

**MICROBIOME-BASED RHIZOSPHERE ENGINEERING
FOR MITIGATION OF SALINITY STRESS
IN *VIGNA RADIATA***

SHUBHAM DUBEY



**DEPARTMENT OF BIOCHEMICAL
ENGINEERING AND BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
NOVEMBER 2023**

© Indian Institute of Technology Delhi (IITD), New Delhi, 2023

**MICROBIOME-BASED RHIZOSPHERE ENGINEERING
FOR MITIGATION OF SALINITY STRESS
IN *VIGNA RADIATA***

by

SHUBHAM DUBEY

Department of Biochemical Engineering and Biotechnology

Submitted

In fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

to the



Indian Institute of Technology Delhi

November 2023

Dedicated
To my Grandparents, who inspired
this and will not read it.....

CERTIFICATE

This is to certify that the thesis entitled “**Microbiome-based rhizosphere engineering for mitigation of salinity stress in *Vigna radiata***” being submitted by **Mr. Shubham Dubey** is worthy of consideration for the award of the degree of Doctor of Philosophy. The thesis has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi, and is a record of the original bonafide research work. The results presented in this thesis have not been submitted in part or full to any other universities or institutes for the award of any other degree or diploma.

Prof. Shilpi Sharma

Professor,
Department of Biochemical Engineering
and Biotechnology,
Indian Institute of Technology Delhi
New Delhi-110016
INDIA

ACKNOWLEDGEMENTS

“वर्णानामर्थसंघानां रसानां छन्दसामपि।
मंगलानां च कर्त्तरौ वन्दे वाणीविनायकौ॥१॥

First Shloka from Sri Ramcharitmanas

By the **Grace of God**, I have successfully completed my **Ph.D. thesis work**. As I stand at the crossroads, on the verge of thesis submission, I am fondly ready to take a walk down memory lane, remembering with gratitude, all the individuals who have supported me throughout this journey and beyond.

Life during Ph.D. can be called a roller coaster ride, but I am immensely fortunate to have been guided by my Supercool Mentor, who has nurtured and cared for me, guarded and shielded me under all circumstances, steered me through the crests and troughs elegantly, and always ensured with her excellent instincts and decisions, that all kinds of problems and worries are at bay. I take this opportunity to sincerely Thank my **Guru**, my **Ph.D. Mentor, Prof. Shilpi Sharma**, for her kind guidance, tireless efforts to set things right for me, constant support, cooperation and understanding. During Ph.D., she has been the major pillar of strength for me and rescued me from all troublesome situations. During this phase, there has been so much to learn from her. Her precision, commitment towards perfection, focused and meticulous work approach, systematic time management, exceptional ability to strike a balance in work and life, have been exemplary. She has been extremely supportive of all my endeavours. It is because of her that I happened to get opportunities to interact with superb researchers in different parts of the world. Her superfast and accurate solutions to all our doubts have rescued us time and again. She is a wonderful Group Leader and amazing Scientist, the Best Mentor, I have known in my life. I cannot thank her enough, but I am ever grateful to her for everything that she has done for me. This journey has become possible because of her patience, her firmness and resilience, her valuable suggestions and constant endeavours to help me become a better version of myself. Whatever I have achieved in my Ph.D. tenure is all because of her continued perseverance. Indeed, I am glad to owe the success of this journey to her.

Due to kind initiatives taken by my Mentor, I was fortunate to have got a chance to pursue a five months' research exchange program at University of Fribourg, Switzerland under the mentorship of **Prof. Laure Weisskopf**. I am grateful to **Prof. Laure** for hosting me in her lab, for

providing me with valuable guidance during my research endeavours and supporting me throughout.

I am grateful to my SRC members, **Prof. Preeti Srivastava**, **Prof. Ravikrishnan Elangovan** and **Prof. P. Hariprasad**, for their valued suggestions and support throughout my Ph.D. tenure. Special thanks to **Preeti Ma'am** for kindly permitting me to set up my final experiments in plant growth chamber in her lab. I thank our Departmental Heads, **Prof. Ritu Kulshreshtha** (present) and **Prof. D. Sundar** (former), for providing us with excellent research facilities in Department of Biochemical Engineering and Biotechnology, at IIT Delhi. I am really grateful and convey my sincere thanks to **Prof. Yashbir S. Shivay**, Principal Scientist at Indian Agricultural Research Institute, for kindly allowing me to conduct some of my crucial experiments in his laboratory. I also thank **Prof. Charaya**, my teacher at Chaudhary Charan Singh University, Meerut, for his valued suggestions and constant encouragement during my research work. I am immensely grateful to **Dr. Manoj Tripathi (Manoj Mama, my Maternal Uncle)** for his valuable advice and kind support at all times, in research and life in general.

I am indeed grateful to my lab members at University of Fribourg, especially my senior colleague **Dr. Floriane L'Haridon** for her kind help and valuable suggestions during my stay there. I was impressed with the disciplined working style of my young intern, **Viviane**, and I thank her for supporting me during my experiments. I also thank **Abhishek Anand** and **Tashi** for their constant support in lab, as well as throughout my stay in Switzerland. Life would have been tough there if such wonderful friends like them were not around. Back home, I am grateful to **Dr. Vijay Wardhan**, my friend at NIPGR for his kind help and valued input in successfully completing my plant growth experiments. I convey my thanks to **Srinivas** and **Keerti Ranjan**, friends at IARI, for helping me in times of need.

I thank **IIT Delhi** for the award of research fellowship and providing financial assistance to attend national and international conferences. I am grateful to all members of the **Department of Biochemical Engineering and Biotechnology, IIT Delhi** for their kind support. I would also like to thank members of the **Central Instrumentation Facility** and **Liquid Nitrogen Facility** for extending their help, whenever required. I sincerely thank **Mukesh Bhaiya** and **Santram Ji** for their help, whenever required. I am really grateful to **Ram Prakash Bhaiya** and especially **Omkar Bhaiya** and associated IIT Delhi nursery staff for helping me to conduct my plant growth experiments under natural conditions during my Ph.D. years.

I am really fortunate to be a part of **Sharma et al.** at **EGL, IIT Delhi**. Lab life has always been fun, with knowledgeable seniors, wonderful batchmates and vibrant young colleagues. Agreed that there have been ups and downs during this long journey, but the best part is that we stood together during challenging and happy moments. When I joined lab in 2017, I was greeted by a group of very cordial lab members. Firstly, I am deeply indebted to **Vasu Goel**, who initiated the rhizosphere engineering work and delivered promising results. His genuine efforts and cooperation in work is really appreciated, as it helped me carry forward the research in this frontier with relative ease. I wholeheartedly thank **Gautam Sir**, for helping and supporting me, and also for patiently teaching me the basics of DGGE after I joined lab. I am really thankful to my seniors and colleagues, **Richa Ma'am**, **Sakshi Ma'am**, **Monica Ma'am**, **Nidhi**, **Prashant** and **Prasun**, who are alumni now. It has been great to have received their support and cooperation, always. I extend my sincere thanks to **Upma Ma'am** and **Swati Ma'am**, for always being there to help me out, whenever I needed their inputs and cooperation. Their goodwill for me is gratefully appreciated. I am grateful to **Late Shiv Bhaiya** (Lab Assistant) for all his help. It's unfortunate that we lost him so early in life, due to his kidney ailments.

I take the opportunity to thank my present EGL colleagues and seniors for their constant support. Many thanks to **Sonal Ma'am**, **Shweta Ma'am**, and **Amitava Sir** for their encouragement and kind cooperation in all my endeavors. It is amazing to work with bright young juniors. I am thankful to **Rashi**, **Salila**, **Rituraj**, **Kanika**, **Soumya**, **Priya**, **Argha**, **Vaibhav**, **Gorika**, **Sakshi**, and **Sriya** for their kind help. It has been a long association with some labmates and friends during my Ph.D. I thank **Priyanka Ma'am**, **Vijal Ma'am**, **Shivani**, **Kanika**, **Salila** and **Anu didi** (**Annapurna Ma'am** in lab), for their motivation and great support. I cherish the times that I spent with **Shivani** and **Vijal Ma'am**, during lab hours as well as the memorable tea breaks at Amul, where we used to share our hearts out. We had great fun moments and candid talks during chai-pe-charcha sessions. On a serious note, I am grateful to **Vijal Ma'am** for enlightening me about the basics of lab work, during my initial phase. **Shivani**, a very good friend of mine, and great batchmate, has been a tremendous support. I cannot thank her enough as words fall short, for the help that she has extended to me, during all the critical stages of my experiments. I am grateful to her for giving valuable suggestions during my work and for motivating me to do my best always. We have been through the best and worst of times in lab together and am really grateful to **Shivani** for her constant support and goodwill. Special mention of gratitude to **Salila**,

for extending her help at all times and carrying out the plant growth experiments for me, when I was away. I would like to specially thank **Dr. Annapurna Bhattacharjee**, my go-to person for all the support and guidance whenever the need be. She helped me with almost all the experiments and planning. This Ph.D. journey would have been really difficult without her support and help. **Anu Didi** has been like the sports physios, who runs around during all the activities on ground to ensure that cricketers are fit enough to perform well and give their best in the matches...:-)

I am very lucky to have been associated with very enthusiastic young B.Tech. and M.Tech. trainees during my tenure in lab. I especially thank **Pratibha** and **Parth** for their unwavering support and affection. I am really thankful to **Abhay**, for his dedicated efforts, kind support and cooperation. My most recent trainees, **Mohita** and **Jaskeen** have been amazing to work with. I am really grateful to all my mentees for their contributions in my research endeavors. They all have been great mentees with whom I got a chance to work. I wholeheartedly thank my **labmates** for extending their help and support whenever I needed.

Beyond lab life, in IIT Delhi, I have been blessed to have had the companionship and support of great friends. **Abhay Tiwari** (CRDT), my very dear friend has been throughout there to boost my morale and hear me out patiently, in most difficult times. I am thankful to **Abhijit** (DBEB), **Durai Sir**, **Himanshu Arora** and **Amir** (CRDT) for always standing by my side, whenever I needed. **Jagat** and **Tanmay** have been very good friends and roommates in my IIT Delhi abode, **Kumaon Hostel**, at different phases of my tenure. Their affection for me is ever cherished. Special thanks and photo credits to **Tanmay** for clicking some wonderful pictures related to my final experiments. I would also like to thank my dear juniors, **Rohit** and **Harsh**, for their continued support and motivation. It has been great being with all of them. They were just one call away and really, we spent some memorable times together. I thank all my **batchmates**, **hostel friends**, and **college friends** for their overwhelming support and goodwill. I would also like to thank my seniors from **Hindu College** (Delhi University), **Swetanshu Shekhar**, **Akashdeep Roy**, **Kunal Kaudan**, **Bharat Khanna**, **Pratyush Rai** and **Vivek Kapoor** for their constant support. I am also grateful to my seniors **Dr. Ashu Tyagi**, **Dr. Amit Saini**, **Gourav Chaudhir**, **Nivedita Vats**, and friend **Prashant Tyagi** at CCS University Meerut for all the support and help.

Friends play a pivotal role in our lives, shaping our present and future in the most beautiful ways. I am especially thankful to my dearest school friend, **Shivam Verma**, for always being there for me. I am also grateful to my best buddies, **Akhilesh Kumar Mishra** and **Deeksha Singh** in

Hindu College, Delhi University, for their staunch support and unflinching faith in me, whenever I have been down and out. They patiently stood by me at all times and motivated me. Their goodwill means a lot to me, and their kind concern is always appreciated.

The support of my **Family** has been tremendous at all times in my life. I wholeheartedly thank all my family members for their blessings and crucial contributions. The blessings and affection of my **Grandparents, Dada Ji-Dadi Ji** and **Nana Ji-Nani Ji**, is ever cherished. Since my childhood, I have nurtured a very special bond with my paternal **Grandmother**. It is because of the love and blessings of my **Dadi Ji (Smt. Prabha Devi)**, that this journey has been so enriching and fulfilling. I am grateful for her affection always. My heart is filled with gratitude for my **beloved Parents, Naresh Kumar Dubey** and **Sadhana Dubey**, for their motivation and constant support. My **Father** has played a key role in smooth execution of my field experiments. My **Mother** has stood by me at all times and her unfathomable love, and faith, keeps me in good stead, always. I can never thank my **Parents** enough, for their unconditional love, blessings and support. My Sisters, **Dr. Soumya Dubey, Shubhangi Dubey (Shubhi Didi)** and **Dr. Annapurna Bhattacharjee (Anu Didi)**, have been incredible support systems for me. Their care and concern for me is ever cherished. I am grateful to **Shubhi Didi** and my Brother-in-Law, **Rajat Dwivedi Ji**, for their encouragement, support and kind understanding. I also thank my **Tauji Vinay Kumar Dubey** and **Chachaji Sanjiv Kumar Dubey** for their guidance and support. My cousins **Prashant, Mayur, Siddharth**, and **Tanu** have all been there to support me during all the ups and downs of my Ph.D. life. It means a lot to me. I am grateful to all my **Buajis, Chachiji, Taiji, Masi** and **Mamajis** for their care and kind consideration. Special thanks to **Tauji Girija Shankar Bhattacharjee** and **Taiji Chhanda Bhattacharjee** for taking good care of me during the last and crucial phase of my Ph.D. Their wisdom has been very helpful and their blessings and affection is cherished always. It is my **Family's** motivation, optimism, and push that has made life easy. I can never thank my **Family** enough for everything that they have done to help me achieve my objectives and goals in life.

Finally, I would like to thank **God** for His divine grace and blessings, for making this journey possible. May All be Well with everyone.

Jai Siya Ram!!

Shubham Dubey

ABSTRACT

The indiscriminate use of chemical fertilizers and pesticides in agriculture, and rising global temperatures (rising sea levels, fluctuations in weather cycles and increase in primary salinization), lead to salt accumulation in the soil, thereby negatively impacting plant physiology and crop yield worldwide. This has significantly affected the global economies, as agriculture is essential for ensuring food security and reducing poverty in many parts of the world. To address this grave issue, researchers have turned to eco-friendly approaches of utilizing beneficial microbes residing in the rhizosphere. However, using single strains or consortia of different strains has often proven ineffective due to issues concerning their ability to colonize roots while facing competition from the native soil microbiome. To combat this challenge, rhizosphere engineering has been employed as a sustainable strategy. It can be categorized into plant-based, metaorganism-based, and microbiome-based methods, wherein the transformation of the non-optimized metaorganism takes place, resulting in an optimized metaorganism that exhibits enhanced fitness. The microbiome-based method can be attained by employing top-down or bottom-up approaches.

In present study, **an indirect plant-assisted microbiome-selection strategy was adopted over several passaging cycles to acclimatize the indigenous microbiome such that it benefitted the growth of *Vigna radiata* plants under salt-stress conditions.** The plant fitness was assessed over successive cycles in terms of plant growth and physiological parameters. Results showed an increased tolerance of plants to salinity stress, as evidenced by enhancement in plant growth attributes and decreased stress marker levels/indicators such as proline, malondialdehyde (MDA), and membrane stability index (MSI). Amplicon sequencing, employing the 16S rRNA and ITS as genetic markers, revealed significant changes in the microbial community inhabiting the rhizosphere of *Vigna radiata* under salinity stress across the passages. A significant increase

in the relative abundance of dominant bacterial phyla such as *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, coupled with a concomitant decrease in the abundance of the fungal phylum *Ascomycota*, was observed in the acclimatized microbiome during successive passages. Moreover, a decline in the abundance of *Mortierellomycota* and *Chytridiomycota* was explicitly noted during the 5th and 13th passages, respectively.

Once the salt stress-adapted rhizospheric microbiome was obtained, the next objective was to understand the mechanism of action in salinity stress mitigation in *Vigna radiata*. Bacterial strains were isolated from stress-acclimatized microbiome using group-specific media to generate a culture bank. As determined by split plate assay, specific bacterial isolates were found to produce volatiles, possibly contributing to salt stress mitigation in *Arabidopsis*. The best-performing plant growth-promoting bacterial strains were selected to analyze their volatilomes by gas chromatography–mass spectrometry (GC-MS). Key metabolites, like 7 methyl-hexadecane, 2-(2-butoxyethoxy) ethanol, sulfur-containing volatiles, etc. were identified after a detailed investigation of volatilomes of stress-acclimatized bacterial strains. These volatiles may promote plant development and stress tolerance. Hence the impact of selected volatiles was then examined on *Vigna radiata* under controlled conditions. In the presence of salinity stress, the volatiles were able to mitigate the effects of stress, promote plant growth and improve their fitness.

To overcome the technical limitations associated with the storage of such an acclimatized microbiome, synthetic microbial communities (SynComs) were designed from the culture bank generated from the acclimatized microbiome. SynComs were generated to perform *at par* with the stress-acclimatized microbiome, while mimicking its functionality in mitigating salinity stress in plants grown in arable land. The salinity stress mitigation potential of these SynComs was assessed

first under control, and subsequently in field conditions, resulting in significant enhancement in the plant growth and stress amelioration under salinity stress condition.

The efficacy of the multi-passaging technique in rhizosphere engineering and the utilization of SynComs has been demonstrated through an extensive investigation conducted in laboratory and field settings. The study presents opportunities for implementing such a unique approach of combining top-down and bottom-up strategies with other crops and under diverse stress regimes. This approach promises an essential and favourable step toward ushering sustainable agricultural practices worldwide.

सार/सारांश

कृषि में रासायनिक उर्वरकों और कीटनाशकों के अत्यधिक उपयोग और बढ़ते वैश्विक तापमान के कारण मृदा में लवण संचयन हो रहा है, जो दुनिया भर में पादप संरचना और फसल की उपज को नकारात्मक रूप से प्रभावित कर रहा है। इसने वैश्विक अर्थव्यवस्थाओं को काफी प्रभावित किया है, क्योंकि दुनिया के कई हिस्सों में गरीबी को कम करने के लिए और खाद्य सुरक्षा सुनिश्चित करने के लिए कृषि आवश्यक है। इस गंभीर मुद्दे को हल करने के लिए शोधकर्ताओं ने मूल परिवेश, राइजोस्फीयर (rhizosphere) में रहने वाले लाभकारी जीवाणुओं के उपयोग के लिए पर्यावरण के अनुकूल तरीकों की ओर रुख किया है। हालांकि, जीवाणुओं के एकल उपयोग या संयुक्त अनुप्रयोग करना अक्सर देशी मिट्टी माइक्रोबायोम से प्रतिस्पर्धा का सामना करते हुए जड़ों को उपनिवेशित करने की उनकी क्षमता से संबंधित मुद्दों के कारण अप्रभावी साबित हुआ है। इस चुनौती का मुकाबला करने के लिए, राइजोस्फीयर इंजीनियरिंग (rhizosphere engineering) को एक स्थायी रणनीति के रूप में अपनाया गया है। इसे पादप-आधारित, मेटाऑर्गेनिज्म (metaorganism)-आधारित और माइक्रोबायोम (microbiome)-आधारित विधियों में वर्गीकृत किया जा सकता है, जिसमें गैर-अनुकूलित मेटाऑर्गेनिज्म का परिवर्तन होता है, जिसके परिणामस्वरूप एक अनुकूलित मेटाऑर्गेनिज्म में परिवर्तित हो जाता है, जो बढ़ी हुई उपयोगिता प्रदर्शित करता

है। माइक्रोबायोम-आधारित विधि को टॉप-डाउन (top-down) या बॉटम-अप (bottom-up) दृष्टिकोणों को अपना करके प्राप्त किया जा सकता है।

वर्तमान अध्ययन में, निवासी माइक्रोबायोम को अनुकूलित करने के लिए बहु पारगमन चक्रों (multi-passaging cycle) पर आधारित एक अप्रत्यक्ष पादप-सहायता प्राप्त माइक्रोबायोम-चयन रणनीति अपनाई गई थी ताकि लवण-तनाव की स्थिति में *विग्ना रेडिएटा* (*Vigna radiata*) पौधों के विकास को लाभ हो। पौधे की फिटनेस का मूल्यांकन पौधे के विकास और शारीरिक मापदंडों के संदर्भ में क्रमिक चक्रों में किया गया था। परिणामों ने लवणता तनाव के लिए पौधों की बढ़ती सहनशीलता दिखाई, जैसा कि पौधे के विकास गुणों में वृद्धि और प्रोलिन (proline), मालोंडिअलडिहाइड (Malondialdehyde, एमडीए), और मेम्ब्रेन स्टैबिलिटी इंडेक्स (Membrane stability index, एमएसआई) जैसे तनाव मार्कर स्तर / संकेतकों में कमी से स्पष्ट है। 16 एस आरआरएनए (16S rRNA) और आईटीएस (ITS) को आनुवंशिक मार्करों के रूप में नियोजित करने वाले एम्प्लिकॉन (amplicon) अनुक्रमण ने पारगमन चक्रों में लवणता तनाव के तहत *विग्ना रेडिएटा* के राइजोस्फीयर में रहने वाले माइक्रोबियल समुदाय में महत्वपूर्ण परिवर्तनों को उजागर किया। प्रोटीओबैक्टीरिया (*Proteobacteria*), एक्टिनोबैक्टीरिया (*Actinobacteria*) और फर्मिक्यूट्स (*Firmicutes*) जैसे प्रमुख बैक्टीरियल फ़ाइला (phyla) की सापेक्ष बहुतायत में एक महत्वपूर्ण वृद्धि, फंगल फाइलम एस्कोमाइकोटा की बहुतायत में सहवर्ती कमी के साथ मिलकर, बहु पारगमन

चक्रों के दौरान अनुकूलित माइक्रोबायोम में देखी गई थी। इसके अलावा, क्रमशः 5 वें और 13 वें चक्र के दौरान मोर्टिएरेलोमीकोटा (*Mortierellomycota*) और चिट्रिडियोमाइकोटा (*Chytridiomycota*) की बहुतायत में गिरावट स्पष्ट रूप से नोट की गई थी। गैस क्रोमैटोग्राफी-मास स्पेक्ट्रोमेट्री (Gas Chromatography-Mass Spectrometry, जीसी-एमएस) द्वारा उनके वोलाटिलोम (volatilome) का विश्लेषण करने के लिए सबसे अच्छा प्रदर्शन करने वाले पौधे के विकास को बढ़ावा देने वाले जीवाणु उपभेदों का चयन किया गया था। प्रमुख मेटाबोलाइट्स (metabolites), जैसे 7 मिथाइल-हेक्साडेकेन (7 methyl-hexadecane), 2-(2-ब्यूटॉक्सीथोक्सी) इथेनॉल (2-(2-butoxyethoxy) ethanol), सल्फर युक्त वाष्पशील, आदि। तनाव-अनुकूलित जीवाणु उपभेदों के ज्वालामुखी की विस्तृत जांच के बाद उनकी पहचान की गई थी। ये वाष्पशील पौधे के विकास और तनाव सहिष्णुता को बढ़ावा दे सकते हैं। इसलिए चयनित वाष्पशील पदार्थों के प्रभाव की जांच तब नियंत्रित परिस्थितियों में विगना रेडिएटा पर की गई थी। लवणता तनाव की उपस्थिति में, वाष्पशील तनाव के प्रभाव को कम करने, पौधे के विकास को बढ़ावा देने और उनकी फिटनेस (fitness) में सुधार करने में सक्षम थे।

एक बार नमक तनाव-अनुकूलित राइजोस्फेरिक माइक्रोबायोम प्राप्त हो जाने के बाद, अगला उद्देश्य विगना रेडिएटा में लवणता तनाव शमन में कार्रवाई के तंत्र को समझना था। कल्चर बैंक (culture bank) उत्पन्न करने के लिए समूह-विशिष्ट मीडिया का उपयोग करके तनाव-अनुकूलित

माइक्रोबायोम से बैक्टीरियल (bacterial) उपभेदों को अलग किया गया था। जैसा कि स्प्लिट प्लेट (split-plate) परख द्वारा निर्धारित किया गया था, विशिष्ट बैक्टीरियल आइसोलेट्स (bacterial isolates) वाष्पशील पदार्थों का उत्पादन करते पाए गए थे, ये वाष्पशील पदार्थ संभवतः एराबिडोप्सिस (Arabidopsis) में नमक तनाव शमन में योगदान देते थे।

इस तरह के एक अनुकूलित माइक्रोबायोम के भंडारण से जुड़ी तकनीकी सीमाओं को दूर करने के लिए, सिंथेटिक माइक्रोबियल समुदायों (सिनकॉम्स, SynComs) को अनुकूलित माइक्रोबायोम से उत्पन्न माइक्रोबियल कल्चर बैंक (microbial culture bank) से डिजाइन किया गया था। कृषि योग्य भूमि में उगाए गए पौधों में लवणता तनाव को कम करने में इसकी कार्यक्षमता की अनुकृति करते हुए, तनाव-अनुकूलित माइक्रोबायोम के बराबर प्रदर्शन करने के लिए सिनकॉम्स को बनाया गया था। इन सिनकॉम्स की लवणता तनाव शमन क्षमता का मूल्यांकन पहले नियंत्रित परिस्थितियों में किया गया, और बाद में वास्तविक कृषि क्षेत्र की स्थितियों में परीक्षण किया गया।

राइजोस्फीयर इंजीनियरिंग (rhizosphere engineering) में बहु पारगमन चक्र तकनीक की प्रभावकारिता और सिनकॉम (SynCom) के उपयोग को प्रयोगशाला और वास्तविक कृषि क्षेत्र में आयोजित एक व्यापक जांच के माध्यम से प्रदर्शित किया गया है। वर्तमान अध्ययन अन्य फसलों तथा विविध तनाव व्यवस्थाओं के उपचार हेतु टॉप-डाउन (top-down) और बॉटम-अप

(bottom-up) रणनीतियों के संयोजन का एक अनूठा दृष्टिकोण लागू करने के अवसर प्रस्तुत करता है। यह दृष्टिकोण दुनिया भर में टिकाऊ कृषि प्रथाओं को शुरू करने की दिशा में एक आवश्यक और अनुकूल कदम का वादा करता है।

LIST OF CONTENTS

ACKNOWLEDGEMENTS	i-v
ABSTRACT	vi-viii
सार/सारांश	ix-xiii
Abbreviations	xxiii-xxv
1. INTRODUCTION AND OBJECTIVES	1-9
1.1. Introduction	1
1.2. Research gaps	7
1.3. Objectives	8
2. REVIEW OF LITERATURE	10-49
2.1. Stress factors affecting agro-ecosystems	10
2.2. Sustainable agriculture: A necessity in the current scenario for stress mitigation	13
2.3. System of study	14
2.4. Eco-friendly approaches for improving plant and soil health	14
2.5. Challenges of bioinoculant application	17
2.6. Rhizospheric microbiome as a hub of microbial activity	18
2.7. Rhizosphere engineering	19
2.7.1. <i>Manipulation of rhizospheric microbiome based on plants</i>	20
2.7.2. <i>Microbial amendments' role in modulating the rhizospheric microbiome</i>	21
2.7.3. <i>Microbiome-based approaches to enhance plant fitness</i>	22
2.7.3.1. <i>Bottom-up approach</i>	23
2.7.3.2. <i>Top-down approach</i>	25
2.8. Plant-mediated rhizosphere engineering as a promising strategy for modulating plant fitness	28
2.8.1. <i>Source of starter microbiome</i>	29
2.8.2. <i>Plant genotype</i>	30
2.8.3. <i>Microbiome's efficiency determined by plant phenotype</i>	31
2.8.4. <i>Selecting microbiome for soil transplantation</i>	32
2.8.5. <i>Transplantation of soil or microbiome</i>	33
2.8.6. <i>Selection rounds</i>	34
2.8.7. <i>Efficacy of optimized microbiome after multiple passaging</i>	36
2.8.8. <i>The need for appropriate controls in plant-mediated microbiome selection</i>	37
2.8.9. <i>Multiple passaging significantly affects the microbiome functionality</i>	38
2.9. Factors governing the evolution of the microbiome in response to environmental change	40
2.10. Limitations of plant-based microbiome selection	42
2.10.1. <i>Concerns related to experimental design</i>	43

2.10.2. Issues pertaining to preservation, dissemination, and transplantation of evolved microbiomes	43
2.11. Road ahead in the field of rhizosphere engineering	46
2.11.1. Proof of concept limited to a few model plants	47
2.11.2. Improving the efficacy of selected microbiome with technical advancements	48
3. MATERIALS AND METHODS	50-79
3.1. Model plant used in the study	50
3.1.1. Characteristics of Pusa Vishal, a mungbean variety for cultivation in India	50
3.1.2. Salt tolerance assessment of <i>Vigna radiata</i> var. Pusa Vishal	51
3.2. Plant growth experiment for acclimatization of the rhizospheric microbiome	51
3.2.1. Soil characterization	52
3.2.2. Soil preparation for multi-passaging experiment	53
3.2.3. Assessment of plants based on biometrics and estimation of levels of stress markers	57
3.2.3.1. Analyzing morphological traits of plants	57
3.2.3.2. Quantifying plant stress marker levels	57
3.2.3.2.1. Chlorophyll estimation	57
3.2.3.2.2. Malondialdehyde (MDA) estimation	58
3.2.3.2.3. Measurement of membrane stability index (MSI)	58
3.2.3.2.4. Proline estimation	59
3.2.3.2.5. Quantification of sodium-potassium (Na^+ - K^+) ions	59
3.3. Culture-independent analysis of the soil microbial communities (bacterial and fungal) by employing molecular tools for studying the rhizospheric microbiome	60
3.3.1. Characterization of microbial diversity of the rhizospheric microbiome by amplicon sequencing	60
3.3.1.1. Bacterial diversity	61
3.3.1.2. Fungal diversity	62
3.4. Culture-dependent analysis of bacterial communities	63
3.4.1. Generation of culture bank from acclimatized microbiome	63
3.4.2. 16S rRNA-based ribotyping and phylogenetic analysis of bacterial strains	65
3.4.3. Testing of plant growth promoting abilities of isolates from culture bank	65
3.4.3.1. Nitrogen fixation	65
3.4.3.2. Phosphate solubilization	65
3.4.3.3. Siderophore production	66
3.4.3.4. Production of indole-3-acetic acid (IAA)	66
3.4.3.5. ACC deaminase production	67
3.4.3.6. Hydrogen cyanide (HCN) production	67
3.4.3.7. Biofilm formation and exopolysaccharide (EPS) production	68
3.4.4. Evaluating the salt tolerance of bacterial isolates	68

3.5. Volatilome analysis of bacterial strains	69
3.6. Validation of identified volatile organic compounds (VOCs) by plant growth experiment	70
3.7. Generation of SynComs	72
3.8. Plant growth experiments to evaluate the impact of bacterial isolates and SynComs in stress tolerance and plant growth promotion	75
3.8.1. Effect of individual bacterial strains in controlled environment	75
3.8.2. Assessment of the impact of SynComs	76
3.8.2.1. SynComs with minimum and maximum strain numbers in a controlled environment	76
3.8.2.2. SynComs under natural conditions (IIT Delhi nursery)	77
3.8.2.3. SynComs under field conditions	78
3.9. Statistical analysis	78
 4. RESULTS	 80-172
4.1 Generation of an acclimatized microbiome using a multi-passaging approach, to help <i>Vigna radiata</i> withstand salinity stress	81
4.1.1. Salt tolerance assessment of <i>Vigna radiata</i> var. Pusa Vishal	81
4.1.2 Effects of acclimatization of microbiome on plant morphological parameters under salinity stress	83
4.1.3 Effects of acclimatization of microbiome on plant stress markers under salinity stress	90
4.1.4. Effects of continued acclimatization of the microbiome on plant morphological parameters and stress markers on further ramping up of salinity stress	95
4.2. Effects of stress acclimatization on the bacterial and fungal composition of the rhizospheric microbiome	99
4.2.1. Rhizospheric bacterial community dynamics	99
4.2.2 Response of rhizospheric fungal communities to passaging under salt stress	104
4.3 Culture-dependent analysis of bacterial communities	125
4.3.1 Isolation of pure bacterial strains from acclimatized microbiome for generation of culture bank	125
4.3.2. Qualitative assessment of multiple plant growth promoting properties of the bacterial strains in the culture bank	128
4.3.3. Volatilome analysis of bacterial strains	136
4.3.3.1 Effect of bacterial volatile organic compounds (VOCs) on plant growth under stress condition	136
4.3.3.2. Identification of the volatiles emitted by bacterial strains mediating salinity stress tolerance in <i>A. thaliana</i>	138
4.3.4 Validation of identified VOCs by plant growth experiment	145
4.4. Employing SynComs in plant growth promotion and stress tolerance	149
4.4.1. Effect of individual bacterial strains on plant growth promotion and stress tolerance in <i>Vigna radiata</i> under controlled conditions	149

4.4.2. <i>Effect of SynComs on plant growth promotion and stress tolerance in Vigna radiata under controlled conditions</i>	155
4.4.3. <i>Effect of SynComs on plant growth promotion and stress tolerance in Vigna radiata under natural conditions (IIT Delhi nursery)</i>	159
4.4.4. <i>Effect of SynComs on plant growth promotion and stress tolerance in Vigna radiata under natural conditions in fields</i>	164
5. DISCUSSION	173-202
5.1. Process of acclimatizing rhizospheric microbiome by multi-passaging approach with ramping up of stress levels	174
5.2. Impact of the acclimatized microbiome on plant, and rhizosphere microbiome	176
5.2.1. <i>Acclimatized rhizospheric microbiome promoted growth attributes of plants</i>	176
5.2.2. <i>Acclimatized rhizospheric microbiome affected levels of plant stress markers reflecting its efficacy in stress tolerance</i>	177
5.2.3. <i>Unravelling the microbiome dynamics during acclimatization: A distinct recruitment of stress-tolerant members was witnessed</i>	179
5.2.3.1. <i>Changes in bacterial community structure</i>	180
5.2.3.2. <i>Response of fungal community</i>	183
5.3. Mechanistic understanding of microbiome-mediated salinity stress tolerance in Vigna radiata	188
5.3.1. <i>Culture bank comprised of several stress-tolerant bacterial isolates exhibiting multiple plant growth promoting attributes</i>	188
5.3.2. <i>Key volatile organic compounds (VOCs) produced by the individual bacterial isolates may have a potent role in stress tolerance</i>	190
5.3.3. <i>Role of shortlisted VOCs in salt stress mitigation validated by treatment of plants with synthetic volatile compounds</i>	193
5.4. Application of potent stress-tolerant bacterial isolates in growth promotion and salt stress amelioration in Vigna radiata	197
5.4.1. <i>Individual bacterial strains exhibited a positive impact on plant growth attributes</i>	197
5.4.2. <i>Synthetic microbial communities (SynComs) assisted plants to withstand salt stress better and promoted plant growth</i>	199
6. SUMMARY AND CONCLUSIONS	203-211
6.1. Summary	203
6.2. Conclusions	210
7. BIBLIOGRAPHY	212-246
<i>Curriculum Vitae</i>	247-250

LIST OF FIGURES

Figure No.	Legend	Page No.
2.1	Rhizosphere engineering facilitates the maintenance of plant and soil health (Khatri et al., 2022).	20
2.2	Host-mediated microbiome selection via multi-passaging approach.	28
2.3	Acclimatization of indigenous microbial communities to adapted microbial communities in a plant's rhizosphere under the influence of plant root exudates (Dubey and Sharma, 2021).	39
2.4	Application of synthetic microbial communities (SynComs) under greenhouse conditions to as an alternative to application of whole microbiome (Dubey and Sharma, 2021).	44
3.1	Outline of the overall workflow of the doctoral study.	50
3.2	The schematic representation of salt treatment administered to <i>Vigna radiata</i> plants across different stages of multi-passaging experiments.	53
3.3	Set up of pots for the plant growth experiment in the IIT Delhi nursery.	55
3.4	Plant-mediated selection of rhizospheric microbiome via multi-passaging approach.	56
3.5	The standard curve obtained by plotting the absorbance of different concentrations of pure L-proline at 520 nm to calculate proline levels in plant samples.	59
3.6	Set up of split plate assay to check the impact of volatile organic compounds (VOCs) released by the bacterial strains.	69
3.7	Experimental set up of closed-loop-stripping analysis (CLSA) system to trap the VOCs produced by bacterial strains.	70
3.8	Outline of pot experiment for investigating the effect of pure VOCs on salt stress tolerance and growth of <i>Vigna radiata</i> .	71
3.9	Outline of the cross-streak assay to check the compatibility of the strains.	73
4.1	Physiological effects of varying concentrations of NaCl on the germination of <i>Vigna radiata</i> seeds.	81
4.2	Effect of acclimatized microbiome on <i>Vigna radiata</i> plants harvested at young developmental stage (30 DAS) during 13 th round of multi-passaging cycle.	83
4.3	Impact of various treatments on the height of plants across the multi-passaging experiments.	84
4.4	Impact of various treatments on fresh weight (a) and dry weight of plants (b) across the multi-passaging experiments.	86

4.5	Impact of various treatments on the number of leaves across the multi-passaging experiments.	87
4.6	Impact of various treatments on the number of root nodules across the multi-passaging experiments.	88
4.7	Impact of various treatments on the amount of total chlorophyll content in leaves, across the multi-passaging experiments.	89
4.8	Heatmaps depicting the levels of stress markers in plants under various treatments across the multi-passaging experiments.	94
4.9	Effect of various treatments on the levels of Na ⁺ and K ⁺ levels and their ratios in plants from the 13 th plant growth cycle.	95
4.10	The impact of acclimatized microbiome post the 20 th passaging round.	97
4.11	Rarefaction curves generated for OTUs obtained for different treatments upon 16S rRNA amplicon sequencing.	100
4.12	The relative abundances of different phyla (expressed as percentages) under specific treatment depicted as stacked bar chart.	101
4.13	The relative abundances of different families (expressed as percentages) under specific treatment depicted as stacked bar chart.	102
4.14	The relative abundances of different genera (expressed as percentages) under specific treatment have been depicted as stacked bar chart.	102
4.15	Violin plots showing the α -diversity of the different treatments over successive passaging, as measured by the Chao 1 (a) and Shannon (b) indices.	104
4.16	Number of amplicon sequence variants (ASVs) identified in the fungal communities from rhizosphere of <i>Vigna radiata</i> in different treatments as compared to initial bulk.	105-106
4.17	Fungal community composition of the rhizosphere soil of <i>Vigna radiata</i> at the phyla level across 5 th and 13 th passaging cycle as compared to initial bulk soil.	108
4.18	Fungal community composition of the rhizosphere soil of <i>Vigna radiata</i> at the family level across 5 th and 13 th passaging cycle as compared to initial bulk soil.	109
4.19	Fungal community composition of the rhizosphere soil of <i>Vigna radiata</i> at the genera level across 5 th and 13 th passaging cycle as compared to initial bulk soil.	110
4.20	Comparison of the α -diversity indices of the fungal communities in the rhizospheres of <i>Vigna radiata</i> across 5 th and 13 th passaging cycle as compared to initial bulk soil.	115

4.21	Principal coordinate analysis (PCoA) plots to assess β -diversity of the fungal communities in the rhizospheres of <i>Vigna radiata</i> across 5 th and 13 th passaging cycle as compared to initial bulk soil.	117
4.22	The boxplot representation of the intra group variability and distance similarity of the fungal community composition with respect to initial bulk samples.	118
4.23	Linear discriminate analysis (LDA) of effect size (LEfSe) to determine preferential fungal taxa in different treatments at various taxonomic levels.	119-120
4.24	Box plots representing the relative abundance of various fungal groups.	121
4.25	Relative abundance of fungal ASVs identified in the rhizospheric soil of <i>Vigna radiata</i> under salinity stress conditions, with and without application of acclimatized microbiome.	123-124
4.26	Phylogenetic tree illustrating the evolutionary relationships among bacterial isolates obtained from the adapted rhizospheric soil of <i>Vigna radiata</i> .	128
4.27	Attributes contributing to biocontrol potential of the isolates strains: hydrogen cyanide (HCN) (a), and hydrogen sulphide (H ₂ S) production (b).	129
4.28	Assessment for production of siderophores (a) and solubilization of phosphates (b) by selected bacterial strains.	130-131
4.29	Exopolysaccharide (EPS) production by selected bacterial strains.	131
4.30	Profiling of salinity stress tolerance in the bacterial strains from culture bank.	132
4.31	Impact of volatile organic compounds (VOCs) produced by bacterial isolates grown in media supplemented with salt on the Arabidopsis seedling leaf area grown under salt stress or control conditions.	137
4.32	Total mass features (mz/rt) determined by one-way ANOVA for untargeted GC-MS analysis of VOCs produced by selected bacterial strains grown on ARE and V8 media supplemented with 50 mM NaCl for 48 h using the closed-loop stripping method.	139
4.33	Score scatter plot depicting variations among different bacterial strains in production of VOCs.	142
4.34	Heatmap showing the VOCs produced by bacterial strain growing on ARE and V8 media.	144
4.35	Impact of pure VOCs on growth attributes of <i>Vigna radiata</i> .	147
4.36	Impact of pure VOCs on stress marker levels in <i>Vigna radiata</i> .	148
4.37	Effect of individual strains on <i>Vigna radiata</i> under non-stress and stress conditions (in plant growth chamber).	151
4.38	Impact of individual bacterial strains on height of plants under salt stress conditions.	152

4.39	Effect of individual bacterial strains on plants' dry weight under salt stress conditions.	153
4.40	Impact of individual bacterial strains on pod numbers per plant under control and salinity stress conditions.	154
4.41	Effect of SynComs on the plant growth parameters under control and stress conditions.	157
4.42	Effect of SynComs on the stress marker levels of plants under control and stress conditions.	158
4.43	Effect of SynComs on plant height under control and salinity stress conditions (nursery experiment).	161
4.44	Effects of SynComs on plant dry weight under control and salinity stress conditions (nursery experiment).	162
4.45	Effects of SynComs on number of pods per plant under control and salinity stress conditions (nursery experiment).	163
4.46	Effect of SynComs on <i>Vigna radiata</i> grown in field under salinity stress.	165
4.47a	Impact of SynComs on plant height under salinity stressed field as compared to control plants and plants amended with rhizospheric microbiome.	166
4.47b	Impact of SynComs on plant dry weight under salinity stressed field, as compared to control plants and plants amended with rhizospheric microbiome.	167
4.47c	Impact of SynComs on the grain yield under salinity stressed field, as compared to control plants and plants amended with rhizospheric microbiome.	168
4.47d	Impact of SynComs on MDA content in plants under stressed field, as compared to control plants and plants amended with rhizospheric microbiome.	169
4.47e	Impact of SynComs on proline content in leaves of plants under stressed field, as compared to control plants and plants amended with rhizospheric microbiome.	170
4.47f	Impact of SynComs on K ⁺ /Na ⁺ ratio in leaves of plants under stressed field, as compared to control plants and plants amended with rhizospheric microbiome.	171
6.1	Summary of the salient findings of this research work	208

LIST OF TABLES

Table No.	Legend	Page No.
2.1	Reports employing bottom-up approach of designing microbial consortia for mitigating abiotic stresses.	24
2.2	Reports employing a top-down approach of acclimatization of microbial communities for mitigating stress by multiple passaging.	27
3.1	Summary of the various treatments used in the study.	57
3.2	Primer pairs used for characterizing microbial diversity of rhizospheric microbiome using amplicon sequencing.	60
3.3	Details of the different SynComs generated using the scoring scheme based on the PGP traits.	73-74
4.1	Proline content in leaves of plants over successive rounds of passaging cycles.	90
4.2	Membrane stability index (MSI) in plant leaves over successive rounds of passaging cycles.	91
4.3	Malondialdehyde (MDA) content in leaves over successive rounds of passaging cycles.	91
4.4	Composition of fungal taxa at phyla level.	112
4.5	Composition of fungal taxa at family level.	113
4.6	Composition of fungal taxa at genera level.	114
4.7	α -diversity indices for the rhizospheric soil fungal communities of <i>Vigna radiata</i> in correlation with treatments.	116
4.8	The taxonomic classification of bacterial strains isolated from the salinity-acclimatized rhizospheric microbiome of <i>Vigna radiata</i> .	125-127
4.9	List of plant growth promoting traits exhibited by bacterial strains isolated from the acclimatized rhizosphere microbiome of <i>Vigna radiata</i> (qualitative tests).	134-135
4.10	List of VOCs identified from selected bacterial strains isolated from stress-acclimatized microbiome, using Gas Chromatography-Mass Spectrometry.	140-141

ABBREVIATIONS

Formula/Abbreviation

$(\text{NH}_4)_2\text{SO}_4$
 ACC
 ARE
 CaCO_3
 CAS
 CMS
 CTAB
 EDTA
 EPS
 FeCl_3
 FeSO_4
 H_2O
 H_2O_2
 H_2S
 HClO_3
 HClO_4
 HCN
 HNO_3
 IAA
 KCL
 KH_2PO_4
 LB
 MDA
 MgCl_2
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
 MS
 Na_2MoO_4
 NaCl
 NaOCl
 NaOH
 NBRIP

Media, Molecules and Chemicals

Ammonium sulphate
 1-aminocyclopropane-1-carboxylate
 Artificial root exudate
 Calcium carbonate
 Chrome-azurol S
 Carboxymethylcellulose
 Cetyl trimethyl ammonium bromide
 Ethylene diamine tetra-acetic Acid
 Exopolysaccharide
 Ferric chloride
 Ferrous sulphate
 Water
 Hydrogen peroxide
 Hydrogen sulphide
 Chloric acid
 Perchloric acid
 Hydrogen cyanide
 Nitric acid
 Indole acetic acid
 Potassium chloride
 Dihydrogen potassium phosphate
 Luria–Bertani
 Malondialdehyde
 Magnesium dichloride
 Magnesium sulphate heptahydrate
 Murashige and Skoog medium
 Sodium molybdate
 Sodium chloride
 Sodium hypochlorite
 Sodium hydroxide
 National Botanical Research Institute's phosphate

Terminology used in the study

AMF	Arbuscular mycorrhizal fungus
ASV	Amplicon sequence variants
CFU	Colony forming unit
CPA	Cryoprotective agents
DAS	Days after sowing
DNA	Deoxyribonucleic acid
DW	Dry weight

EC	Electrical conductivity
FAO	Food and Agriculture Organization
FW	Fresh weight
GC-MS	Gas Chromatography-Mass Spectrometry
HMME	Host mediated microbiome engineering
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
ITS	Internal transcribed spacer
LC/MS	Liquid Chromatography-Mass Spectrometry
LDA	Linear discriminant analysis
MCT	Microcentrifuge tube
MSI	Membrane stability index
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
NIST	National Institute of Standards and Technology
OUT	Operational taxonomic unit
PCA	Principal component analysis
PCD	Programmed cell death
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PERMANOVA	Permutational multivariate analysis of variance
PGPB	Plant growth promoting bacteria
PGPR	Plant growth promoting rhizobacteria
QIIME	Quantitative insights into microbial ecology
qPCR	Quantitative polymerase chain reaction
RO	Reverse osmosis
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
SRA	Sequence read archive
SynComs	Synthetic microbial communities
TOC	Total organic carbon
USDA	United States Department of Agriculture
VOCs	Volatile organic compounds

Elements

N	Nitrogen
P	Phosphorus
K	Potassium
C	Carbon
Na	Sodium
Cl	Chlorine

Units

%	Percent
Cm	Centimetre
dS/m	DeciSiemens per metre
g	Grams
Kg	Kilogram
°C	Degrees Celsius
Cl	Chlorine
L	Litre
M	Metre
M	Molar
mL	Millilitre
nm	Nanometre
min	Minutes
rpm	Revolutions per minute
S	Seconds
v/v	Volume by volume
RT	Retention time
μL	Microlitre
μM	Micromolar
q/h	Quintals per Hectare