S	Seconds

Molecules and Chemicals

ACC	Aminocyclopropane-1-carboxylic acid
$(NH_4)_2SO_4$	Ammonium sulfate
$CaCl_2$	Calcium chloride
CMC	Carboxymethyl cellulose
$CoCl_2$	Cobalt (II) chloride
CTAB	Cetyltrimethylammonium bromide
DCM	Dichloromethane
ddH_2O	Double-distilled water
DNS	Dinitro-salicylic acid
EDTA	Ethylenediaminetetraacetic acid
FCP	Folin–Ciocalteu Phenol
$FeSO_4 \cdot 7H_2O$	Ferrous sulfate heptahydrate
H_2O_2	Hydrogen peroxide
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
KH_2PO_4	Potassium dihydrogen phosphate
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$MnSO_4$	Manganese (II) sulfate
Na_2CO_3	Sodium carbonate
$Na_2HPO_4 \cdot 7H_2O$	Disodium hydrogen phosphate heptahydrate
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
NBT	Nitrobluetetrazolium
PVP	Polyvinylpyrrolidone
TBA	Thiobarbituric acid

TCA	Trichloroacetic acid
$ZnCl_2$	Zinc chloride