DEVELOPMENT AND EVALUATION OF PROMISING BACTERIAL CONSORTIA FOR IMPROVING GROWTH AND YIELD OF RICE

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B.Sc. (Hons) Ag.

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(AGRICULTURAL MICROBIOLOGY)

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BY

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THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

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2023

DECLARATION

I, **ANGALA SRIJA**, hereby declare that the thesis entitled, "**DEVELOPMENT AND EVALUATION OF PROMISING BACTERIAL CONSORTIA FOR IMPROVING GROWTH AND YIELD OF RICE**" submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **Master of Science in Agriculture** in the major field of **Agricultural Microbiology**, is the result of original research work done by me. I also declare that no material contained in the thesis or any part thereof has not been published earlier in any manner.

Date: **(ANGALA SRIJA)**

Place: Hyderabad **I.D. No. RAM/2020-101**

CERTIFICATE

Ms. ANGALA SRIJA has satisfactorily prosecuted the course of research and the thesis entitled "**DEVELOPMENT AND EVALUATION OF PROMISING BACTERIAL CONSORTIA FOR IMPROVING GROWTH AND YIELD OF RICE**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date: **(Dr. P. C. Latha)**

Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"DEVELOPMENT AND EVALUATION OF PROMISING BACTERIAL CONSORTIA FOR IMPROVING GROWTH AND YIELD OF RICE"** submitted in partial fulfillment of the requirements for the degree of **"Master of Science in Agriculture"** of the **Professor Jayashankar Telangana State Agricultural University**, **Hyderabad** is a record of the bonafide research work carried out by **Ms. ANGALA SRIJA** under our guidance and supervision.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been duly acknowledged by the author of the thesis.

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Date:

LIST OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF IILISTRATIONS

APPENDICES

LIST OF SYMBOLS AND ABBREVATIONS

%	:	Per cent
±	:	Plus or minus
∞	:	Infinity
◦C	:	Degree celsius
AAS	:	Atomic absorption spectroscopy
bp	:	Base pair
$CaCO_3$	:	Calcium carbonate
CAS	:	Chromo azurol sulfonate
cm	:	Centimeter
CRD	:	Completely randomised design
$CuSO_4$	:	Copper sulphate
CV	:	Coefficient of variation
DNA	:	Deoxyribonucleic acid
ds m^{-1}	:	Desisiemens per meter
DTPA	:	Diethylenetriamine pentaacetate
EC	:	Emulsifiable concentrate
EC	:	Electrical conductivity
EDTA	:	Ethylenediamine tetraacetic acid
F	:	Forward
FDA	:	Fluorescein diacetate
Fe	:	Iron
g	:	Gram
G	:	Granular
GA	:	Gibberellic acid
g/l	:	Gram per litre
ha	:	Hectare
HCN	:	Hydrogen Cyanide
$HgCl_2$	:	Mercuric chloride
hrs	:	Hours
H_2SO_4	:	Sulphuric acid
IAA	:	Indole acetic acid
IPM	:	Integrated pest management
K	:	Potassium
kb	:	Kilo base
KCl	:	Potassium chloride
Kg/ha	:	Kilogram per hectare
$KMnO_4$	:	Potassium permanganate
K_2O	:	Potassium oxide

K_2SO_4	:	Potassium sulphate
LB agar	:	Luria Bertani agar
Mg^{+2}	:	Magnesium ion
mg L^{-1}	:	Milligram per litre
mins	:	Minutes
ml	:	Millilitre
mm	:	Millimeter
mM	:	Milli molar
MOP	:	Muriate of potash
MPa	:	Mega pascal
N	:	Normality
N	:	Nitrogen
NA	:	Nutrient agar
NaCl	:	Sodium chloride
NaHCO3	:	Sodium hydrogen carbonate
NaOH	:	Sodium hydroxide
NB	:	Nutrient broth
NCBI	:	National centre for biotechnology information
ng/µl	:	Nanograms per microlitre
nm	:	Nanometer
OD	:	Optical density
P	:	Phosphorus
PBS	:	Phosphate buffered saline
PCR	:	Polymerase chain reaction
PEG	:	Polyethylene glycol
PGP	:	Plant growth promotion
PGPB	:	Plant Growth Promoting Bacteria
PGPR	:	Plant Growth Promoting Rhizobacteria
pH	:	Pussancea hydrogen
pmoles/ µl	:	Pico moles per micro litre
P_2O_5	:	Phosphorus pentaoxide
ppm	:	Parts per million
PSB	:	Phosphate solubilizing bacteria
PSM	:	Phosphate solubilizing microorganisms
R	:	Reverse
RDF	:	Recommended dose of fertilizers
rpm	:	Revolutions per minute
r RNA	:	Ribosomal RNA

RNA	:	Ribonucleic acid
SC	:	Suspension concentrate
SE	:	Solubilization efficiency
sec	:	Seconds
SI	:	Solubilization index
Sp.	:	Species
SSP	:	Single super phosphate
t/ha	:	Ton per hectare
TPF	:	Triphenyl formazan
TTC	:	Tetrazolium chloride
TY agar	:	Tryptone yeast agar
U/ µl	:	Unit per microlitre
µg/ml	:	Microgram per millilitre
µL	:	Micro litre
µL/ml	:	Micro litre per milli litre
V	:	Voltage
WG	:	Wettable granules
w/v	:	Weight by volume
w/w	:	Weight by weight
YMD	:	Yeast malt dextrose
Zn	:	Zinc
$ZnCo_3$	:	Zinc carbonate
ZnO	:	Zinc oxide
ZSB	:	Zinc solubilizing bacteria

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ABSTRACT

Rice (*Oryza sativa*) is the most important cereal crop in the world, feeding more than 50% of the world's population. To meet the world's demand for rice, it is imperative to find environmentally sound ways that supplement the need for fertilizers. The use of microbial inoculants is a desirable option since they can complement the fertilizer use. Microbial formulations developed using two or more beneficial plant growth promoting rhizobacteria, colonize the rhizosphere, plant roots and aids in plant development. Therefore, inoculating the plants with microbial consortia produces positive results because microbial consortia can generally accomplish tasks by mutual synergism thus performing better than individual strains.

The present study “Development and evaluation of promising bacterial consortia for improving growth and yield of rice” was carried out to identify a potential consortia for rice. A total of 32 (IIRRSS22-1 to 27, P1, R1, M1, O1 and P2) bacteria isolated from the rhizosphere of rice were screened and scored for plant growth-promoting traits (phosphate, potassium, zinc solubilization, siderophore production, indole acetic acid production and HCN production) under *in vitro* conditions. Three potential (P1, IIRRSS22-1 and IIRRSS22-6) isolates having highest score with regard to plant growth promoting traits were selected and the bacterial performance was evaluated further by conducting germination test.

All the isolates were then screened for abiotic stress tolerance such as salinity, temperature and drought tolerance. Scores were assigned to the isolates based on their ability to grow at different NaCl concentrations, temperature of 45°C and water potential of -0.73MPa and three isolates (IIRRSS22-3, R1 and IIRRSS22-7) with highest score for abiotic stress tolerance traits were selected for *in vitro* germination studies.

Agrichemical compatibility of bacterial isolates with fertilizers (Urea, Single super phosphate and Muriate of potash), insecticides (Cartap, Ferterra, Thiamethaxom), herbicides (Pretilachlor, Bispyribac sodium) and fungicides (Carbendazim, Mancozeb) used for rice cultivation was assessed by disc diffusion method. Based on the tolerance

exhibited by the bacterial isolates, IIRRSS22-5, IIRRSS22-2 and IIRRSS22-4 with the highest tolerance score were selected for further experimentation.

Nine isolates, 3 from each category (plant growth promoting traits, abiotic stress tolerance and compatibility with agrichemicals) were grouped into consortia (Consortium-1, 2 and 3) containing 3 bacterial isolates in each. These bacterial combinations were evaluated for compatibility among each other by cross streak method. All the isolates were compatible with each other and these consortia combinations and individual bacteria were evaluated for germination under *in vitro* conditions with Telangana sona (RNR-15048) as test variety. Consortium-1 exhibited highest germination percentage, seedling length, vigour index I and II when compared with control, individual inoculations and other consortia (C-2 and C-3).

Based on the *in vitro* germination assay, consortium-1 was selected for further evaluation under pot culture conditions with different treatments tested at 100% RDF except the control treatment. The pot culture experiments were taken up during *rabi* 2021-22, the plant morphological traits and yield traits of rice were recorded. The root length, shoot length, leaf area and plant biomass at active tillering and at the harvest stage were found to be highest with treatment T14 (100% RDF + Bacterial consortia (Seed treatment + Seedling root dip). The yield parameters of rice such as tiller number, panicle length, filled grains per panicle, test weight and grain yield as affected by different treatments were found to be highest in treatment T14 (100% RDF + Bacterial consortia (Seed treatment + Seedling root dip). The soil available nutrients and the nutrient uptake by the plants were also highest in the treatment T14 (100% RDF + Bacterial consortia (Seed treatment + Seedling root dip).

The molecular characterization of bacterial partners in C-1 by 16S rRNA gene sequencing revealed that the bacterial isolates were *Pseudomonas stutzeri* (P1)*, Stenotrophomonas* sp. (IIRRSS22-3)*, Achromobacter* sp (IIRRSS22-5). The isolates in consortium-2 and 3 were identified as *Achromobacter insuavis* (IIRRSS22-6), *Rhizobium* sp, (IIRRSS22-1), *Stenotrophomonas maltophilia* (IIRRSS22-2), *Ochrobacter anthropi* (IIRRSS22-4). *Achromobacter xylosoxidans* IIRRSS22-7.

Based on the results obtained, the present study showed that the consortia (C-1) which consisted of rhizobacterial isolates (*Pseudomonas stutzeri, Stenotrophomonas* sp*, Achromobacter* sp) with multiple plant beneficial traits along with tolerance to eco-physiological stresses has enhanced rice plant growth and yield parameters under pot culture conditions. This study also suggests that the use of plant-beneficial bacteria as a consortium results in better plant performance rather than as a single strain.

INTRODUCTION

Chapter I

INTRODUCTION

Rice (*Oryza sativa*) is the most important cereal crop in the world, feeding more than 50% of the world's population. It is regarded as the oldest and most significant crop in the entire world, particularly in Asia. In India, rice is grown over an area of 450.67 lakh hectares with a total production of 122.27 million tonnes (Annual report, 2021). Telangana is one of the major rice-growing states in the country which covers approximately 27.4 lakh hectares of paddy cultivation, yielding 13 million tonnes (Anonymous, 2021).

Agriculture nowadays is becoming more and more reliant on chemical pesticides and fertilizers from the period of the introduction of the green revolution to meet the need of the increasing population. But excessive and unbalanced usage of these synthetic inputs have negative impacts, and overuse of chemical fertilizers, and pesticides in agricultural production reduces the quality of the soil by altering its physical, chemical, and biological properties (Prashar and Shah, 2016).

Therefore, to meet the world's demand for rice, it is imperative to find environmentally sound ways that supplement the need for fertilizers. The use of microbial inoculants is a desirable option since they can complement fertilizer use and reduce its negative effects, resulting in a healthier environment. The best alternative to chemical fertilizers is biofertilizers, which are live cells of microbes that supply nutrients to plants through their roots, control numerous soil borne diseases spread through contaminated soil, and enhance the quality of the soil (Bhardwaj *et al*., 2014; Kour *et al*., 2020).

Plant Growth Promoting Rhizobacteria (PGPR) are bacteria that colonize the rhizosphere, plant roots and promote plant development. PGPR's have demonstrated beneficial impacts on a variety of plant growth parameters, including seed germination rate, drought tolerance, shoot and root weight, nutrient uptake, yield and plant growth (Kloepper *et al*., 2004; Kokalis-Burelle *et al*., 2006). PGPR benefits plants by producing a range of secondary metabolites, growth regulators, siderophores and organic acids. It also aids in nitrogen fixation and phosphorus (P) solubilization to assist plants in absorbing nutrients from the soil (Tabassum *et al*., 2017).

Numerous beneficial bacteria, such as *Pseudomonas* sp., *Sphingomonas*, *Bacillus* sp., *Blastocatella, Thiobacillus, Bryobacter, Anaeromyxobacter, Streptococcus and Staphylococcus* have been identified in the rhizosphere of rice plants (Purwanto *et al*., 2019; Osman *et al*., 2017). One option for restoring soil biological fertility and soil health is the application of biofertilizer formulated using microorganisms isolated from local rice fields.

Although agricultural and environmental sustainability are in the forefront of challenges faced in agriculture, the use of agrochemicals is a necessity in the foreseeable future as they are indispensable for controlling pests and diseases required to achieve the high yields for feeding the growing population. Hence, there is a need to study the compatibility between inoculants and pesticides (Santos *et al*., 2021). In addition, the identification and utilization of abiotic stress-tolerant inoculants are the need of the hour to achieve climate-resilient crop production (Valliere *et al*., 2020).

Microbial formulations are usually developed using single strain of bacterial isolate, but because of their difference in the functioning of these PGPR under *in vitro* conditions and in the field, due to the prevailing abiotic stress conditions like high temperature, salinity, drought stress, *etc* (Kumar *et al*., 2014), considerable importance is currently being given towards developing microbial consortium which consists of a combination of two or more compatible microbial species/strains, that can live and grow together.

A bacterial consortium may include bacteria of different species or strains of the same species that behave in a synergistic manner for plant growth promotion. The efficiency of applying microbial consortia to promote plant growth is determined by the interaction and compatibility of the mixture of rhizobacteria. The application of the bacterial consortium is expected to bring about multifaceted effects through synergies, nutrient supply, removal of inhibitory products and interaction between bacteria through mutual stimulation by physical and biochemical activities (Purwanto *et al*., 2021).

The application of different plant growth promoting species with diverse mechanisms of action will proffer a wide range of benefits for the plant, like direct growth stimulation and providing for protection from pathogens while simultaneously increasing production. Santoyo *et al*. (2021) have reported that phytobeneficial properties of the bacteria in the consortia can bring about a decrease or prevent diseases caused by

pathogens in plants. In bio-fertilizers application, co-inoculation of *Azospirillum*, *Azotobacter*, P-solubilizing bacteria and fluorescent pseudomonads has been found to be significantly better than their single inoculation (Macik *et al*., 2020; Bargaz *et al*., 2018).

Most studies of bioinoculants are based on interactions of single microorganisms with plants, evaluating different parameters of growth, plant health and yields. It has been advocated that because of several microbe-microbe, microbe-host plant and microbe-environment interactions that happen when single bacterial isolate are inoculated in the field, reliable beneficial results in terms of facilitating plant growth are not always achieved (Babalola, 2010). However, inoculating plants with microbial consortia containing two or more beneficial microorganisms have been known to produce positive results because microbial consortia (Sharma *et al*., 2020; Zhang *et al*., 2018) can generally accomplish tasks better than individual strains.

Therefore, the aim of present study is to characterize the rhizospheric bacteria for PGPR traits, to screen the bacterial isolates for abiotic stress tolerance and compatibility with agrichemicals for the development of a promising bacterial consortium which could promote the growth and yield of rice.

Objectives of investigation:

1. Screening of bacteria for eco-physiological stress tolerance.
2. Consortia development by selection of compatible microbial isolates.
3. Molecular identification of the members of promising bacterial consortia.
4. Evaluation of the developed consortia in pot culture studies.

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

The present study for development and evaluation of a promising bacterial consortium for improving growth and yield of rice was carried out at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad.

Rice (*Oryza sativa*) is one of the important cereal crops, feeding half of the world's population. In India, rice is grown over an area of 450.67 lakh hectares with total production of 122.27 million tonnes (Annual report, 2021-22). Telangana is one among the major rice growing states in the country with approximately 27.4 lakh hectares under paddy cultivation, yielding 13 million tonnes (Anonymous, 2021).

In the face of a growing demand for food due to the increasing population, and the changing global climate, one of the major challenges in rice cultivation is to obtain high rice yields with a limited cultivable area. So to increase the yields, the use of excess chemical fertilizers has become a practice. The usage of chemical fertilizers causes harmful environmental impacts (Schindler and Hecky, 2009). Therefore, to meet the world's demand for rice it is imperative to find environmentally sound ways that supplement the need for fertilizers. The use of microbial inoculants is an attractive option because they can complement the use of the fertilizers and mitigate their undesirable effects thereby ensuring a healthier environment.

Soil microorganisms play an essential role in ecosystem function and fertility maintenance by regulating several crucial biogeochemical processes (Fierer, 2017). Plants rely heavily on these microorganisms for nutrient uptake and protection against stresses (Backer *et al*., 2018). The rhizosphere of rice harbors many microorganisms, some of which aid in plant growth by biological N fixation, solubilization of nutrients, production of siderophores, production of growth stimulants, and acting as biocontrol agents. All of these together are called plant growth-promoting rhizobacteria.

Hence, these bacteria can be used as biofertilizers for rice production. But, there is difference in performance of these PGPRs under lab and field conditions due to several abiotic factors and also due to interaction with agrichemicals applied to the rice crop (Kumar *et al*., 2014). Therefore, screening of PGP bacteria for abiotic stress tolerance and compatibility with agrichemicals becomes necessary.

Microbial consortia in agriculture have become an area of interest for use as eco-friendly plant growth promoters. A combination of two or more PGPB have been reported to increase plant growth, combat stress, and control pathogens (Olanrewaju and Babalola, 2019) better than single culture inoculations. Therefore, application of different plant growth-promoting species with diverse mechanisms of action will provide a wide spectrum of benefits for the plant, including direct stimulation of its growth and health, as well as production increase.

2.1 CHARACTERIZING BACTERIAL ISOLATES FOR PLANT GROWTH-PROMOTING TRAITS

2.1.1 Phosphorus solubilization

Phosphorus (P) is one of the mineral fertilizers which aids in the growth and development of plants. Soluble phosphorus is the limiting mineral nutrient for biomass production as the applied phosphatic fertilizers become unavailable to the plant immediately after application as it gets fixed in the soil as insoluble forms (Sharma *et al*., 2013). Phosphorous solubilizing microorganisms (PSMs) by the process of chelation, acidification, and exchange reactions can convert the insoluble phosphorous into soluble forms (Rodriguez *et al*., 2004) thus aiding in normal plant growth and development.

Jha *et al*. (2009) isolated fluorescent pseudomonad strains from rhizospheric soil samples of rice. The phosphate-solubilizing potential of these isolates was tested by streaking the pure cultures on Pikovskaya agar medium and incubating the cultures at 28 ℃ for 3 days. The formation of halo zones around the culture indicated phosphorous solubilization. Out of 80 pseudomonad strains, 3 strains were identified as phosphate solubilizers.

Hussein *et al*. (2015) collected soils from the rhizosphere of apple for isolation of phosphate solubilizing microorganisms by inoculating the bacteria on Pikovskaya media and observing the plates for the formation of halo zone. The strain *Pseudomonas* isolate An-15-Mg showed highest P-solubilization in plate assay (46 mm). Further, these isolates were screened for other PGPR traits like IAA production, ammonia production, and HCN production.

Pande *et al*. (2017) isolated phosphate solubilizing bacteria from rhizospheric soil of maize. These isolates were screened for their ability to solubilize tricalcium phosphate on Pikovskaya agar plate. The isolates were spot inoculated on media plates and incubated

at 28 ℃ for 7 days. In this study, a total of 8 phosphate solubilizing bacteria have been isolated. Among them, 3 isolates have shown higher phosphate solubilization index ranging from 4.88 ± 0.69 to 4.48 ± 0.30.

Chakdar *et al*. (2018) screened 5 bacterial isolates for phosphate solubilization based on solubilization index (SI) and solubilization efficiency (SE) on Pikovskaya agar media. It was found that *Bacillus* sp. AH9 had maximum SI (3.5) and SE (250%), while *Pantoea* sp. A34 had minimum SI (1.8) and SE (75%).

Prasad *et al*. (2020) screened the bacterial isolates for phosphate solubilization by plate assay using Pikovskaya agar media. Bacterial colonies with a clear halo zone around them were considered phosphorous solubilizers. The isolates NR-1, NR-2, and NR-5 have produced a halo zone of about 12mm in diameter.

2.1.2 Potassium solubilization

The third most important mineral nutrients that play a crucial role in plant growth and development is potassium. Generally, the reserves of soil potassium are high, but the major portion of K is present as an insoluble form in the soil. Certain rhizobacteria have the capability to transform the insoluble forms such as feldspars and micas into soluble potassium thus making the minerals available to the plants for uptake. These rhizobacteria are known as potassium solubilizing bacteria and they can be used for enhancing the availability of potassium to the plants.

Prajapati *et al.* (2012) collected soil samples from insoluble potassium mineral-containing soils. The soil samples were inoculated on Aleksandrov agar media and were incubated at 37 ℃ for 1week. The bacterial colonies exhibiting clear zone were selected as potassium solubilizers. Among the selected 14 isolates, 5 isolates with the highest potassium solubilization were selected for further screening.

Parmar and Sindhu (2013) isolated rhizobacteria on Aleksandrov medium by supplementing it with mica powder as source of potassium. They isolated 130 strains of bacteria and screened them for potassium solubilization and found that twenty bacterial strains significantly solubilized potassium on the Aleksandrov medium and the range of K released by isolated bacterial strains was 15 to 48 mg L^{-1}. They also observed the highest K solubilization when the bacterial isolates were grown at pH 7.0 in a broth medium containing KCl as a source of potassium.

Rajawat *et al*. (2016) screened 85 bacterial isolates for potassium solubilization by qualitative assay on Aleksandrov agar media by spot inoculation of the isolated bacteria. Based on the formation of halo zones around the inoculum, 11 isolates were selected after a quantitative assay of solubilization by growing the isolates in Aleksandrov broth.

Fatharani *et al*. (2018) isolated rhizospheric bacteria from rice. The selected isolates were subjected to grow on Aleksandrov agar media for assessment of potassium solubilization of the isolates. After inoculation, the isolates were incubated at 30℃ for 4 days and the zone of solubilization was noted. 7 bacterial isolates were able to solubilize potassium, the strain LJK2 showed the highest ability to solubilize potassium.

Sun *et al*. (2020) isolated potassium solubilizing bacteria from rhizospheric soil by using Aleksandrov agar media and incubating the plates at 30 ℃ for 7 days. The zones of solubilization of the bacteria were recorded and used for the calculation of potassium solubilization efficiency. The results suggested that these bacteria could be used for potassium solubilization and uptake, aiding in plant growth and development.

2.1.3 Zinc solubilization

Zinc is the essential micronutrient required for optimum plant growth. Plants uptake Zn in the form of a divalent cation, but a major portion of zinc is available as an insoluble form and is less available for the plants, creating Zn deficiency. Few rhizobacteria such as Zinc solubilizing microorganisms help in the acquisition of minerals, thus making Zn available to plants for uptake.

Mishra *et al*. (2017) isolated 9 rhizobacterial isolates (ZN 1 to Zn 9) from the soil collected from rhizosphere of rice and screened them for the zinc solubilizing bacteria (ZSB) which were further evaluated for their plant growth promotion. They observed that the isolates competently solubilized the zinc compounds that are insoluble (ZnO and ZnCO3) and out of 9 isolates, Zn 3 (62.48 mg/l) showed maximum zinc solubilization. They further identified that the isolates belong to 4 different genera i.e., *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Ralstonia picketti* and *Burkholderia cepacia* based on 16 S rRNA gene sequencing. The results of pot experiment conducted by them showed the isolates promoted the growth of rice crop and also increased the Zn content in grain.

Kamran *et al*. (2017) studied the effect of zinc solubilising organisms on the growth and zinc content of wheat. Out of 24 strains, EPS 1 (*Pseudomonas fragi)*, EPS 6 (*Pantoea dispersa)*, EPS 13 (*Pantoea agglomerans)*, PBS 2 (*E. cloacae)* and LHRW1 (*Rhizobium* sp.) have been selected based on their zinc solubilizing potential and PGP traits. The results showed that there was a significant increase in root dry weights and maximum zinc content was recorded for *Pseudomonas fragi* (EPS 1) inoculated plants.

Mumtaz *et al.* (2017) isolated rhizobacteria from maize rhizosphere and screened them to check their zinc oxide solubilizing ability. They observed that there was enhancement in growth of maize when inoculated with zinc solubilizing rhizobacteria. They also reported that isolates ZM20, ZM31, ZM63, and S10 showed improved plant growth attributes and were further identified as *Bacillus aryabhattai* (ZM31 and S10), *Bacillus sp.* (ZM20), and *Bacillus subtilis* (ZM63).

Dinesh *et al*. (2018) isolated and screened a number of rhizobacterial isolates for Zinc solubilization activity. They conducted *in-vitro* studies and reported that among the six potential Zinc solubilizing bacteria (ZSB), the strain ZnSB2 (*Bacillus megaterium*) had shown the highest potential to solubilize Zinc. They also conducted a quantitative study and found that the total amount of Zinc solubilized by ZnSB2 was considerably more than the other Zinc solubilizing bacteria

Yasmin *et al*. (2021) conducted experiments to isolate, characterize and evaluate the Zn solubilizing potential of the isolated rhizobacteria. Among the 10 isolates, the strain RY2 identified as *Pseudomonas protegens was found most promising isolate. When chickpea seeds were inoculated with RY2 strain, there was an increase in root and shoot dry weight, and lengths when compared with uninoculated control.*

2.1.4 Siderophore production

Iron is the essential micronutrient for plants and microorganisms, involved in many biological processes. Siderophores are low molecular weight molecules produced by microbes which have high affinity for Fe molecules. The release of siderophores by bacteria stimulates plant growth, thereby improving nutrition (direct effect) or inhibiting the establishment of phytopathogens (indirect effect) through the sequestration of Fe from the environment.

Chaudhary *et al*. (2017) selected 11 bacterial isolates, which were isolated from garden soil and characterized them for PGPR traits. The isolates NT1, NT2, NT3 and G1

has produced siderophores within 48 hrs of incubation. These four isolates were further screened for siderophore production at different conditions. The results have shown that siderophore production by bacterial isolates was maximum at pH 9, with 2 % NaCl concentration and in a medium containing glycerol.

Ghavami *et al*. (2017) isolated rhizobacteria from the rhizosphere of canola (*Brassica napus*) and screened them for the presence of siderophore production. They isolated 45 distinct isolates which were able to produce siderophore on chrome azurol sulfonate (CAS)-agar plate assay. They reported that out of the 45 isolates, ten isolates were potent strains on the basis of maximum halo diameter. The isolates were further identified as *Stenotrophomonas chelatiphaga* and *Micrococcus yunnanensis* on the basis of 16S rRNA sequencing. They also reported that *Stenotrophomonas chelatiphaga* and *Micrococcus yunnanensis* notably increased iron content of roots and shoots and the weight of the grain.

Kumar *et al*. (2017) performed a qualitative estimation of siderophore for the bacterial strains isolated from rhizospheric soils of the Chikkamagaluru district of Karnataka. The isolates were screened for siderophore production by CAS Agar plate assay. The formation of orange zone around the bacterial isolate confirmed the siderophore production. Therefore, this study concludes that 2 strains VITVK5 and VITVK6 could be promising isolates producing siderophore.

Ghazy *et al*. (2020) evaluated six strains of *B. subtilis, B. circulance, B. coagulanse, B. licheniformis, P. fluroscence* and *P. koreensis* for siderophore production on CAS agar plate. All the strains have produced orange halo after 5 days of incubation at 30 ℃. Among the 6 strains, the orange halo diameter ranged from 1.62 cm to 0.36 cm. *B. subtilis* and *P. koreensis* were the highest producers of iron chelators among the other bacterial strains. The results showed that, when these 2 strains were inoculated individually or in combination has induced disease resistance in maize for wilt caused by *C. maydis* and there was also an increase in growth and yield.

Fahsi *et al*. (2021) isolated 9 phosphate solubilizing bacteria from the rhizosphere of jujube. All these isolates were screened for siderophore production by the CAS agar plate assay. The results showed that all the isolates produced siderophore, the best strain was *P. moraviensis*, the six strains (*Pseudomonas* sp. J10, J153 & J154, *P. moraviensis* J12 & J15, and *P. xylanexedens* J155 produced intermediate levels, whereas the lowest production was seen in both *B. megaterium* J11 and *B. cereus* J156 strains.

2.1.5 Indole acetic acid production

Auxins provide a variety of plant growth-promoting properties, including the development of roots, promoting cell division, cell extension and cell differentiation. Tryptophan which is the precursor of IAA is released as root exudates by many plants and is used by the rhizobacteria for biosynthesis of IAA (Glick *et al*., 1995). The synthesis of these growth regulators by rhizobacteria promotes root growth, boosts water and nutrient absorption and ultimately increases plant longevity.

Mohite (2013) screened 10 bacterial strains isolated from rhizospheric soil for IAA production by inoculating the isolates in yeast malt dextrose broth (YMD broth) for 4 days at 28 ℃. After incubation, the culture was centrifuged and the supernatant was separated. To 1ml of supernatant, 2ml of Salkowski reagent was added and the optical density of the solution was recorded at 530 nm after 30 mins. Among the 10, 5 isolates have shown efficient IAA production. These isolates were further used in pot assay to study their effect on plant growth in wheat. The results obtained showed that these rhizospheric isolates significantly affected the plant growth and shoot length of wheat seedlings.

Baggam *et al*. (2017) isolated bacteria from soil samples collected in forest areas. About 6 strains were isolated and they screened the isolates for IAA production. All 6 isolates produced IAA in different concentrations. The strains I4 and I5 have produced the highest IAA when the conditions for IAA were optimized.

Anosike *et al*. (2018) isolated and characterized the rhizospheric bacterial isolates for IAA production. A total of 6 isolates were able to produce IAA and these were identified as *Bacillus, Micrococcus, Pseudomonas sp*. The effect of these isolates on the growth of maize was studied. The results showed that there was an increase in germination, root and shoot length in maize.

Wagi *et al*. (2019) isolated 10 bacterial isolates from rhizosphere of *Solanum nigrum.* IAA production potential of these isolates was screened in the presence and absence of precursor molecule tryptophan in the culture media. The strains of *Bacillus cereus* and *Bacillus subtilis* have shown the highest IAA production in presence of tryptophan which is 35.8 and 36.6 µg/ml respectively when compared with the values in the absence of tryptophan.

Widawati (2020) collected soil samples from peat lands and isolated bacteria, screened them for IAA production by inoculating the bacteria in tryptone soy broth and YEMB media with tryptophan and optimization of IAA production was done at different incubation temperatures, tryptophan concentrations. The results showed that *Bacillus siamensis* produced the highest IAA after 96 hrs of incubation time. And supplementing the YEMB media with 250 ppm tryptophan was also the best medium to produce IAA.

2.1.6 Ammonia production

Many rhizospheric bacteria convert complex nitrogenous compounds present in soil into ammonia, which can be taken up by plants as a nitrogen source and also increase the nitrogen content in the soil. Ammonia production causes alkalization which indirectly promotes plant growth by inhibiting the growth of fungal pathogens (Weise *et al*., 2013).

Geetha *et al*. (2014) isolated PGPR bacteria from roots and rhizosphere of green gram. A total of 80 isolates were screened for various PGPR traits. Based on antagonist activity 6 potential isolates were selected for further characterization. All the 6 isolates have produced ammonia in peptone water. Ammonia production by PGPR bacteria indirectly affects plant growth.

Kifle *et al*. (2016) isolated diazotrophic bacteria from the rhizosphere, roots, leaves of wheat and maize plants. All the isolates were analyzed for ammonia production by inoculating in the peptone water. The results showed that all the isolates were able to produce ammonia in peptone water. When these isolates were inoculated to the maize seedlings, there was improvement in growth parameters when compared with the control seedlings.

Rodrigues *et al*. (2016) collected leaf, rhizosphere and stem samples of sugarcane for isolation of endophytic bacteria. All the isolates were screened for plant growth-promoting characters, including ammonia production by inoculating the bacterial isolates in peptone water for 96 hrs. The culture was centrifuged at 10000 rpm for 10 mins and to the supernatant 0.5ml of Nessler's reagent was added for brown color development. Ammonia was produced in 45% of the samples. Ammonia production by bacterial isolates helps in catering to the nitrogen needs of the host plant and when the ammonia was produced in excess it helps in the reduction of colonization of pathogens.

Kanchan *et al.* (2018) isolated endophytic bacterial isolates from the root and shoot of cauliflower. These bacterial isolates were characterized for biocontrol activity

such as ammonia production by inoculating the bacterial isolates in peptone water and incubating at 28 ℃ for 48-72 hrs. The addition of 0.5 ml Nessler's reagent develops brown color which was the indicator for ammonia production. Among the 47 bacterial isolates tested for ammonia production, 28 isolates showed the production of ammonia.

Mahidi *et al*. (2021) isolated phosphate solubilizing bacteria (PSB) from soil samples of 3-month-old quinoa plants. All these PSB's produced ammonia, indole acetic acid and siderophores. Ammonia production assay was performed by inoculating the bacterial cultures in peptone water and incubating at 28 ℃ for 48 hrs. After incubation, 0.5ml Nessler's reagent was added to the culture. The development of brown color indicated the production of ammonia. The PSB isolates S6 and S7 have produced the highest values of ammonia production.

2.1.7 Hydrogen cyanide production

Several microorganisms produce HCN as the secondary metabolite. It is highly toxic to phytopathogens, indicating that it can be utilized widely as a biocontrol agent to manage pathogenic diseases in agriculture.

Lanteigne *et al*. (2012) evaluated the antagonistic ability of HCN produced by *Pseudomonas sp.* on *Clavibacter michiganensis subsp. michiganensis*. They found that screening of the bacteria *Pseudomonas spp.* under *in vitro* conditions appreciably inhibited the development of *Clavibacter michiganensis subsp. michiganensis*. They further conducted *in vivo* study and reported that *Pseudomonas sp.* reduced the disease occurrence and the population of the pathogen in the rhizosphere suggesting that production of HCN was involved in the biocontrol of bacterial canker of tomato.

Reetha *et al*. (2014) isolated indigenous *Psuedomonas* and *Bacillus spp* from the rhizosphere of sunflower. These isolates were screened for hydrogen cyanide production by inoculating the isolates in King's B medium amended with 4.4 g/l glycine and placing the filter paper saturated with alkaline picrate solution in the upper lid of the petriplate. The color change on the filter paper was observed from yellow to light brown or reddish-brown which confirms the production of hydrogen cyanide. The selected strains of bacteria were screened for biocontrol activity against *Macrophomina phaseolina* and reduction in growth of the pathogen was observed.

Apastambh *et al*. (2016) isolated 8 strains of fluorescent *Pseudomonas* (Yps1- 8) and 4 strains of *Bacillus* (Yb1- 4) from rhizospheric soil of banana collected from Nanded,

Maharashtra. They further characterized these strains morphologically and biochemically and studied for their biocontrol traits such as siderophore production and HCN production. They reported that maximum HCN production was exhibited by Yps1 and Yps7. They further tested these strains for antifungal activity against *Fusarium oxysporum* and *Alternaria solani* and found that maximum inhibition against *Fusarium oxysporum* was exhibited by Yps1.

Rahman *et al*. (2019) isolated 39 rhizobacteria from economic plants and screened them for HCN production. Among all isolates, 6 isolates have shown production of HCN, and 16S rRNA analysis revealed that all 6 isolates belong to the genera *Pseudomonas*. These isolates when inoculated with tomato plants have decreased the population and number of galls produced by Meloidogyne incognita when compared with control.

2.2 SCREENING OF BACTERIAL ISOLATES FOR ABIOTIC STRESS TOLERANCE

Plant growth-promoting rhizobacteria are the group of organisms that are used for plant growth enhancement and as biocontrol agents for the management of diseases in plants. The difference in the performance of these PGPR under *in vitro* conditions and in the field is due to the prevailing abiotic stress conditions like high temperature, salinity, drought stress, *etc* (Kumar *et al*., 2014). Therefore, screening of bacterial isolates for abiotic stress tolerance would be beneficial for showing the desirable effects on plants by promoting the plant's growth under adverse conditions.

2.2.1 High-temperature tolerance

Pandey *et al*. (2014) isolated 28 bacterial cultures from hot springs of Uttarakhand and these bacterial isolates have been characterized for temperature tolerance by inoculating the cultures on TY agar plates and incubating at different temperatures ranging from 15°C-90°C with the interval of 5°C. The results have shown that all the isolates could grow at optimum temperatures of 55-65°C. Therefore, these organisms have been classified as mesophilic-thermophiles.

Sharma *et al*. (2018) isolated bacteria from water samples collected from hot springs in Jammu and Kashmir. The optimum temperature for the growth of these bacterial isolates was assessed by streaking these bacterial isolates on nutrient broth with 2% agar. The different temperature ranges used in this study were 40, 50, 55, 60, 65, and

70°C. The results obtained showed that these bacterial isolates could grow at the temperature range of 60-65°C.

Tsegaye *et al*. (2019) performed screening tests for bacterial isolates for abiotic stress tolerance including temperature tolerance by incubating the bacterial isolates at different temperatures such as 4℃, 20℃, 30℃, 40℃, 50℃, 60℃. The results showed that all the isolates have grown well at 20℃ and 30℃. At 60℃, 7 isolates have grown well. These isolates have also shown some PGPR traits like IAA production, phosphate solubilization and ammonia production. Thus, these isolates can enhance crop growth in semi-arid and arid regions.

Khan *et al*. (2020) screened bacterial isolates for various traits including temperature tolerance by inoculating the isolated cultures on LB agar media and incubating the plates at different temperatures such as 25℃, 30℃, 35℃, 40℃ and 45℃. The results showed that all the isolates have grown normally till 35℃ and there was reduction in growth after 40℃. One of the isolates SN1 has grown at high temperatures, thus this isolate was selected as heat stress-tolerant and it can be used in crop production for mitigation of heat stress.

Prashad *et al.* (2020) isolated halophilic bacteria from water and tested these isolates for temperature tolerance by streaking these cultures on nutrient agar plates and incubating them at different temperatures such as 10°C, 37°C, 45°C for 48 hrs and the growth of bacteria was observed. The results showed that the optimum temperature for the growth of halophilic bacteria was found to be 37°C-45°C. Thus, they have classified these bacteria as mesophilic.

2.2.2 Salinity tolerance

Damodaran *et al*. (2013) conducted experiments for determining salt tolerance of isolated bacteria from rhizospheric soils of halophilic plants grown in Raiberaily, Uttar Pradesh. The isolated bacteria were streaked on nutrient agar supplemented with 0.5, 5, 7.5 and 10% NaCl. The ability of bacteria to grow at different NaCl concentrations, confirms the salinity tolerance. Among the 16 salt-tolerant bacteria, 5 isolates have grown luxuriously at 7.5% NaCl concentration.

Ramadoss *et al.* (2013) isolated 84 halotolerant bacteria from saline habitats and screened them for growth on NA media amended with different NaCl concentrations (5,10, 15, 20, and 25 % w/v). All the isolates had grown well at 5% NaCl and only 25%

of the isolates showed growth at 20 % NaCl concentrations. Inoculation of 5 halotolerant bacteria to wheat seedlings to overcome salt stress (80,160,320 Mm) has induced an increase in root length of 71.1% when compared with uninoculated wheat seedlings. *Hallobacillus* and *Bacillus halodenitrificans* have produced 90% increase in root elongation and a 17.4% increase in dry weight when compared with uninoculated wheat seedlings at 320 mM NaCl concentration.

Kumar *et al*. (2017) studied the effect of salt-tolerant bacteria on the growth of rice under salt stress conditions. In this experiment, they isolated a total of 82 bacterial isolates from the rhizosphere and evaluated the growth of these bacterial isolates on NA media amended with different salt concentrations [5,10, 15 and 20 % (w/v)]. All the isolates have grown well at 5% NaCl concentration and 12 isolates had grown well at 15% NaCl concentration. And they have selected the bacterial isolates which grow at 15 % NaCl concentrations for further experiments.

Karnwal (2019) screened bacterial isolates for salinity tolerance by streaking the isolates on nutrient agar media amended with varied concentrations of NaCl- 5, 7, 8.5, 10, 12 % and incubated at 28 ℃ for 48 hrs. And the isolates which have grown in NaCl concentration greater than 5% were considered salt tolerant. The salt-tolerant bacteria were further examined for plant growth-promoting traits.

Sharma *et al*. (2021) isolated bacteria from salt-affected soils of Haryana. These isolates were screened for salinity tolerance, ranging from 2-10 % NaCl concentration. About 13 isolates were screened as salt-tolerant bacteria and the strains HB6P2 and HB6J2 have shown maximum salinity tolerance up to 10 % of NaCl concentration. The results of this experiment indicate that these isolates can be used to alleviate the salt stress in plants by enhancing plant growth and yield.

2.2.3 Drought tolerance

Kumar *et al*. (2014) collected soil samples across India and isolated *Pseudomonas* and *Bacillus spp* and screened these isolates for drought resistance by growing the bacterial isolates in TSB medium amended with 32.6% PEG 6000 and incubated at 28℃ for 24 hrs. The OD was recorded at 600 nm using a spectrophotometer and uninoculated media as blank. The OD of 0.1 for bacterial isolates is considered stress-tolerant. A total of 14 isolates showed tolerance at –1.2 MPa osmotic pressure. Therefore, these stress-tolerant stains can be used in extreme environments for protecting the plants from drought, high temperatures, etc.

Niu *et al*. (2018) isolated bacteria from root samples of foxtail millet which is a drought-tolerant crop. Screening of bacterial isolates was done by inoculating 1% bacterial culture in trypticase soy broth amended with PEG-6000 for creating the water potential of 0, -0.05, -0.30, -0.73 Mpa and incubated at 28°C. The growth of bacteria is estimated every 3 hours at 600 nm using a spectrophotometer. The results showed that the growth of all bacteria was affected by PEG 6000. And they have identified that out of 14 isolates, 4 isolates were able to grow at lower water potential (-0.03 Mpa). Thus, the results show that these bacteria can be used for mitigating drought conditions by improving seed germination and seedling growth.

Susilowati *et al*. (2018) isolated bacteria from the rhizosphere of soybean and screened the purified isolates for drought tolerance. To create drought conditions the bacterial cultures were inoculated in NB that has been amended with PEG 6000. The cultures are incubated at 38°C for 24 hrs and the optical density readings were taken with a spectrophotometer at 570 nm. The bacterial isolates having OD value greater than 0.5 have been selected as drought-tolerant isolates. The results showed that 11 isolates produce exopolysaccharides and thus are drought tolerant.

Ashry *et al*. (2021) isolated 20 bacterial isolates from harsh soils and evaluated the drought tolerance potential of these isolates by growing them in broth amended with poly ethylene glycol PEG 6000. The selected isolates were screened for plant-promoting characteristics and *in vitro* germination tests were conducted in maize in presence of PEG 6000. The results showed that one bacterial isolate has shown an increase in IAA, GA, siderophore and exopolysaccharide production in presence of stress conditions. When the two selected strains were inoculated in combination for *in vitro* germination, the germination percentage and the total seedling length were highest. Therefore the 2 strains in combination can be used to alleviate drought stress in plants growing in dry lands.

Kumar *et al*. (2021) isolated bacteria from soils of drought-prone areas of Andhra Pradesh state. These isolates were screened for drought tolerance by growing isolates in tryptic soy broth amended with PEG at different concentrations creating varied osmotic potentials (-0.05, -0.15, -0.30, -0.49, and -0.73 MPa). After incubation for 24 hrs, the growth of the bacterial isolates was measured at 600 nm using a spectrophotometer. Thus these strains can be used to mitigate the drought stress, enhance the plant and soil health.

2.3 COMPATIBILITY OF BACTERIAL ISOLATES WITH AGRICHEMICALS

Use of agrochemicals has become a part of conventional agriculture to increase the crop yield. Agrichemicals include synthetic fertilizers, insecticides, herbicides, which are applied to plants for providing nutrients to plants, kill insects and weeds respectively. So when the bacterial inoculants such as PGPR's applied to crops, they interact with agrichemicals and this may affect the bacterial survival. Hence the compatibility testing between the agrichemicals and bacterial isolates must be done, for the integrated use of bacterial isolates along with agrichemicals.

Verma *et al*. (2016**)** collected rhizosphere and non-rhizosphere soil samples from IIVR, Varanasi where the vegetable fields were applied with chlorpyriphos for insect control. A total of 50 microbes were isolated, out of which 14 were selected for their plant growth-promoting properties and biochemical characteristics. All the selected isolates are checked for compatibility with chlorpyriphos at different concentrations using the disc diffusion method. The sterile filter paper discs are dipped in different concentrations of pesticide chlorpyriphos (0, 2, 4, 6 and 20µl/ml) and are placed on a nutrient agar plate spread uniformly with pure culture of selected organisms. The plates are incubated at 30°C for 48hrs and the zones of inhibition around the discs are observed. The results show that strains IESDV3 and IESDV11 are tolerant at 6 µl/ml concentration of chlorpyrifos. Thus, these pesticide tolerant strains can be used for plant growth promotion in vegetable fields.

Basha *et al*. (2018) studied the compatibility of native *Pseudomonas fluorescence* strains with agrochemicals like fungicides, herbicides, and insecticides by disc diffusion method. The solutions of different fungicides, insecticides, and herbicides are prepared by dissolving them in water, based on recommended dose. The filter paper discs dipped in different concentrations of agrochemicals was placed on KB agar plate spread with 100µl of bacterial culture. The filter paper disc dipped in sterile distilled water is used as control. The plates are incubated at 28°C for 2-3 days and observed for inhibition zones. The results showed that the pseudomonas strains were not compatible with copper oxychloride and mancozeb. Thus, these strains which are agrochemical tolerant can be used for integrated disease management.

Verma *et al*. (2018) studied the effect of organophosphate insecticides like chlorpyriphos and monocrotophos on the growth of *Bacillus* strains. Different concentrations (Control, 1X, 2X and 10X) of these insecticides are prepared based on the

recommended dose. The sterile discs dipped in different concentrations of chemicals are kept on nutrient agar plate with respective microbial culture and the plates are incubated at 30°C for 48 hrs. After 48 hrs the growth of bacteria and inhibition zones around the discs is observed. The results showed that the bacterial strains BHUJP-H1, BHUJP-H2, and BHUJP-H3 showed no inhibition zone against monocrotophos that means these strains are more tolerant. The strains BHUJP-H1 and BHUJP-H2 showed a zone of inhibition at 1x, 2x, and 3x concentration chlorpyrifos, and these strains are more susceptible.

Kiran *et al*. (2018) evaluated the compatibility of fungicides with bacterial biocontrol agents by disc diffusion method by spreading the overnight grown *Bacillus* culture on a nutrient agar plate and placing the disc dipped in different fungicidal concentrations. The control disc was dipped in normal water. The zone of inhibition around the discs was observed and compared with the control. The results showed that copper oxychloride was incompatible with *Bacillus spp.*

Mansotra *et al*. (2022) screened 50 *Mesorhizobium spp* for tolerance to 4 recommended pesticides of chickpea by disc diffusion method. Highest growth of bacteria was observed with pendimethalin when compared with other pesticides and decline in growth was observed at double dose for all pesticides. Highest inhibitory effect on the growth of *Mesorhizobium* was observed with chlorpyrifos. About 20 strains of *Mesorhizobium* were selected as pesticide tolerant strains in this experiment.

2.4 *IN VITRO* COMPATIBILITY STUDIES BETWEEN BACTERIAL ISOLATES FOR CONSORTIA DEVELOPMENT

Prasad *et al*. (2017) checked the compatibility of bacterial isolates by streaking the isolates on nutrient agar plate in such a way that center has one bacterial isolate and other bacterial isolates are streaked perpendicular to the center culture. These plates are incubated at 37°C for 48 hrs and the zone of inhibition around the cultures was observed. The results have shown that *Rhizobium* has shown growth inhibition of other bacteria. A consortia was formulated with only compatible bacterial isolates for further pot culture studies in groundnut.

Oljira *et al.* (2018) conducted *in vitro* compatibility test between three potential bacterial isolates by cross streak method. The isolates are streaked across diametrically on nutrient agar plates and were kept for incubation at room temperature or at 37°C overnight. The results of the study showed that all the isolates were compatible with each

other. Therefore, these three bacterial isolates can be used in combination rather than individual strain.

Daghari *et al*. (2020) tested compatibility between bacteria by cross streak assay. The results showed that all the bacterial isolates showed compatibility with each other. These bacterial isolates were also assessed for antagonistic activity with *Trichoderma viridae*, all the isolates were found compatible.

Cumpa *et al*. (2021) conducted a compatibility test between selected phosphate solubilizing bacteria and *Rhizobia* strain by cross streak method. The 2 strains were streaked perpendicular to each other on TY agar plate and incubated for 5 days at 28 ℃. The zones of inhibition forms at the intersection when the strains are incompatible. The results showed that both the strains have not produced any inhibition zone, suggesting that both the isolates are compatible and can be used in combination.

Denaya *et al*. (2021) performed compatibility tests for PGPB so that these bacterial isolates can be used as consortia instead of single strain inoculation. The method followed for compatibility testing was cross streak method. In this study, one bacterial isolate was streaked horizontally and the other vertically on LB agar media and incubated for 48hrs. The lysis or inhibition zones of bacterial isolates are observed when the two cultures are not compatible and no inhibition zone was formed if they are compatible. The results showed that all the isolates were compatible with each other as no inhibition zones or lysis has occurred.

2.5 EFFECT OF BACTERIAL CONSORTIA ON PLANT GROWTH UNDER *IN VITRO* CONDITIONS

Numavo *et al*. (2013) assessed the effect of 3 plant growth-promoting rhizobacteria on maize under *in vitro* conditions. The highest germination percentage, leaf area, vigour index, number of leaves and higher volume of aerial biomass were obtained when the seeds were treated with a combination of *Pseudomonas fluorescens and Pseudomonas putida.* Therefore, these results suggest that PGPR in combination enhances germination and biomass in maize.

Mishra *et al*. (2015) has studied the effect of PGP bacteria on growth promotion in *Sorghum bicolor*. The PGP bacteria were inoculated singly and in combination under *in vitro* conditions to study the parameters of root length, shoot length and biomass.

Application of consortia resulted in a 2-fold increase in growth parameters in sorghum with 23% increase in chlorophyll content when compared with control.

Akintokun *et al*. (2016) studied the effect of bacterial isolates and their consortia on seed germination of tomato. The surface-sterilized tomato seeds were soaked in individual culture as well as in their consortia for 30 mins and were placed in Petri plates for 7 days at 28℃. The number of seeds germinated and seedling lengths were taken from 7^{th} day. The results showed that there was significant difference in vigor index between the consortia and the single strain.

Hashmi *et al*. (2019) studied the effect of individual strains as well as consortia on germination of oat in *in vitro* conditions. Five seeds inoculated with bacterial culture were kept for germination in each replication with 5 replications for each treatment. Uninoculated seeds are kept as control and these seeds were incubated at 22℃ in dark for 10 days. After 10 days the number of seeds that have germinated was counted and the germination percentage is calculated. The *in vitro* germination assay has shown that both the individual strains and the consortia has increased seed germination when compared with control.

Singh *et al*. (2021) conducted *in vitro* seed germination assay by agar plate method to study the effect of consortia on the growth of urad bean and mung bean. In this experiment, seed treatment was done by submerging the seeds in microbial culture having 10^{-8} cells for overnight. The treated seeds were kept on semi solid agar plate for germination at 23℃ for 12 days. The number of seeds germinated is observed daily and root length, shoot length were taken on 12^{th} day. The results showed that the seeds treated with microbial consortia showed the highest germination percentage when compared with control seeds.

2.6 MOLECULAR IDENTIFICATION OF MEMBERS OF BACTERIAL CONSORTIA

Naveed *et al*. (2014) collected rhizospheric soil and root nodules for isolating plant growth-promoting rhizobacteria. Molecular identification of the isolates was done by 16S rRNA sequence analysis and the isolates belonged to the genera *Ensifer, Bacillus, Pseudomonas, Leclercia, and Rhizobium*.

Islam *et al*. (2019) isolated 24 indigenous bacterial isolates from the rhizosphere of rice, jute, bean, and sesbania. Out of 24 isolates, 2 isolates were selected for molecular

identification after initial screening for antagonistic activity and biochemical characteristics. The molecular identification by using the 16S rRNA gene and subsequent NCBI-BLAST analysis revealed that the isolate Prb2 showed 100% similarity with *Brevundimonas olei strains and Prb1 showed 100% identity to Bacillus methylotrophicus* strains.

Patel *et al*. (2019) isolated 226 bacterial isolates from the sugarcane rhizosphere. On the basis of dual culture assay, biochemical, PGP traits, 26 isolates were selected. The selected isolates were identified by 16S rRNA sequence analysis, it was found that the isolates belonged to Proteobacteria, Firmicutes, and Bacteriodes.

Kalam *et al*. (2020) isolated 60 bacterial isolates from tomato rhizosphere, out of which 7 isolates were selected based on their plant growth-promoting characteristics. The 16S rDNA sequence analysis of these 7 isolates revealed that all these isolates belong to genus *Bacillus*, sharing 99- 100 % similarity with the members.

Oo *et al*. (2020) isolated 102 bacterial isolates from the rhizosphere of different crop fields. The selected isolates were identified by 16S rRNA sequencing as B3-*Pseudomonas plecoglossicida*, S2-*Stenotrophomonas maltophilia*, S3-*Achromobacter insolitus*, S4-*Pseudomonas aeruginosa*.

2.7 EFFECT OF BACTERIAL CONSORTIA ON GROWTH AND YIELD OF RICE

Kumar *et al*. (2016) carried out experiments on the effect of four rhizobacterial strains for the growth promotion of basmati rice. The bacterial strains when applied individually showed an increase in plant height, shoot and root dry weight. And when these rhizobacterial strains were applied as consortia, the uptake of N and P was maximum than the control. Hence these strains when applied as consortia have enhanced the growth promotion and nutrient uptake in basmati rice.

Paneerselvam *et al.* (2020) studied the development of effective microbial consortia using native microbial strains with insecticidal, fungicidal properties with plant growth promotion abilities for sustainable agriculture production under Sikkim conditions. They have prepared a biofertilizer consortium using microbial strains viz., *A. chroococcum, B. megaterium* and *P. putida* and microbial consortia using *B. luciferensis, B. subtilis, B. luciferensis* which were evaluated in local rice variety under field condition. The results showed that the combined application of these two products significantly

increased rice grain yield (2.6 - 4.5 t/ha) as compared to un-inoculated control (1.9 - 3.1 t/ha).

Pratiwi *et al*. (2021) isolated bacterial strains from rhizospheric soil of rice fields. The bacterial isolates were selected based on their plant growth promoting characteristics. When these cultures were applied as consortium, the inorganic fertilizer use efficiency and the rice production has increased. Hence these consortia could be used as bioferlizer for the cultivation of rice.

Purwanto *et al*. (2021) studied the effect of the PGPR consortium on nutrient uptake and yield of rice. The consortium was applied by immersing the rice seedlings in a 1% consortium solution before planting and after planting spraying 1% consortium solution. The results showed that the consortium was able to increase root length, nutrient uptake and plant biomass in rice. Therefore, this study recommends the use of this consortium as biofertilizer formulation in cultivation of rice.

Sherpa *et al*. (2021) isolated 8 rhizospheric bacteria from organic paddy fields. Three liquid microbial consortia were formulated using these 8 isolates in different combinations. These 3 consortia were used in rice for improving the growth and yield of rice under green house and field conditions. The studies have shown that consortia 3 has improved the agronomic characteristics of rice.

MATERIAL AND METHODS

Chapter III

MATERIAL AND METHODS

The present study was carried out at ICAR-Indian Institute of Rice Research to develop and evaluate a promising bacterial consortia for improving growth and yield of rice.

3.1 COLLECTION AND PURIFICATION OF BACTERIAL ISOLATES

The bacterial isolates (32) were collected from ICAR-IIRR (Indian Institute of Rice Research), Rajendranagar, Hyderabad. They were purified by quadrant streaking method on nutrient agar media and the cultures were used to evaluate for Plant growth promoting traits, eco-physiological stress tolerance and evaluation of promising isolates under *in vitro* and pot culture experiments.

3.2 CHARACTERIZATION OF BACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING TRAITS

3.2.1 Qualitative assay for phosphate solubilization: (Pikovskaya, 1948)

The pure cultures of bacterial isolates were inoculated in nutrient broth and incubated at 28°C for 48 hrs. 10 µl of cultures were used for study of phosphate solubilization on Pikovskaya's agar medium and incubated for 4 days at 28 ºC and the zone of solubilization formed around the colonies were recorded.

The solubilization index of the microorganisms was calculated by using following formula:

Solubilizing index (S.I) = C + Z / C

where,

Z = Solubilization zone (mm)

C = Colony diameter (mm)

Based on solubilization index, the isolates were assigned scores. The isolates with solubilization index < 0.1 were given a score of 0, solubilization index range 1.0-2.0 were given score 1, solubilization index from 2.0-3.0 were given score 2, solubilization index from above 3.0 were given score 3.

3.2.2 Plate assay for potassium releasing activity

Potassium solubilization assay was performed by the method given by Hu *et al.* (2006). The bacterial isolates were inoculated on Aleksandrov agar media by spot inoculation of 10 µl culture and the plates were incubated at 28 ℃ for 72 hrs. The diameters of clearing zone around the colonies were recorded. Solubilization index was calculated and scores were assigned as described for phosphate solubilization.

3.2.3 Plate assay for zinc solubilization

The isolates were inoculated on tris minimal media supplemented with ZnO and incubated at 28ºC for 72 hrs. The standard method developed by Saravanan *et al*. (2004) was followed to study the zinc releasing ability of the bacterial isolates. The diameter of the clearing zone around the colonies was measured. Solubilization index was calculated and the isolates were assigned scores by using the same procedure as described for phosphate solubilization.

3.2.4 Siderophore production

Siderophore production of bacterial isolates was tested by chromo-azurol-S (CAS) plate assay method given by Schwyn and Neilands (1987). The cultures were spot inoculated on CAS agar plates and were incubated at 28 ℃ for 72 hrs. Formation of orange zone around the colonies indicated the production of siderophore. The diameter of the colour formation around the colonies was recorded. Siderophore producing index was calculated as described earlier for phosphorus solubilization. Based on change in colour the isolates were given scores. The isolates with index range from 0 – 2 were given score 0, index from 2– 3 were given score 1, index from 3-4 were given score 2 and index above 4 were given score 3.

3.2.5 Indole acetic acid production

Indole acetic acid production by bacterial isolates was performed by the method given by Gordon and Weber (1951). The bacterial cultures were grown in a nutrient broth amended with tryptophan (5 mM) for 7 days. The cultures were centrifuged at 10,000 rpm for 20 min at 4°C and two ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of Salkowski reagent. The tubes were then incubated at room temperature for 25 min and based on the intensity of colour, the bacterial isolates were classified into 4 groups viz., -, +, ++, +++ and the scores given were 0, 1, 2 and 3 respectively.

3.2.6 Ammonia production

The ammonia production was performed by the method given by Cappucino and Sherman (1992). The isolates were inoculated in peptone water and incubated for 4 days at 28 ℃ and centrifuged at 10,000 rpm for 15 min at 4°C. To the supernatant, 0.5 ml of Nessler's reagent was added for development of brown to yellow colour which is indicator of ammonia production. Based on the intensity of colour, scores were assigned to bacterial isolates from 0-3.

3.2.7 Hydrocyanic acid production (HCN)

The HCN production by bacterial isolates was done by the method given by Castric (1983). Bacterial culture (0.1ml) was taken and spread on nutrient agar plate supplemented with 4.4 g per litre of glycine. On the upper lid of petriplate, a filter paper impregnated with 0.5 % picric acid (w/v) in 1% sodium carbonate solution was placed under aseptic conditions and the plate was incubated at 28 ℃ for 3-4 days. The change of colour of the filter paper from yellow to light brown or reddish brown indicates HCN production. Based on the intensity of colour, scores were assigned to bacterial isolates from 0-3.

3.3 SCREENING OF BACTERIA FOR ECO-PHYSIOLOGICAL STRESS TOLERANCE

3.3.1 Screening of Isolates for Abiotic Stress Tolerance

The isolates taken from Indian Institute of Rice Research were screened for abiotic stresses such as high temperature, salinity and drought tolerance.

3.3.1.1 Screening for Temperature tolerance

Screening of isolates for temperature tolerance was done by taking a loopful of pure culture and streaking the cultures on tryptic soy agar media (Sharma *et al*., 2018). Initially the petri plate was divided into four equal parts and each culture was streaked separately in each part and labelled properly. After streaking the culture on tryptic soy agar, the plates were incubated at different temperature such as 4, 15, 25 and 45℃ separately. And the growth of bacterial isolates was observed after 3 days. Based on the growth of bacteria at different temperatures the scores were given as 0- no growth, 1- less growth, 2- moderate growth, 3- good growth.

3.3.1.2 Screening for salinity tolerance

Screening of bacterial isolates for salt tolerance was performed according to Kumar *et al*. (2017). The bacterial isolates were screened for different salt concentrations like 4, 6, 8% NaCl w/v. For this experiment tryptic soy agar was prepared by adding salt of different concentrations and autoclaved. Later the media was melted and poured into petri plates. The bacterial isolates were then streaked on trypic soy agar plates containing different salt concentrations separately and the plates were incubated at 28°C and the growth of isolates was checked after 3 days. Based on growth, the scores were given like 0- no growth, 1- less growth, 2- moderate growth, 3- good growth. For selecting bacterial isolates for consortia development, the isolates exhibiting growth at all three concentrations were given score 3, isolates showing growth at 4% and 6% NaCl were scored 2, isolates growing at only 4% were scored as 1 and isolates not showing growth at all three concentrations were given a score of 0.

3.3.1.3 Screening for drought tolerance

The bacterial isolates were screened for drought by inoculating the bacterial cultures in tryptone soy broth amended with polyethylene glycol at different concentrations (Niu *et al*., 2018). The concentrations of PEG 6000 - 0, 8, 35, 64 g/L was used to create the water potential of 0, -0.05, -0.30, -0.73 MPa in the media. The growth of the bacterial isolates was observed by taking spectrophotometer readings at 600nm after 24 hrs. The bacterial isolates were classified for drought tolerance based on OD values at -0.73MPa as highly sensitive-OD <0.3, sensitive- OD 0.3-0.4, tolerant- OD 0.4-0.5, Highly tolerant OD>0.5. For highly sensitive bacteria score was given as 0, sensitive-1, tolerant-2 and highly tolerant-3 (Alikhani and Mohamadi, 2010).

3.3.2 Compatibility of bacterial isolates with agrichemicals

Effects of different concentrations of agrichemicals on microbial growth was observed on media plate by using filter paper disc technique (Verma *et al.,* 2016). The dosage of agrichemicals used in this experiment was C-control, C1-50% of recommended dose, C2-100% of recommended dose and C3-150% of recommended dose (Table 3.1) normally used in paddy cultivation. Therefore, the solutions of agrichemicals were prepared accordingly and were used to wet the filter discs. And the filter discs with different concentrations of agrichemicals were placed on nutrient agar media plate which has the bacterial culture of 0.1 ml spread across the plate. The plates were incubated at

ambient temperature and growth of bacteria was observed for over 1 week and appearance of inhibition zones around the filter discs was noted. Based on the formation of inhibition zones, the compatibility of bacterial isolates with agrichemicals was checked. The isolates growing at all the concentrations of agrichemicals were given a score of 3, the isolates growing at C, C1 and C2 were given a score of 2, the isolates growing at C and C1 were given a score of 1 and the isolates not showing growth at all concentrations of agrichemicals were given a score of 0.

Table 3.1 Agrichemicals and the recommended dose generally used in rice cultivation

Fertilizers/pesticides	**Recommended dose**
Urea	260 kg/ha
Single super phosphate	375 kg/ha
Muriate of potash	67 kg/ha
Cartap hydrochloride	18.75 kg/ha (4% G)
Ferterra (Chlorantraniliprole)	10 kg/ha (0.4% G)
Thiamethaxom	0.5 kg/ha (25% WG)
Pretilachlor	400 ml/acre (50% EC)
Bispyribac sodium	0.5 ml/lit (10% SC)
Carbendazim	0.25 kg/ha
Mancozeb	1.5 g/l

3.4 SELECTION OF EFFECTIVE CONSORTIA PARTNERS AND IDENTIFICATION OF THE PROMISING ISOLATES

3.4.1 Selection framework for identification of efficient rhizobacteria

A scale was generated similar to that described by Hazarika *et al*. (2021) and Krechel *et al.* (2002) to select the best bacterial isolates for consortia formulation. PGP traits, abiotic stress tolerance and compatibility of isolates with agrichemicals were the three categories employed for preparing the scale and scoring of isolates, following which three isolates with the highest score in each category were selected. The isolates were grouped into three consortia with each consortium consisting of one bacterial isolate from each category.

3.4.2 *In vitro* compatibility studies between isolates for consortium development

Compatibility of bacterial isolates with each other was evaluated by cross streak method (Prasad *et al.,* 2017). The bacterial isolates in each consortia were evaluated for cross compatibility with each other. After incubation for atleast a week the cultures were checked for the development of inhibition zones. Development of inhibition zone indicates the incompatibility with the test isolate. Based on compatibility three consortia combinations were selected and the performance of these consortia were evaluated by *in vitro* germination studies.

3.4.3 Consortia formulation and *invitro* germination assay

A loopful of bacterial culture was inoculated in nutrient broth and allowed to grow for 3-5 days. Then the culture was centrifuged, and pellet was washed with phosphate buffered saline (PBS), and it was made up to 25 ml with PBS. The optical density reading was taken for the pellet dissolved in PBS at 600 nm with O.D of 1 ($1x10^7$ CFU/ml). The seeds were surface sterilized with 0.15% $HgCl_2$ for 1min, followed by 70% ethanol for 1min and finally washed with distilled water. The seeds were soaked in the culture and its consortia (containing three organisms in equal proportions and having OD-1 at 600 nm) for 24 hrs at 28℃. The overnight soaked seeds were placed on water agar and kept for incubation at 28℃ for 7 days. The data such as germination percentage, seedling length and vigor index (I and II) was collected. Based on the above data collected the promising isolates were selected for pot culture experiments.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds kept for germination}} \text{X } 100$$

Vigor index I = Germination (%) x seedling length (cm)

Vigor index II = Germination (%) x Dry weight of seedlings (g)

3.5 MOLECULAR IDENTIFICATION OF THE MEMBERS OF COMPATIBLE PROMISING BACTERIAL CONSORTIA.

The elite strains selected for microbial consortia studies were identified using 16S rRNA gene sequencing.

3.5.1 Extraction of microbial genomic DNA

Extraction of microbial genomic DNA was done by using GSURE® bacterial genomic DNA isolation kit (GCC BIOTECH). The overnight grown culture in nutrient broth was pelleted down by centrifugation in microfuge tube. The supernatant was discarded and 250 µl GDB1 solution was added to cell pellet. Resuspension of the pellet was done by vigorous vortexing. After resuspension the tube was incubated at 70 ℃ for

15 mins and added with 250 µl GDB2 buffer. Again the tube containing GDB2 buffer was placed at 70 ℃ for another 15 min. Then, 350 µl GDB3 buffer was added to the tube and it was shaken vigorously to mix the solutions. After the GDB3 buffer was added, the tubes were centrifuged for 10 mins at 13,000 rpm. The supernatant was transferred to the GMini spin column by pipette and centrifuged at 13,000 rpm for 30-60 s. The flow through was discarded. The column was washed by adding 600 µl membrane wash buffer and centrifuged for 30-40 s. The flow through was discarded again and the washing step was repeated. The column was centrifuged for additional 2 mins for removal of residual wash buffer in the membrane. The GMini spin column was kept in a fresh 1.5 ml microcentrifuge tube and to the column, 50 µl nuclease free water was added and it was left undisturbed for 1 min. The column was discarded after centrifugation for 1 min at maximum speed and the eluted DNA was collected in microcentrifuge tube.

3.5.2 Amplification of 16S rRNA gene

Gene amplification of 16S rRNA was carried out in BioRad T100 thermocycler (BioRad, USA) using 2 primers developed in-house for amplification of 16S Rrna. The primers used were mentioned in Table 3.2. The amplification was carried out in PCR tubes with 20 µl reaction volume consisted of following components given in Table 3.3.

Table 3.2 Base sequences of 16S rRNA gene primers

Two primers which amplifies the entire length of rRNA (8-1492 bp)

Primer name	Primer sequence	Product length (kb)
16S rRNA-I		
16S_8F_F	AGAGTTTGATCCTGGCTCAG	899 bp
16S_907R_R	CCGTCAATTCCTTTRAGTTT	
16S rRNA-II		
16S_785F_F	GGATTAGATACCCTGGTA	707 bp
16S_1492R_R	CGGTTACCTTGTTACGACTT	

Table 3.3 Components of PCR

Reagent (µl)	Volume (µl)
DNA template (50 ng/µl)	2.0 µl
Forward primer (10 pmoles/µl)	0.6 µl
Reverse primer (10 pmoles/µl)	0.6 µl
10 mM dNTP Mix (µl)	1.6 µl
10 X PCR buffer (Mg^{+2})	2.0 µl
Taq DNA polymerase (5 U/µl)	0.2 µl
RNase free water	13.0 µl
Total	20 µl

Table 3.4 PCR (Thermal cycler) gene amplification

S. NO	Steps	Temperature	Duration	Cycles
1	Initial denaturation	94 ℃	5 min	1
2	Denaturation	94 ℃	30 sec	30
3	Annealing	54 ℃	30 sec	
4	Extension	72 ℃	1 min	
5	Final extension	72 ℃	7 min	1
6	Hold	12 ℃	∞	-

3.5.3 Agarose gel electrophoresis of amplified PCR products

The amplified PCR products were resolved using 1 % agarose gel electrophoresis in 1 X Tris acetate EDTA buffer. The PCR product was mixed with ethidium bromide before loading the amplified product in wells. The gel was run for 45 mins at 120 V. The gel image was captured using gel documentation system.

3.5.4 Sequencing and data analysis

PCR products of 16S rRNA gene of seven efficient bacterial isolates were obtained through amplification and the products were sequenced using sanger sequencing platform (Eurofins, Hyderabad).

3.5.5 16S rRNA gene sequence analysis

The 16S rRNA sequences (Eurofins, Hyderabad) were aligned and contigs were created using Cap3 Bioedit Software. The sequences obtained for the bacterial isolates

were compared against the sequences of 16S rRNA of bacterial isolates available in the National Centre for Biotechnology Information NCBI Gene Bank Nucleotide Database (http://www.ncbi.nih.gov/blast) and the isolated bacteria was identified based on maximum percentage of similarity of the sequences.

3.6 POT CULTURE STUDIES

3.6.1 Location of the experiment

Pot culture experiment was carried out at ICAR-Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad.

3.6.2 Characteristics of experimental soil

Soil samples from ICAR-IIRR were collected before sowing and analysed for the chemical properties by adopting standard procedures.

Table 3.5 Initial characteristics of soil

Soil properties	Values	Method adopted
pH	7.75	Jackson, 1973
EC	0.65 ds m^{-1}	Jackson, 1973
Available N	267.34 kg/ha	Subbiah and Asija, 1956
Available P	38.34 kg/ha	Olsen,1954
Available K	306.23 kg/ha	Jackson, 1973
Iron	6.89 ppm	Lindsay and Norvell, 1978
Zinc	0.89 ppm	Lindsay and Norvell, 1978
Dehydrogenase activity	84 µg TPF g^{-1} soil $24h^{-1}$	Casida *et al.* (1964)
Fluorescein diacetate (FDA) hydrolysis	45 µg FDA g^{-1} soil $0.5h^{-1}$	Schnurer and Rosswall, (1982)

3.6.2.1 Soil reaction (pH)

pH of the soil samples was determined in 1:2.5 soil water suspension by using pH meter with glass electrode (Jackson, 1973).

3.6.2.2 Electrical conductivity (EC)

The electrical conductivity was determined in 1:2.5 soil water extract with help of a digital conductivity meter and results were expressed in dS m^{-1} (Jackson, 1973).

3.6.2.3 Available nitrogen

The available nitrogen content in the soil was estimated by alkaline permanganate method (Subbiah and Asija, 1956). A known amount of soil (5 g) was added to 0.32 % alkaline $KMnO_4$ and 2.5 % NaOH and the contents were distilled. The ammonia liberated was absorbed in boric acid and mixed indicator solution. Soil nitrogen was estimated by titrating the above mixture against standard sulphuric acid till the bluish green colour of the solution changed to pink colour. Available N was expressed in kg ha^{-1}.

3.6.2.4 Available phosphorus

For assessment of available phosphorus, the soil was extracted with Olsen's extractant (0.5 N NaHCO3 with pH 8.5). The phosphorus content in the extract was recorded with spectrophotometer at 420 nm and were expressed in kg P_2O_5 ha^{-1}.

3.6.2.5 Available potassium

The available potassium (K) in the soil was estimated by extracting the soil with 1N neutral ammonium acetate and potassium content in the extract was obtained by using flame photometer (Jackson, 1973). The quantity was calculated and expressed as kg K_2O ha^{-1}.

3.6.2.6 Iron and zinc

The available micronutrients (Fe, Zn) were extracted from the soil sample by using Diethylene triamine penta acetic acid (DTPA) extraction solution. The soil to solution ratio taken was 1:2 and the mixture was shaken for 2 hours (Lindsay and Norvell, 1978). The mixture was filtered and the clear aliquot was used to determine available Fe and Zn with the help of Atomic Absorption Spectrophotometer (AAS). The results were expressed in ppm.

3.6.2.7 Soil dehydrogenase activity

Estimation of soil dehydrogenase activity was carried out by using 2,3,5-triphenyl tetrazolium chloride (TTC) as substrate solution as described by Casida *et al.* (1964). To

5 g of soil sample, 0.2 g $CaCo_3$, 1ml of 3% TTC solution and 2.5 ml of distilled water were added and incubated for 30℃ for 24 hrs. Later, 10 ml of methanol was added and the contents were filtered using filter paper by adding methanol until the red colour disappears on the filter paper. The intensity of the red color was measured using spectrophotometer at 485 nm. TPF produced was determined quantitatively by using TPF dissolved in methanol as standard.

3.6.2.8 Fluorescein diacetate (FDA) hydrolysis

Fluorescein diacetate hydrolysis was carried by the method given by Schnurer and Rosswall, (1982). To 1 g of soil sample 7.5 ml of 60mM potassium phosphate buffer of pH 7.6 was added followed by addition of 0.1 ml of FDA solution. The contents were kept on shaker for 30 mins at 25 ℃ and to it 7.5 ml of extractant (chloroform: methanol; 2:1) was added. The soil suspension was centrifuged at 8000 rpm for 5 min. The supernatant was taken in the cuvette and the absorbance of filtrate was read with spectrophotometer at 490 nm wavelength.

3.6.3 Inoculation of bacterial consortium to rice seeds

3.6.3.1 Seed treatment

The seeds of rice were surface sterilized with 0.15% $HgCl_2$ for 1min, followed by 70% ethanol for 1 min and finally washed with distilled water. The seeds were then soaked in the culture and its consortia [containing three organisms in equal proportions and having optical density which was set to OD-1 ($1x10^7$ CFU/ml)] at 600 nm for 24 hrs. Later the culture was drained from seeds and the seeds were sown in trays filled with soil for germination.

3.6.3.2 Seedling root dip

After 21 days, the seedlings were removed from trays and washed properly to remove soil adhering to roots. The roots of the seedlings were dipped in culture (same as discussed in 3.6.3.1) for 30 mins and these seedlings were later planted in pots with 2-3seedlings per pot.

3.6.4 Details of the pot culture experiment

Crop	:	Rice (*Oryza sativa*)
Variety	:	Telangana sona (RNR-15048)
Sowing date	:	25th February 2022
Harvest date	:	21st July 2022
Pot size	:	15 x 15 cm
Design	:	Completely randomized (CRD)
Replications	:	3
Treatments	:	14

Treatments

T1: Control

T2: 100% RDF

T3: 100% RDF + Bacterial consortia partner 1 (Seed treatment)

T4: 100 % RDF + Bacterial consortia partner 2 (Seed treatment)

T5: 100% RDF + Bacterial consortia partner 3 (Seed treatment)

T6: 100% RDF + Bacterial consortia partner 1(Seedling root dip)

T7: 100% RDF + Bacterial consortia partner 2(Seedling root dip)

T8: 100% RDF + Bacterial consortia partner 3 (Seedling root dip)

T9: 100% RDF + Bacterial consortia partner 1(Seed treatment + Seedling root dip)

T10: 100% RDF + Bacterial consortia partner 2 (Seed treatment + seedling root dip)

T11: 100% RDF + Bacterial consortia partner 3 (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

3.6.5 Plant morphological traits and yield parameters

3.6.5.1 Root length

Root length at active tillering and at harvest was measured by uprooting the plants from the pots of all replications and was expressed in cm.

3.6.5.2 Shoot length

The shoot length was calculated by measuring the total height of the plant from the base of plant to the uppermost leaf at active tillering and at harvest was expressed in cm.

3.6.5.3 Leaf area

Leaf area of each treatment at active tillering and harvest was measured with LICOR-3100 automatic leaf area meter and the values were presented as cm^2.

3.6.5.4 Plant biomass

Fresh weights of shoot and root were measured by uprooting plants from the pots of all replications at active tillering and harvest. Dry weights of plants were taken after drying the plants in oven till constant weights were obtained and were expressed in g/plant.

3.6.5.5 Tiller number per hill

In each replication the number of tillers per plant were counted and were expressed as tiller number per hill.

3.6.5.6 Panicle length

Panicle length of each plant was measured with scale and was expressed in cm.

3.6.5.7 Filled grain per panicle

Number of filled grains per panicle were counted in each treatment.

3.6.5.8 Test weight

1000 grains weight was taken after drying till constant weight was obtained and expressed in grams.

3.6.5.9 Grain yield

Grain weights were taken for each treatment after proper drying and expressed as per plant basis.

3.6.6 Plant NPK

3.6.6.1 Nitrogen (N)

The nitrogen content in plant samples was estimated by digestion with conc. H_2SO_4 for 2-3 hours followed by distillation with NaOH using Kjeldhal distillation unit. Finely powdered plant sample 0.2 g was taken into a 300ml Kjeldhal tube into which 10 ml of conc. H_2SO_4 and 3g of catalyst mixture (K_2SO_4 + $CuSO_4$ in a ratio of 5:1) was added. The tubes were placed in Kelplus digestion unit and heated at 410^oC for 2-3 hours till digestion was completed. The Kjeldhal tubes were removed from the digestion chamber and cooled to room temperature.

The Kjeldhal tube with digested contents was placed in the distillation unit. 25 ml of 4% boric acid containing mixed indicator was taken in a 250 ml conical flask and placed it under the receiver tube (dipped the receiver tube end into the boric acid). Then 15 ml of 40% NaOH was added and distillation process (running steam) was carried out for 8 minutes. After completion of distillation, conical flask was taken out titrated against 0.01 N H_2SO_4 till the colour changed from bluish green to pink colour (Piper, 1966). Nitrogen content was calculated from the titre value and was expressed in percent.

3.6.6.2 Digestion of plant samples

One gram of processed plant sample was digested with 10 ml of diacid mixture (9:4 mixture of nitric acid and perchloric acid) on a hot plate. The clear digested residue was cooled, 20 ml of distilled water was added to it and filtered. The filtrate was used for estimating P, K, Fe and Zn.

3.6.6.3 Phosphorous (P)

In the filtrate, phosphorous content was determined by Vanado-molybdo phosphoric yellow colour method using Spectrophotometer at 420nm as described by Piper (1966) and P content was expressed in percent.

3.6.6.4 Potassium (K)

Potassium content in diacid extract was determined by using flame photometer (Piper, 1966) and was expressed in percent.

3.6.6.5 Iron and Zinc

Fe and Zn content in diacid mixture was determined by using Atomic absorption Spectrophotometer (AAS) and was expressed in ppm.

3.6.7 Soil available nutrients after harvest

The soil samples were analysed for available nutrients, dehydrogenase activity and Fluorescein diacetate (FDA) hydrolysis after harvest by following the procedures same in 3.62.

3.7 STATISTICAL ANALYSIS

The data obtained was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1967), Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

Plant growth promoting bacterial strains which were isolated from rhizosphere of rice were collected from ICAR-Indian Institute of Rice Research, Rajendranagar. The isolates were purified and maintained on nutrient agar plates (Plate 4.1). The isolates were examined for their functional plant growth-promoting traits and were screened for tolerance towards various eco-physiological stress and compatibility with agrichemicals. The best isolates were evaluated for their effect on the rice plant growth, yield and the results obtained on these aspects are presented under the following headings.

4.1 CHARACTERIZING BACTERIAL ISOLATES FOR PLANT GROWTH PROMOTING PROPERTIES

4.1.1 Phosphate solubilization

Phosphate solubilization ability of bacterial isolates was performed by spot inoculating the cultures on Pikovskaya's agar media containing tricalcium phosphate (Plate 4.2) as insoluble phosphate. Among the 32 isolates tested for phosphate solubilisation, 29 isolates were able to solubilize tricalcium phosphate and the solubilisation index (S.I) and the related scores are depicted in Table 4.1. Majority of the isolates (18) had a score of 2 indicating moderate phosphate solubilizing ability while only one isolate had the score of 3 (Fig. 4.1). The highest solubilization index of 3.1 and a score of 3 was recorded by IIRRSS22-18. The maximum halo zone of 30 mm was produced by the isolate IIRRSS22-18. The least solubilization index (score of 1) was recorded by IIRRSS22-9 and R1 with S.I of 1.6. The isolates with S.I range 2-3 with score of 2 with moderate phosphate solubilization were IIRRSS22-3, 4, 11, 15, 5, 6, 22, 23, 24, P1. The isolates IIRRSS22-8, 9, 2, 12, 13, 14, 16, 17, 19, 20, 21, 7, 25, 26, 27, R1, M1, O1 showed less solubilization and were grouped in S.I range 1-2, with a score of 1. The isolates which showed no solubilization with S.I range 0-1 and a score of 0 were IIRRSS22-1, 10 and P2.

Gupta *et al.* (2022) followed a similar method for screening of isolates from rhizosphere of rice for phosphate solubilization viz., formation of halo zone around the spot of pure culture on media plates containing Pikovskaya's agar. Of the 37 isolates, similar to this study, they identified 12 isolates that produced halo zone of varying diameter indicating the phosphate solubilizing activity. Four isolates with highest solubilizing index were selected as the highest phosphate solubilizers in this study. The

main mechanism involved in solubilization of P by PSB could be the production of organic acids such as malic, acetic, succinic, lactic and citric acid (RodriGuez and Fraga, 1999).

4.1.2 Potassium solubilization

Potassium solubilization efficiency of bacterial isolates was performed by spot inoculating the bacterial cultures on Aleksandrov agar media (Plate 4.3). The bacterial isolates having the ability to solubilize insoluble K, forms halo zone around the colonies. Among the 32 isolates, 9 isolates exhibited potassium solubilization efficiency (Table 4.1). Most of the isolates (23) have not produced the halo zone indicating no potassium solubilization were given a score of 0 (Fig 4.1). The isolates which have not produced zone of solubulization with score 0 were IIRRSS22-1, 8, 9, 3, 10, 4, 11, 12, 13, 14, 16, 17, 20, 6, 7, 23, 24, 25, 26, 27, M1, O1, P2. The isolates with S.I range 1-2 with score 1 were IIRRSS22-2, 15, 18, 19, 5, 21, 22, R1 showed less solubilization. The isolates with S.I range 2-3 with score of 2 were P1 which showed moderate solubilization. The P1 has also produced maximum halo zone of 19 mm.

Potassium solubilizing bacteria (KSB) dissolve K- minerals such as illite, mica, orthoclase mainly by secreting organic acids (Friedrich *et al*., 1991). Similar results were obtained by Fatharani *et al*. (2018) who characterized the bacteria isolated from paddy rhizosphere for potassium solubilization on Aleksandrov agar media. Similarly, based on solubilization index, which is the value of clear zone around each colony, Premono *et al*. (1996), were able to select 7 isolates as potassium solubilizers.

4.1.3 Zinc solubilization

The bacterial isolates were screened for zinc solubilization by spot inoculation of culture on tris minimal media (Plate 4.4). Out of 32 isolates, 20 isolates exhibited zinc solubilization efficiency (Table 4.1). The isolates with S.I range 0-1 with score 0 were IIRRSS22-11, 14, 15, 16, 17, 20, 7, 23, 25, 26, 27 and P2 have not shown solubilization of zinc in media. A total of 15 isolates had a score of 1 with solubilization index ranging from 1-2 showed less solubilization (Fig. 4.1). The isolates with S.I range 1-2 were IIRRSS22-8, 9, 2, 3, 10, 4, 12, 13, 18, 19, 5, 6, 24 and M1, O1. The isolates IIRRSS22-1, 21, 22 and R1, P1 have shown moderate solubilization with S.I range of 2-3 (score of 2). The isolate P1 has recorded maximum halo zone of 12 mm.

Gandhi *et al*. (2014) has isolated Zn solubilizing bacteria from rhizosphere of rice on tris minimal salts media. From a total of 240 isolates, 15 isolates were selected as potential zinc solubilizing strains based on the halo zone formation around the colonies. Related results were also obtained by Upadhyay *et al*. (2022) when the bacterial isolates were inoculated on tris minimal media for formation of halo zone. Out of 62 isolates, they identified 2 isolates based on solubilizing potential.

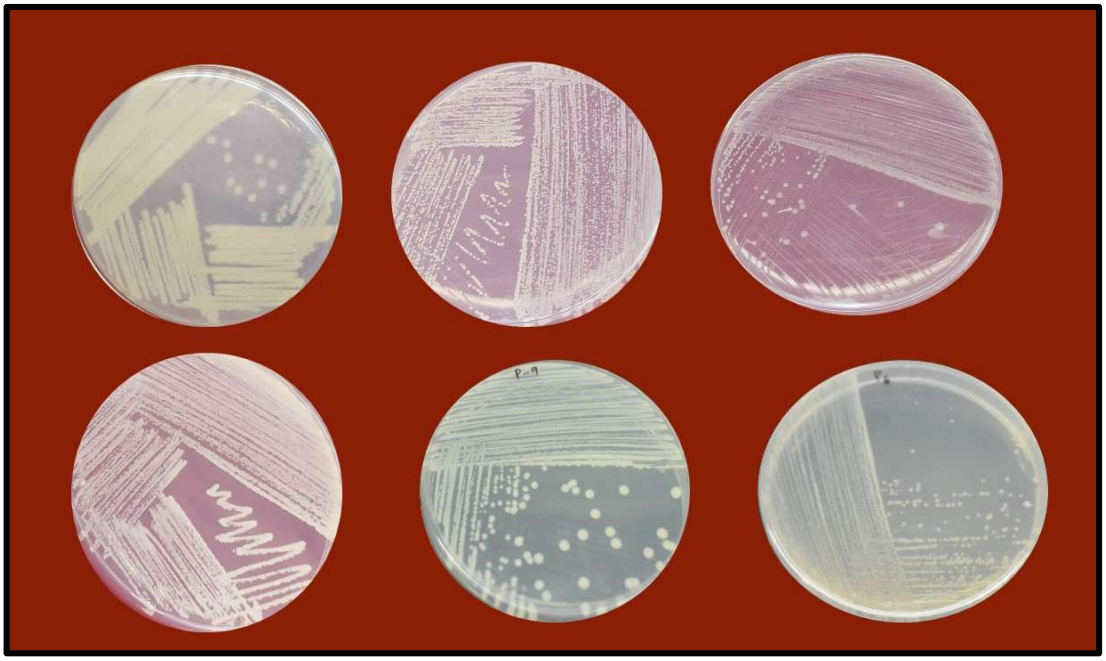


Plate 4.1 Pure cultures of bacterial isolates

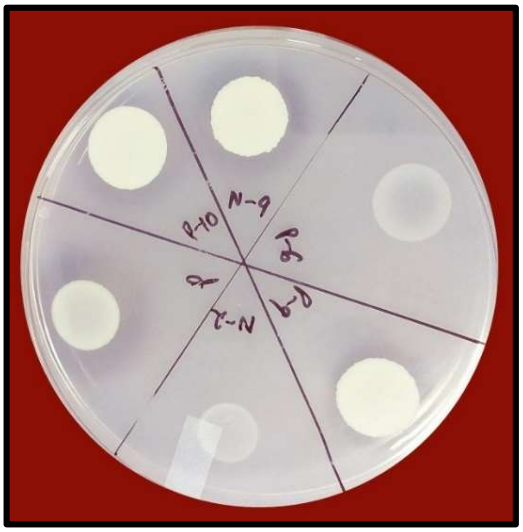


Plate 4.2 Phosphate solubilization potential of isolates on Pikovskaya's agar media

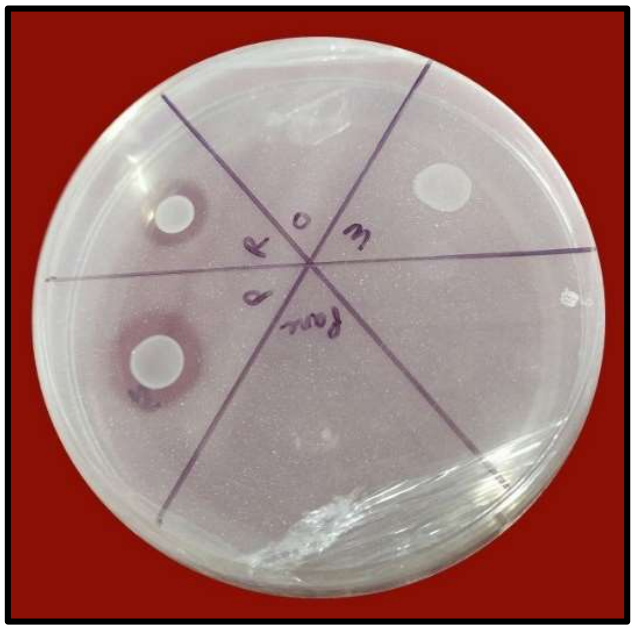


Plate 4.3 Potassium solubilization potential of isolates on Aleksandrov agar media

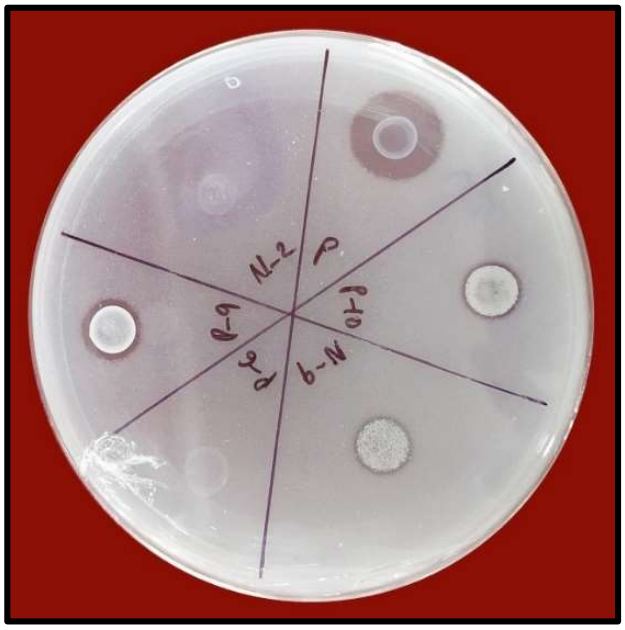


Plate 4.4 Zinc solubilization potential of isolates on tris minimal agar media

Table 4.1 Solubilization of insoluble phosphate, potassium and zinc solubilization by bacterial isolates

Isolate ID	Phosphate Halo zone (mm)	Phosphate solubilization index (S.I) *	Score	Potassium halo zone (mm)	Potassium solubilization (S.I) *	Score	Zinc halo zone (mm)	Zinc solubilization (S.I) *	Score
IIRRSS22-1	0	-	0	0	-	0	8	2.4	2
IIRRSS22-8	21	2	1	0	-	0	6	1.9	1
IIRRSS22-9	18	1.6	1	0	-	0	6	1.8	1
IIRRSS22-2	21	1.7	1	14	1.4	1	6	1.7	1
IIRRSS22-3	24	2.1	2	0	-	0	2	1.4	1
IIRRSS22-10	0	-	0	0	-	0	8	2	1
IIRRSS22-4	21	2.4	2	0	-	0	8	1.9	1
IIRRSS22-11	24	2.1	2	0	-	0	0	-	0
IIRRSS22-12	25	2	1	0	-	0	8	2	1
IIRRSS22-13	19	2	1	0	-	0	6	1.7	1
IIRRSS22-14	18	1.9	1	0	-	0	0	-	0
IIRRSS22-15	20	2.1	2	12	1.5	1	0	-	0
IIRRSS22-16	21	2	1	0	-	0	0	-	0
IIRRSS22-17	22	1.9	1	0	-	0	0	-	0
IIRRSS22-18	30	3.1	3	14	1.8	1	6	1.8	1
IIRRSS22-19	19	1.8	1	13	1.9	1	6	1.7	1
IIRRSS22-20	16	1.7	1	0	-	0	0	-	0
IIRRSS22-5	22	2.9	2	14	1.8	1	6	1.9	1
IIRRSS22-21	24	1.9	1	13	1.9	1	8	2.2	2
IIRRSS22-6	17	2.6	2	0	-	0	4	1.5	1
IIRRSS22-7	20	1.7	1	0	-	0	2	-	0
IIRRSS22-22	29	2.4	2	15	1.7	1	8	2.4	2

IIRRSS22-23	21	2.2	2	0	-	0	0	-	0
IIRRSS22-24	20	2.1	2	0	-	0	6	1.7	1
IIRRSS22-25	19	2	1	0	-	0	0	-	0
IIRRSS22-26	17	1.8	1	0	-	0	0	-	0
IIRRSS22-27	18	1.7	1	0	-	0	0	-	0
R1	18	1.6	1	13	1.5	1	10	2.3	2
P1	20	2.1	2	19	2.1	2	12	2.5	2
M1	18	1.9	1	0	-	0	2	1.3	1
O1	19	1.8	1	0	-	0	2	1.3	1
P2	0	-	0	0	-	0	0	-	0

Values are the means of three replicates

*S.I- Solubilization index

4.1.4 Siderophore production

Siderophore production of bacterial isolates was performed on CAS agar media by spot inoculating the cultures on media plates (Plate 4.5). Siderophore production of bacterial isolates was indicated by formation of yellow to orange zones around the colonies. Among 32 isolates, 23 isolates have shown siderophore production activity (Table 4.2). Majority of the isolates (14) had a score of 1 exhibiting lower siderophore production and only 2 isolates scored 3 indicating higher siderophore production (Fig.4.1). The isolates with S.I range 0-2 with score 0 were IIRRSS22-13, 19, 21, 24, 26, 27 and M1, O1, P2 showed no formation of orange zones. The isolates with S.I range 2-3 with score 1 were IIRRSS22-1, 8, 9, 2, 3, 10, 4, 16, 17, 18, 20, 5, 22, 23 have shown less production of orange zones around the colonies. The isolates with S.I range 3-4 with score 2 were IIRRSS22-11, 12, 14, 15, 25, R1, P1 showed moderate solubilization. The isolates IIRRSS22-6, 7 have exhibited high production of orange zones with S.I range above 4 (score of 3).

In the same way, Abirammi *et al*. (2018) identified siderophore producting isolates from 64 bacteria isolated from soils of different crops based on formation of yellow to orange zones around bacterial colonies on CAS media plates. The siderophore production index of these isolates ranged from 1.03 to 1.70.

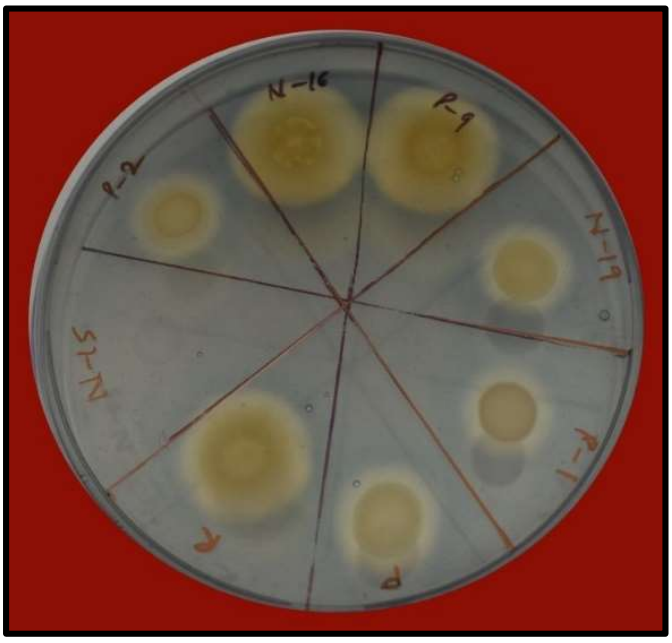
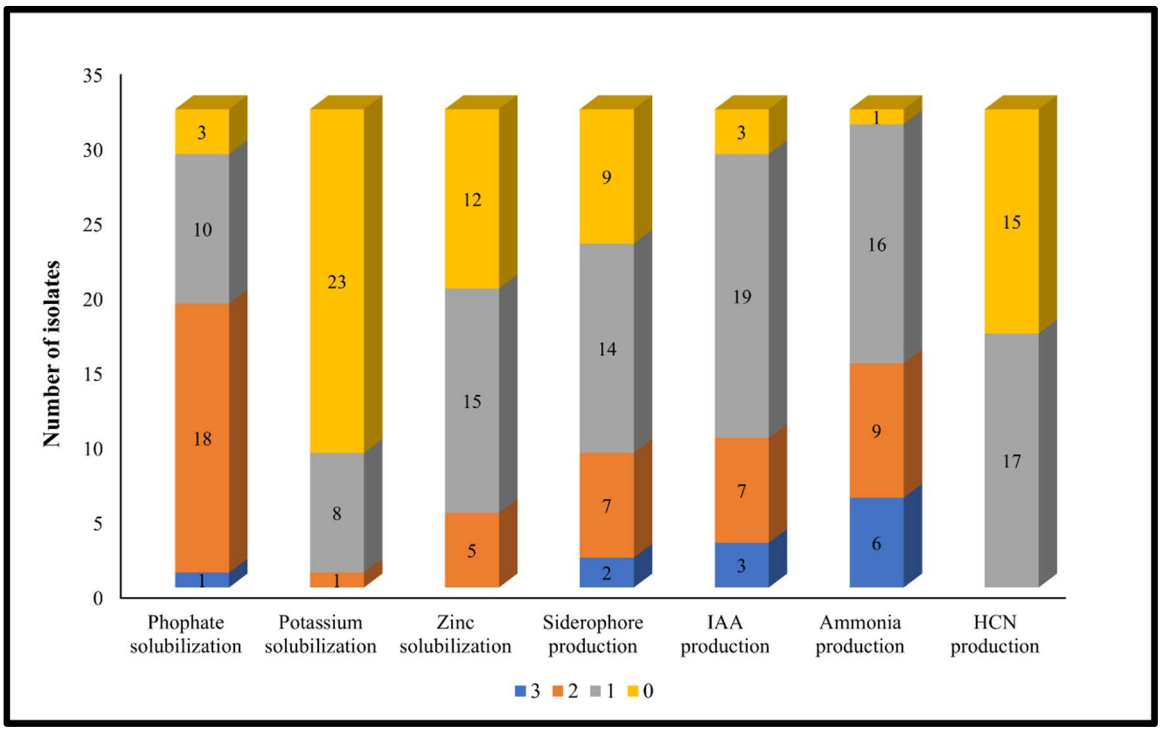


Plate 4.5 Siderophore production potential of isolates on CAS agar media



Legend 3, 2 1 and 0 are scores indicating high, moderate, low and no PGP activity of isolates

Fig. 4.1 Number of isolates exhibiting plant growth promoting (PGP) traits

Table 4.2 Siderophore production by bacterial isolates

Isolate ID	Yellow zone (mm)	Siderophore production (S.P.I) **	Score
IIRRSS22-1	20	3	1
IIRRSS22-8	17	2.2	1
IIRRSS22-9	22	2.5	1
IIRRSS22-2	20	2.9	1
IIRRSS22-3	12	2.5	1
IIRRSS22-10	19	2.5	1
IIRRSS22-4	14	2.8	1
IIRRSS22-11	23	3.2	2
IIRRSS22-12	17	3.4	2
IIRRSS22-13	0	-	0
IIRRSS22-14	28	3.8	2
IIRRSS22-15	23	3.1	2
IIRRSS22-16	17	2.9	1
IIRRSS22-17	16	3	1
IIRRSS22-18	17	2.9	1
IIRRSS22-19	0	-	0
IIRRSS22-20	17	2.6	1
IIRRSS22-5	16	3	1
IIRRSS22-21	0	-	0
IIRRSS22-6	31	4.1	3
IIRRSS22-7	29	4.3	3
IIRRSS22-22	18	2.9	1
IIRRSS22-23	16	2.6	1
IIRRSS22-24	0	-	0
IIRRSS22-25	23	3.3	2
IIRRSS22-26	0	-	0
IIRRSS22-27	0	-	0
R1	30	4	2
P1	25	3.3	2
M1	0	-	0
O1	0	-	0
P2	0	-	0

Values are the means of three replicates

*S.P.I- Siderophore production index

4.1.5 IAA production

The bacterial isolates were screened for IAA production by inoculating the isolates in nutrient broth amended with tryptophan. After incubation, orthophosphoric acid and Salkowski reagent were added for pink colour development (Plate 4.6). Majority of the isolates (19) had produced IAA in low quantity and only 3 isolates have not developed pink colour indicating no IAA production with score 0 (Fig. 4.1). Based on the intensity of pink colour the scores were given for IIRRSS22-1, 17, 19 as 3 (high production), for isolates IIRRSS22-8, 10, 4, 5, 7, 23, 26 the scores were given as 2 (moderate production). The isolates with score 1 were IIRRSS22-9, 2, 3, 11, 12, 13, 14, 15, 16, 18, 20, 21, 6, 24, 25, 27 and R1, P1, M1 showed less production. Three isolates IIRRSS22-22 and O1, P2 have shown negative results for IAA production (Table 4.3).

The microorganisms isolated from the rhizosphere of various crops have the ability to produce IAA as secondary metabolites due to the rich supply of substrates exuded by the roots of plants. Indole acetic acid helps in the production of longer roots with more root hairs and lateral roots which are involved in nutrient uptake (Datta and Basu, 2000).

Comparable results of IAA production by rhizobacterial isolates were obtained by Anisoke *et al*. (2018). All the bacterial isolates produced varied concentrations of IAA which were indicated by the intensity of pink colour development. These IAA producing organisms were identified as *Staphylococcus* sp, *Pseudomonas* sp, *Micrococcus* sp and *Bacillus* sp.

4.1.6 Ammonia production

The bacterial isolates were characterized for ammonia production by inoculating the isolates in peptone water and adding Nessler's reagent after 72 hrs for change of colour from yellow to brown (Plate 4.7). Among the 32 isolates, 31 isolates have produced ammonia which was indicated by colour change (Table 4.3). Most of the isolates (16) produced less change of colour and only 1 isolate has not produced any colour change upon addition of Nessler's reagent (Fig.4.1). The isolates IIRRSS22-1, 9, 2, 24, 25, 26 have produced ammonia in high quantity and were given scores as +++(3). The isolates IIRRSS22-3, 10, 13, 14, 15, 22 and P1, M1, O1 have produced ammonia in moderate quantity were given a score of ++(2). The isolates with score + (1) were IIRRSS22-8, 4, 11, 16, 17, 18, 19, 20, 5, 21, 6, 7, 23, 27 and R1, P2 have produced ammonia in less quantity. One isolate IIRRSS22-12 has tested negative (-) for ammonia production.

Ammonia plays an important role in the biocontrol of wide range of fungal pathogens causing diseases in plants. It acts as metabolic inhibitor and at the same time provides nitrogen directly to the plants (Orhan *et al*., 2016)

Similar results of ammonia production by bacteria isolated from the rhizosphere of rice were recorded by Sahu *et al*. (2017). Of the 101 isolates which were screened for ammonia production, 78 isolates produced ammonia in varying quantities.

4.1.7 HCN production

HCN production of bacterial isolates was performed by inoculating them in nutrient agar media containing glycine and a sterilized filter paper saturated with solution of picric acid (0.5%) and sodium carbonate (2%) was placed on upper lid of petri plate. The indication of the hydrogen cyanide production was the change of filter paper color from yellow to light brown or reddish brown (Plate 4.8). A majority of the isolates (17) have developed light brown colour on filter paper indicating less production of HCN and 15 isolates have not produced any HCN hence scored as 0 (Fig.4.1). Out of 32 isolates the isolates IIRRSS22-1, 8, 3, 4, 11,12 13, 14, 16, 17, 19, 21, 6, 7, 24, 25, 27 has produced HCN in minimal quantity as the colour change of filter paper was less and these isolates were given score 1. The isolates IIRRSS22- 9, 2, 10, 15, 18, 20, 5, 22, 23, 26, R1, P1, M1, O1, P2 has not produced any colour change on filter paper, hence the score was given as 0 (Table 4.3).

HCN is an organic compound and a major component of antibiotics produced by bacteria which is a suppressor of weeds (Pettan *et al*., 2002) and insects (Glick., 1995).

Related results were obtained by Adnan *et al*. (2016) who isolated various plant growth-promoting bacteria from the rhizosphere of rice. These isolates when screened for HCN production, only 4 isolates (CG4, CG9, CG11 and CG18) have produced HCN out of total 22 isolates.

Plate 4.6 Evaluation of Indole acetic acid production potential of isolates

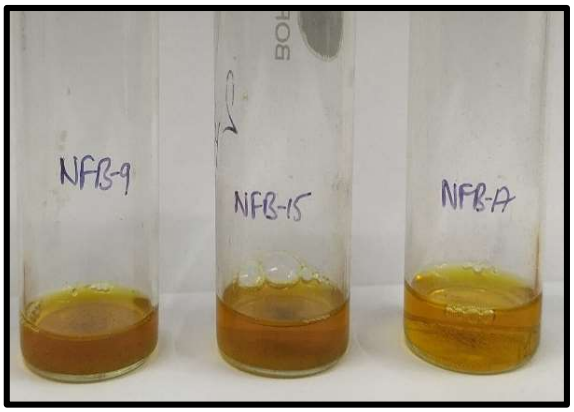


Plate 4.7 Ammonia production potential of isolates in peptone broth

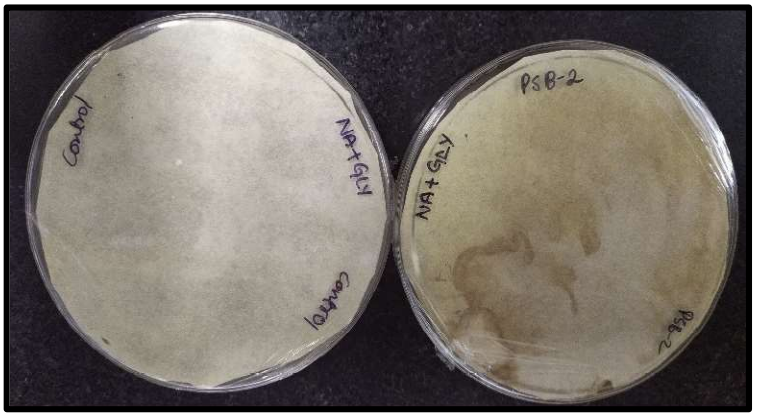


Plate 4.8 Assessment of hydrogen cyanide production potential of bacterial isolates

Table 4.3. Screening of bacterial isolates for IAA, Ammonia production and HCN production

S.No	Isolate ID	Indole acetic acid production	Score	Ammonia production	Score	HCN production	Score
1	IIRRSS22-1	+++	3	+++	3	1	1
2	IIRRSS22-8	++	2	+	1	1	1
3	IIRRSS22-9	+	1	+++	3	-	0
4	IIRRSS22-2	+	1	+++	3	-	0
5	IIRRSS22-3	+	1	++	2	1	1
6	IIRRSS22-10	++	2	++	2	-	0
7	IIRRSS22-4	++	2	+	1	1	1
8	IIRRSS22-11	+	1	+	1	1	1
9	IIRRSS22-12	+	1	-	0	1	1
10	IIRRSS22-13	+	1	++	2	1	1
11	IIRRSS22-14	+	1	++	2	1	1
12	IIRRSS22-15	+	1	++	2	-	0
13	IIRRSS22-16	+	1	+	1	1	1
14	IIRRSS22-17	+++	3	+	1	1	1
15	IIRRSS22-18	+	1	+	1	-	0
16	IIRRSS22-19	+++	3	+	1	1	1
17	IIRRSS22-20	+	1	+	1	-	0
18	IIRRSS22-5	++	2	+	1	-	0
19	IIRRSS22-21	+	1	+	1	1	1
20	IIRRSS22-6	+	1	+	1	1	1
21	IIRRSS22-7	++	2	+	1	1	1
22	IIRRSS22-22	-	0	++	2	-	0
23	IIRRSS22-23	++	2	+	1	-	0
24	IIRRSS22-24	+	1	+++	3	1	1

25	IIRRSS22-25	+	1	+++	3	1	1
26	IIRRSS22-26	++	2	+++	3	-	0
27	IIRRSS22-27	+	1	+	1	1	1
28	R1	+	1	+	1	-	0
29	P1	+	1	++	2	-	0
30	M1	+	1	++	2	-	0
31	O1	-	0	++	2	-	0
32	P2	-	0	+	1	-	0

+++ - Good growth, ++ moderate growth, + less growth, - no growth

4.2 CHARACTERIZATION OF BACTERIAL ISOLATES BASED ON ECO-PHYSIOLOGICAL STRESS TOLERANCE

4.2.1 Screening of bacterial isolates for abiotic stress tolerance

4.2.1.1 Temperature tolerance

The bacterial isolates were screened for temperature tolerance by incubating the bacterial cultures at different temperatures 4, 15, 25, 45℃ for 3 days (Plate 4.10). Based on the growth of bacteria at each temperature, scores were assigned (Table 4.3).

At 4℃, 12 isolates not exhibiting growth were given a score of 0 and only 4 isolates showed moderate growth with a score of 2 (Fig. 4.2). Out of 32 isolates, 20 cultures were able to grow at 4℃ (Table 4.4). Nine isolates IIRRSS22-2, 3, 12, 20, 5, 6, 23, 24, 26 exhibited good growth, the isolates IIRRSS22-14, 25, P1, O1 exhibited moderate growth and the isolates IIRRSS22-8, 10, 11, 16, 19, 7, R1 have shown less growth. The isolates IIRRSS22-1, 9, 4, 13, 15, 17, 18, 21, 22, 27, M1, P1 have not shown any growth at 4℃.

Most of the isolates (14) have shown growth were given a score of 3 and only 4 isolates each have exhibited moderate and less growth at 15℃ with scores of 2, 1 respectively (Fig. 4.2). The isolates IIRRSS22-1, 9, 2, 3, 10, 4, 13, 16, 20, 5, 6, 22, 25, P1 have shown good growth, the isolates IIRRSS22-8, 7, R1, O1 has exhibited moderate growth and the isolates IIRRSS22-11, 12, 14, 19 exhibited less growth. A total of 10 isolates IIRRSS22-15, 17, 18, 21, 23, 24, 26, 27, M1, P2 have not shown any growth at 15℃ (Table 4.4).

All the 32 isolates have grown at 25℃ (Table 4.4 and Fig. 4.2).

At 45℃, 20 isolates which have not shown growth were given a score of 0 and only 2 isolates exhibiting good growth were given a score of 3 (Fig. 4.2). Among the 32 isolates, 12 isolates were able to grow at 45℃ (Table 4.4). The isolates IIRRSS22-8 and O1 have exhibited good growth, the isolates IIRRSS22-10, 11, 6 have shown moderate growth and the isolates IIRRSS22-9, 3, 4, 7, 24, 27, R1 have shown less growth. And about 20 isolates IIRRSS22-1, 2, 12, 13, 14, 15, 16, 17, 18, 19, 20, 5, 21, 22, 23, 25, 26, P1, M1, P2 have not shown any growth at 45℃.

Tsegaye *et al*. (2019) recorded relatable results when the bacteria isolated from the rhizosphere of tef (*Eragrostis tef*) crop were subjected to temperature tolerance by incubating the bacterial isolates at 4℃, 20℃, 30℃, 50℃, 60℃. Out of total 200 isolates,

all isolates have grown at 20℃ and 30℃, 27% isolates have grown at 4℃, 25.5% grew at 40℃, 3.5% isolates grew at 50℃ and 60℃.

4.2.1.2 Salinity tolerance

Salinity tolerance of bacterial isolates was assessed by streaking the isolates on tryptone soy agar plates containing NaCl at different concentrations 4, 6, 8% NaCl (Plate 4.9). Based on the growth of bacterial isolates at all three concentrations, scores were assigned to isolates (Table 4.4).

Majority of the isolates (12) with score 0 have not exhibited any growth and only 3 isolates have shown less growth at 4% NaCl concentration (Fig.4.2). Out of 32 isolates, 20 isolates IIRRSS22-1, 8, 9, 2, 3, 10, 4, 11, 12, 16, 20, 5, 6, 7, 24, 25, 27, R1, P1, O1 have grown at 4% NaCl concentration (Table 4.4). The isolates IIRRSS22-13, 14, 15, 17, 18, 19, 21, 22, 23, 26, M1, and P2 have not shown any growth at 4% NaCl concentration.

At 6% NaCl concentration, 17 isolates have exhibited no growth with a score of 0 and only 2 isolates have exhibited good growth with a score of 3 (Fig. 4.2). Among 32 isolates screened for salinity tolerance, 15 isolates (IIRRSS22-1, 8, 2, 3, 10, 11, 12, 5, 6, 7, 24, 27, R1, P1, O1) have shown growth at 6% NaCl concentration (Table 4.4). A total of 17 isolates IIRRSS22- 9, 4, 13, 14, 15, 16, 17, 18, 19, 21, 20, 22, 23, 25, 26, M1, P2 have shown no growth at 6% NaCl concentration.

Most of the isolates (24) have not shown growth and were given score of 0 and only 1 isolate has shown good and moderate growth at 8% NaCl concentration (Fig. 4.2). At 8% NaCl concentration, 8 isolates (IIRRSS22-8, 3, 10, 12, 6, 7, 27, R1) were able to grow (Table 4.4). The isolates IIRRSS22-1, 9, 2, 4, 11, 13, 14, 15, 16, 17, 18, 19, 20, 5, 21, 22, 23, 24, 25, 26, P1, M1, O1, P2 has not exhibited any growth at 8% NaCl concentration.

Related results of salinity tolerance were obtained by Naser *et al*. (2022) who screened the bacteria isolated from rhizospheric soils of rice plants by inoculating bacteria in media amended with different salt concentrations (2-12% NaCl). Among 41 isolates, three isolates have shown better growth at 10 and 12% NaCl concentrations were selected.

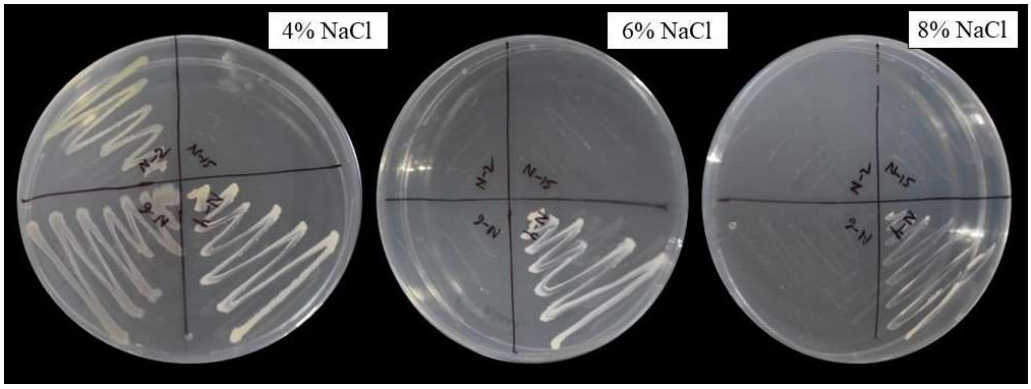


Plate 4.9 Screening for salinity tolerance of bacterial isolates at 4%, 6% and 8% NaCl concentrations

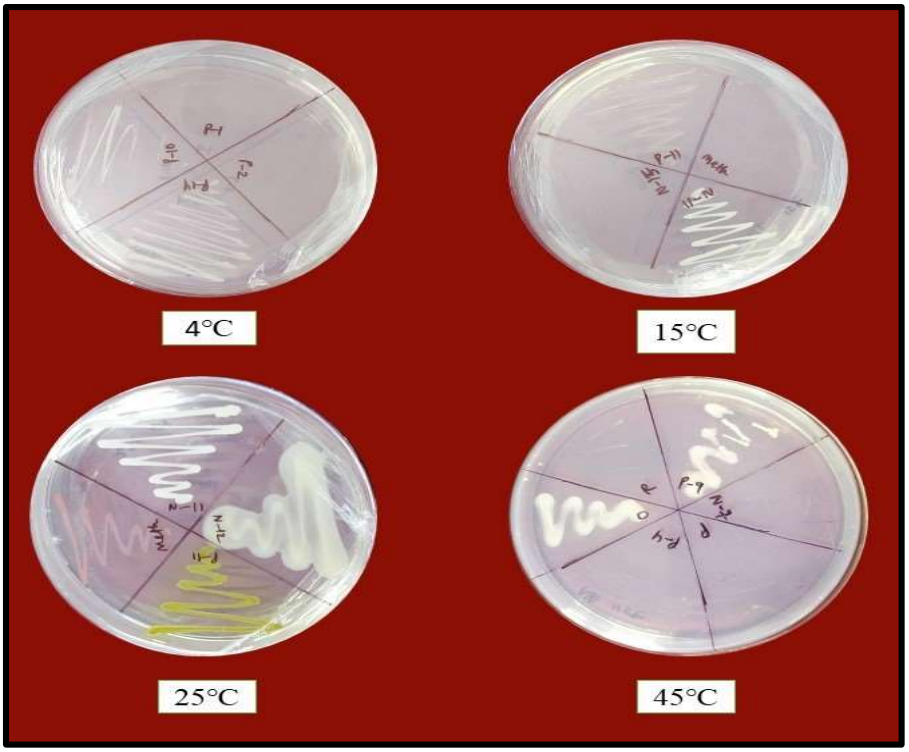


Plate 4.10 Screening for temperature tolerance of bacterial isolates at 4, 15, 25 and 45°C.

Table 4.4. Screening of bacterial isolates for temperature tolerance and salinity tolerance

S.No	Isolate ID	Temperature tolerance				Salinity tolerance			
		4℃	15℃	25℃	45℃	4%	6%	8%	Score for salinity tolerance
1	IIRRSS22-1	0	3	3	0	+	+	-	2
2	IIRRSS22-8	1	2	3	3	+	+	+	3
3	IIRRSS22-9	0	3	3	1	+	-	-	1
4	IIRRSS22-2	3	3	3	0	+	+	-	2
5	IIRRSS22-3	3	3	3	1	+	+	+	3
6	IIRRSS22-10	1	3	3	2	+	+	+	3
7	IIRRSS22-4	0	3	3	1	+	-	-	1
8	IIRRSS22-11	1	1	3	2	+	+	-	2
9	IIRRSS22-12	3	1	3	0	+	+	+	3
10	IIRRSS22-13	0	3	3	0	-	-	-	0
11	IIRRSS22-14	2	1	3	0	-	-	-	0
12	IIRRSS22-15	0	0	3	0	-	-	-	0
13	IIRRSS22-16	1	3	3	0	+	-	-	1
14	IIRRSS22-17	0	0	3	0	-	-	-	0
15	IIRRSS22-18	0	0	3	0	-	-	-	0
16	IIRRSS22-19	1	1	3	0	-	-	-	0
17	IIRRSS22-20	3	3	3	0	+	-	-	1
18	IIRRSS22-5	3	3	3	0	+	+	-	2
19	IIRRSS22-21	0	0	3	0	-	-	-	0
20	IIRRSS22-6	3	3	3	2	+	+	+	3
21	IIRRSS22-7	1	2	3	1	+	+	+	3
22	IIRRSS22-22	0	3	3	0	-	-	-	0
23	IIRRSS22-23	3	0	3	0	-	-	-	0
24	IIRRSS22-24	3	0	3	1	+	+	-	2

25	IIRRSS22-25	2	3	3	0	+	-	-	1
26	IIRRSS22-26	3	0	3	0	-	-	-	0
27	IIRRSS22-27	0	0	3	1	+	+	+	3
28	R1	1	2	3	1	+	+	+	3
29	P1	2	3	3	0	+	+	-	2
30	M1	0	0	3	0	-	-	-	0
31	O1	2	2	3	3	+	+	-	2
32	P2	0	0	3	0	-	-	-	0

Temperature tolerance: 3 - Good growth, 2 - moderate growth, 1 - less growth, 0 - no growth

Salinity tolerance: the isolates exhibiting growth at all three concentrations were given score 3, isolates showing growth at 4% and 6% NaCl were scored 2, isolates growing at only 4% were scored as 1 and isolates not showing growth at all three concentrations were given a score of 0.

4.2.1.3 Drought tolerance

Drought tolerance of bacteria was assessed by inoculating the bacterial isolates in nutrient broth amended with polyethylene glycol (PEG) at different concentrations for creating varied osmotic potential of 0Mpa, - 0.05 Mpa, - 0.30Mpa and - 0.73 MPa. The results obtained shows that with increase in PEG concentration there was decrease in optical density of the bacterial cultures (Table 4.5). Based on the optical density at -0.73MPa, bacterial isolates were classified as highly sensitive, sensitive, tolerant and highly tolerant (Alikhani and Mohamadi, 2010).

Most of the isolates (12) have shown high sensitivity for the PEG and have not shown any growth were given a score of 0 and only 3 isolates have shown less growth with a score of 1 (Fig. 4.2). Among the 32 isolates screened for drought tolerance, 12 isolates IIRRSS22-8, 10, 4, 17, 19, 23, 24, 26, 27, M1, O1, P2 were classified as highly sensitive (O.D- <0.3), the isolates IIRRSS22-1, 6, 22 were classified as sensitive (O.D-0.3-0.4) and the isolates IIRRSS22-11, 13, 14, 15, 18, 21, 25 have been classified as tolerant (O.D-0.4-0.5). The highly tolerant isolates were IIRRSS22-9, 2, 3, 12, 16, 20, 5, 7, R1, P1 with O.D values (>0.5) (Table 4.5).

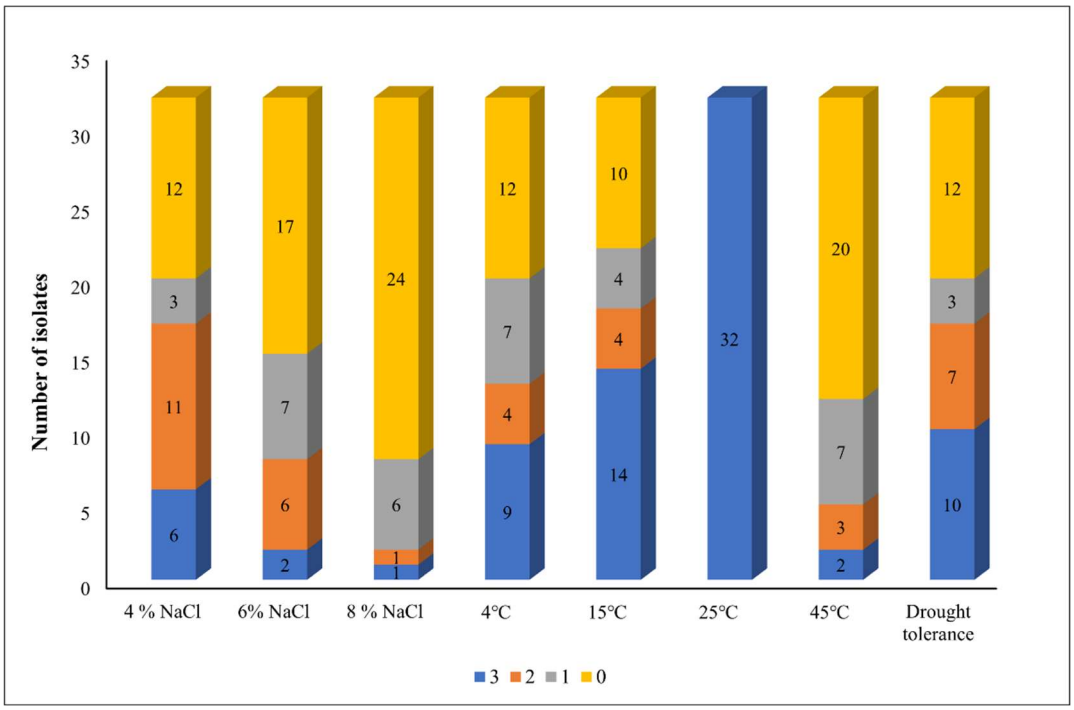
Drought stress conditions under *in vitro* conditions can be created by adding polyethylene glycol to the bacterial culture medium at varied concentrations for creation of osmotic potential (Michel *et al*., 1973). Tolerance of bacterial isolates for drought conditions in this study may be due to production of exopolysaccharide or formation of biofilm (Kavamura *et al*., 2013). A similar study was conducted by Susilowati *et al*. (2018) who isolated rhizospheric bacteria from soils of soybean and screened 11 isolates for drought tolerance, among which 9 isolates were highly tolerant.

Table 4.5 Drought tolerance of bacterial isolates at different PEG concentrations

S.No	Isolates	Water potential				Category	Total score
		0 MPa	- 0.05 Mpa	- 0.30Mpa	- 0.73 MPa		
1	IIRRSS22-1	0.428	0.431	0.417	0.347	Sensitive	1
2	IIRRSS22-8	0.079	0.049	0.026	0.016	Highly sensitive	0
3	IIRRSS22-9	1.784	1.664	1.687	1.165	Highly tolerant	3
4	IIRRSS22-2	1.551	1.349	0.901	0.864	Highly tolerant	3
5	IIRRSS22-3	1.183	0.803	0.794	0.737	Highly tolerant	3
6	IIRRSS22-10	0.178	0.12	0.054	0.055	Highly sensitive	0
7	IIRRSS22-4	0.177	0.208	0.174	0.155	Highly Sensitive	0
8	IIRRSS22-11	0.681	0.668	0.52	0.43	Tolerant	2
9	IIRRSS22-12	0.762	0.588	0.507	0.501	Highly tolerant	3
10	IIRRSS22-13	0.561	0.653	0.542	0.44	Tolerant	2
11	IIRRSS22-14	0.876	0.5	0.77	0.495	Tolerant	2
12	IIRRSS22-15	0.986	0.893	0.54	0.459	Tolerant	2
13	IIRRSS22-16	0.577	0.956	0.804	0.78	Highly tolerant	3
14	IIRRSS22-17	0.288	0.327	0.298	0.249	Highly sensitive	0
15	IIRRSS22-18	0.588	0.547	0.499	0.487	Tolerant	2
16	IIRRSS22-19	0.373	0.365	0.347	0.259	Highly sensitive	0
17	IIRRSS22-20	0.828	0.723	0.637	0.575	Highly tolerant	3
18	IIRRSS22-5	0.706	0.701	0.677	0.553	Highly tolerant	3
19	IIRRSS22-21	0.64	0.544	0.483	0.421	Tolerant	2
20	IIRRSS22-6	0.464	0.421	0.389	0.351	Sensitive	1
21	IIRRSS22-7	0.7	0.744	0.658	0.548	Highly tolerant	3
22	IIRRSS22-22	0.338	0.193	0.319	0.313	Sensitive	1
23	IIRRSS22-23	0.19	0.169	0.211	0.183	Highly Sensitive	0

24	IIRRSS22-24	0.542	0.226	0.23	0.197	Highly Sensitive	0
25	IIRRSS22-25	0.678	0.623	0.455	0.43	Tolerant	2
26	IIRRSS22-26	0.225	0.258	0.197	0.195	Highly Sensitive	0
27	IIRRSS22-27	0.194	0.156	0.112	0.089	Highly Sensitive	0
28	R1	1.234	1.123	1.048	0.991	Highly tolerant	3
29	P1	1.589	1.345	1.227	0.979	Highly tolerant	3
30	M1	0.034	0.031	0.029	0.022	Highly Sensitive	0
31	O1	0.255	0.251	0.163	0.156	Highly Sensitive	0
32	P2	0.056	0.089	0.065	0.051	Highly Sensitive	0

O.D<0.3- highly sensitive-0, O.D 0.3-0.4- sensitive-1, O.D 0.4-0.5- tolerant-2, O.D >0.5- highly tolerant-3



Legend 3, 2 1 and 0 are scores indicating high, moderate, low and no tolerance of isolates to abiotic stresses

Fig. 4.2 Number of isolates exhibiting abiotic stress tolerance

4.2.2 Compatibility of bacterial isolates with agrichemicals

Plant growth promotion using bacterial isolates will be successful when these bacterial isolates are compatible with agrochemicals that are used commonly for cultivation under field conditions. Therefore, the present study was undertaken to evaluate the compatibility of PGPR isolates with agrichemicals like fertilizers (urea, single super phosphate (SSP) and murate of potash (MOP), insecticides (Cartap, Ferterra and Thiamethaxom), herbicides (Pretilachlor, Bispyribac sodium) and fungicides (Carbendazim and Mancozeb) at C-0, C1-50%, C2-100% and C3-150% of the recommended dose under *in vitro* condition.

4.2.2.1 Compatibility with fertilizers

The PGPR isolates were screened for their compatibility with three commonly used fertilizers - Urea, SSP, MOP using disc diffusion method (Plate 4.11).

Out of 32 isolates, a total of 22 isolates with a score of 3 have not shown any inhibition of growth and only 2 isolates showing less tolerance to urea were given a score of 1 (Fig. 4.3). The isolates IIRRSS22-1, 8, 9, 2, 3, 10, 4, 11, 12, 14, 16, 20, 5, 6, 7, 22, 24, 25, R1, P1, M1, O1 did not have inhibition zones at all concentrations of urea, hence they were considered as highly tolerant. The isolates IIRRSS22- 13, 19 have produced inhibition zones at C_2 and C_3 concentrations and were therefore classified as less tolerant. The isolates IIRRSS22- 15, 17, 18, 21, 23, 26, 27, P2 have shown inhibition zones at C1, C2 and C3 concentrations of urea, therefore they were classified as not tolerant/susceptible (Table 4.6).

Most of the isolates (19) had a score of 3 showing high tolerance and only 2 isolates with a score of 2 showing moderate tolerance to SSP is presented in Fig.4.3. Among 32 isolates, 19 isolates IIRRSS22-1, 8, 9, 2, 3, 10, 4, 11, 12, 14, 20, 5, 6, 7, 24, 25, R1, P1, O1 have not shown any inhibition zone against SSP; therefore, these isolates were grouped as highly tolerant. The isolates IIRRSS22-17, 22 have recorded inhibition zone formation at C3 concentration of SSP and are therefore grouped as moderately tolerant. The isolates IIRRSS22- 13, 15, 16, 18, 19, 21, 23, 26, 27, M1, P2 have produced inhibition zone at C1, C2 and C3 concentration of recommended dose of SSP and were hence grouped as not tolerant (Table 4.6).

Out of 32 isolates screened for compatibility with MOP, 18 isolates IIRRSS22-1, 9, 2, 3, 10, 4, 11, 12, 14, 20, 5, 6, 7, 24, 25, R1, P1, O1 have shown high tolerance at all

concentrations of fertilizer were given score 3 (Fig. 4.3). The isolate IIRRSS2-8, 16 have shown moderate growth as there was formation of inhibition zone at C3 concentration. The isolates IIRRSS22- 13, 15, 17, 18, 19, 21, 22, 23, 26, 27, M1, P2 have not shown growth at C1, C2 and C3 concentrations hence grouped as not tolerant (Table 4.6).

Related results of compatibility of plant growth-promoting bacteria to different concentrations of inorganic fertilizers such as urea, rock phosphate and muriate of potash which were commonly used in tea plantations were studied by Bagyalakshmi *et al*. (2012). In this study, PGPR such as *Azospirillum lipoferum*, *Psuedomonas putida*, *Burkholderia cepacia* were used for compatibility studies. At higher concentrations of the chemicals used in this study, they reported a decrease in growth of bacteria.

4.2.2.2 Compatibility with insecticides

The PGPR isolates were screened for compatibility with insecticides cartap, ferterra, thiamethaxom using disc diffusion method (Plate 4.12).

When the bacterial isolates were screened for compatibility with insecticide cartap, 19 isolates IIRRSS22- 8, 9, 2, 3, 10, 4, 11, 12, 14, 16, 20, 5, 6, 7, 23, 24, 27, P1, O1 showed high tolerance (Score-3) to all concentrations of cartap (Fig. 4.3). The isolates IIRRSS22- 1, 21, 26, and R1 exhibited moderate tolerance (Score-2), as these isolates have formed inhibition zone around disc at C3 concentration. The isolate IIRRSS22-19 has shown inhibition zone at C2 and C3 concentration hence grouped as less tolerant (Score-1). A total of 8 isolates IIRRSS22- 13, 15, 17, 18, 22, 25, M1, and P2 have not shown growth at all concentrations of cartap and hence grouped as not tolerant (Score-0) (Table 4.6).

Out of 32 isolates, 13 isolates IIRRSS22-1, 8, 2, 3, 4, 16, 5, 6, 23, 27, R1, P1, and O1 have shown compatibility with all concentrations of ferterra; therefore, all these isolates were grouped as highly tolerant with score 3 (Fig. 4.3). The isolates IIRRSS22-20, 21, 7, 24, and 26 were grouped as moderately tolerant as these isolates have produced inhibition zone at C3 concentration of chemical. The isolates IIRRSS22- 9, 10, 11, 12, and 14 were grouped as less tolerant as there was inhibition of bacterial growth at C2 and C3 concentration. And 9 isolates IIRRSS22- 13, 15, 17, 18, 19, 22, 25, M1, P2 have not shown any growth at all concentrations of ferterra (Table 4.6).

The isolates IIRRSS22- 2, 4, 12, 20, 5, 27 (6) were highly tolerant at all concentrations of thiamethaxom as no inhibition zone was produced by bacterial isolates (Fig. 4.3). Nine isolates IIRRSS22-1, 8, 9, 14, 6, 7, 23, P1, and O1 have recorded

moderate growth at C3 concentration and the isolate IIRRSS22- 3, 16, R1 has recorded less growth at C2 and C3 concentration. The isolates IIRRSS22- 10, 11, 13, 15, 17, 18, 19, 21, 22, 24, 25, 26, M1, P2 have exhibited inhibition zones around the discs at C1, C2 and C3 concentrations (Table 4.6).

4.2.2.3 Compatibility with herbicides

The isolates were screened for their compatibility with commonly used herbicides in rice such as pretilachlor, and bispyribac sodium by disc diffusion method (Plate 4.13).

Out of 32 isolates tested for compatibility with pretilachlor, 19 isolates IIRRSS22- 1, 9, 2, 3, 10, 4, 11, 12, 14, 16, 17, 5, 6, 7, 24, 25, 27, R1, P1 have shown compatibility with the chemical. Therefore, these isolates were grouped as highly tolerant with score 3 (Fig. 4.3). The isolates IIRRSS22-13, 15, and 21 have exhibited less growth at C2 and C3 concentrations. The isolate O1 has shown moderate tolerance. A total of 9 isolates IIRRSS22- 8, 18, 19, 20, 22, 23, 26, M1, and P2 have not exhibited any growth at all concentrations of pretilachlor (Table 4.6).

The isolates IIRRSS22- 1, 9, 3, 10, 11, 12, 14, 16, 17, 20, 5, 6, 7, 24, 25, R1, P1 have shown growth at all concentrations of bispyribac sodium. Hence these isolates were grouped as highly tolerant with score 3 (Fig.4.3). The isolates IIRRSS22- 2, 4, 13, 15 and O1 have shown moderate tolerance at C2 and C3. One isolate IIRRSS22-8 have shown less tolerance at C3 concentrations. The isolates IIRRSS22-18, 19, 21, 22, 23, 26, 27, M1 and P2 have recorded no growth at all concentrations of bispyribac sodium (Table 4.6) and hence are not compatible with the herbicide.

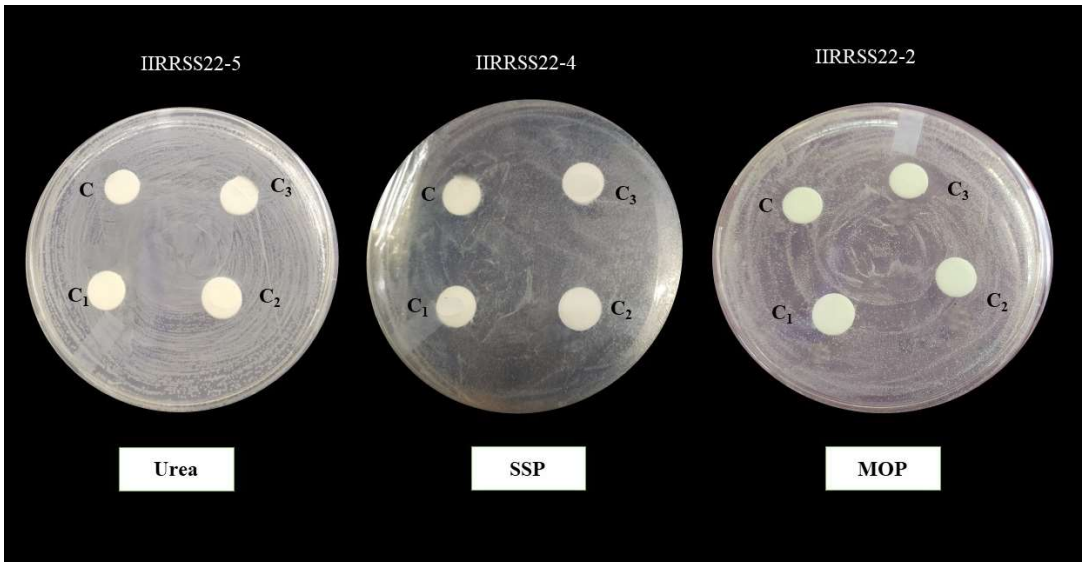
4.2.2.4 Compatibility with fungicides

The bacterial isolates were screened for their compatibility with commonly used fungicides in rice such as carbendazim and mancozeb by disc diffusion method (Plate 4.14).

The isolates (20) IIRRSS22- 8, 9, 2, 3, 10, 4, 12, 14, 15, 16, 20, 5, 6, 7, 23, 24, 27, R1 P1, O1 were highly tolerant as these isolates have grown at all concentrations of carbendazim and were given a score of 3 (Fig. 4.3). Seven isolates IIRRSS22- 1, 11, 13, 17, 18, 22, 25 were moderately tolerant as there was formation of inhibition zone at C3 concentration. The isolates IIRRSS22- 19, 21 were less tolerant because there was inhibition of growth at C2 and C3 concentration. Three isolates IIRRSS22-26, M1, and P2 have recorded no growth at all concentrations of carbendazim (Table 4.6).

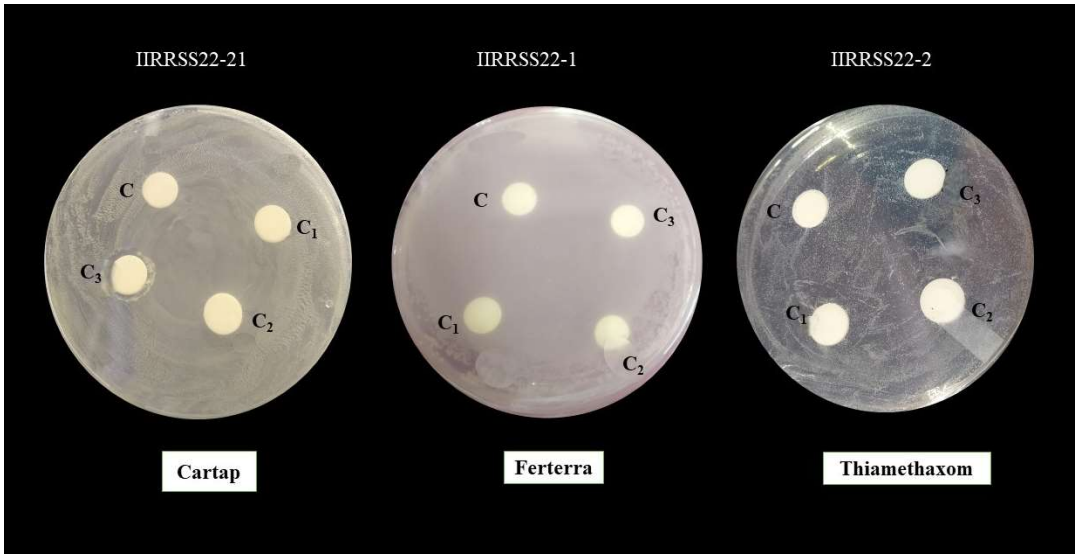
When tested for compatibility with fungicide mancozeb, the isolates revealed that no isolate was highly tolerant to the chemical. The isolates IIRRSS22- 1, 4, R1 have exhibited moderate tolerance and were given a score of 2 (Fig. 4.3). Nine isolates IIRRSS22-9, 2, 3, 10, 20, 5, 23, 24, O1 have recorded less tolerance due to formation of inhibition zone at 2x and 3x concentrations of the chemical. The isolates IIRRSS22- 8, 11, 12, 13, 14, 15, 16, 17, 18, 19 21, 6, 7, 22 25, 26, 27, P1, M1, P2 have shown no growth at all concentrations of mancozeb (Table 4.6).

Similar results of compatibility of bacteria *Mesorhizobium sp* with pesticides (Herbicide - Pendimethalin, Insecticide - Chlorpyrifos, Fungicide - Carbendazim) commonly used in chickpea were tested with single and double the recommended doses by Manasotra *et al.* (2022) by disc diffusion method. There was decrease in bacterial growth when the isolates were inoculated with 2x recommended doses of the pesticides. Among the 50 strains screened for compatibility, 20 strains were found to have the potential to be selected as pesticide tolerant strains.



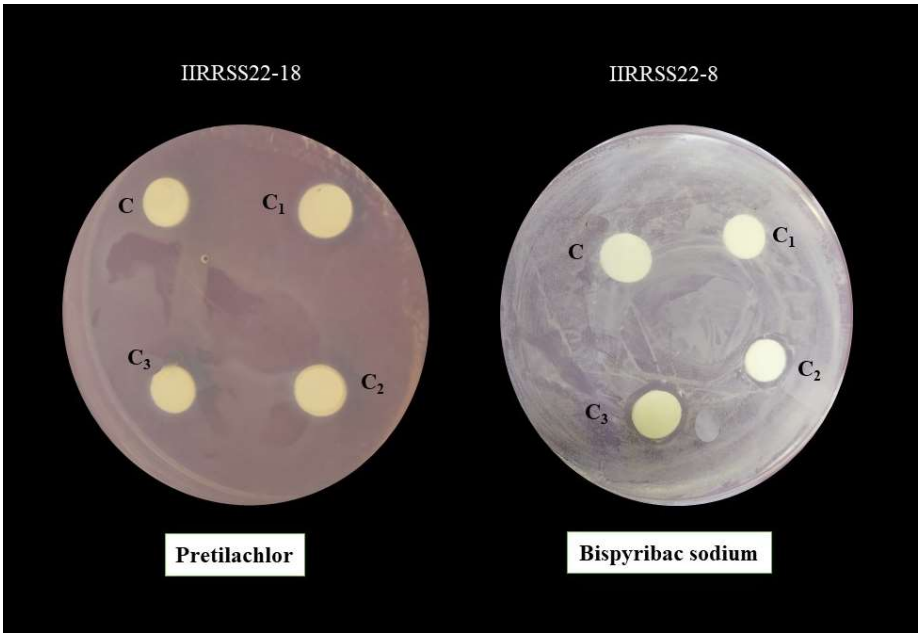
C- Control, C1-50% of the recommended dose, C2-100% of the Recommended dose, C3-150% of the recommended dose

4.11 Identification of bacterial isolates sensitive and tolerant to fertilizers using disc diffusion method



C- Control, C1-50% of the recommended dose, C2-100% of the Recommended dose, C3-150% of the recommended dose

Plate 4.12 Identification of bacterial isolates sensitive and tolerant to insecticides using disc diffusion method



C- Control, C1-50% of the recommended dose, C2-100% of the Recommended dose, C3-150% of the recommended dose

Plate 4.13 Identification of bacterial isolates sensitive and tolerant to herbicides using disc diffusion method

C- Control, C1-50% of the recommended dose, C2-100% of the Recommended dose, C3-150% of the recommended dose

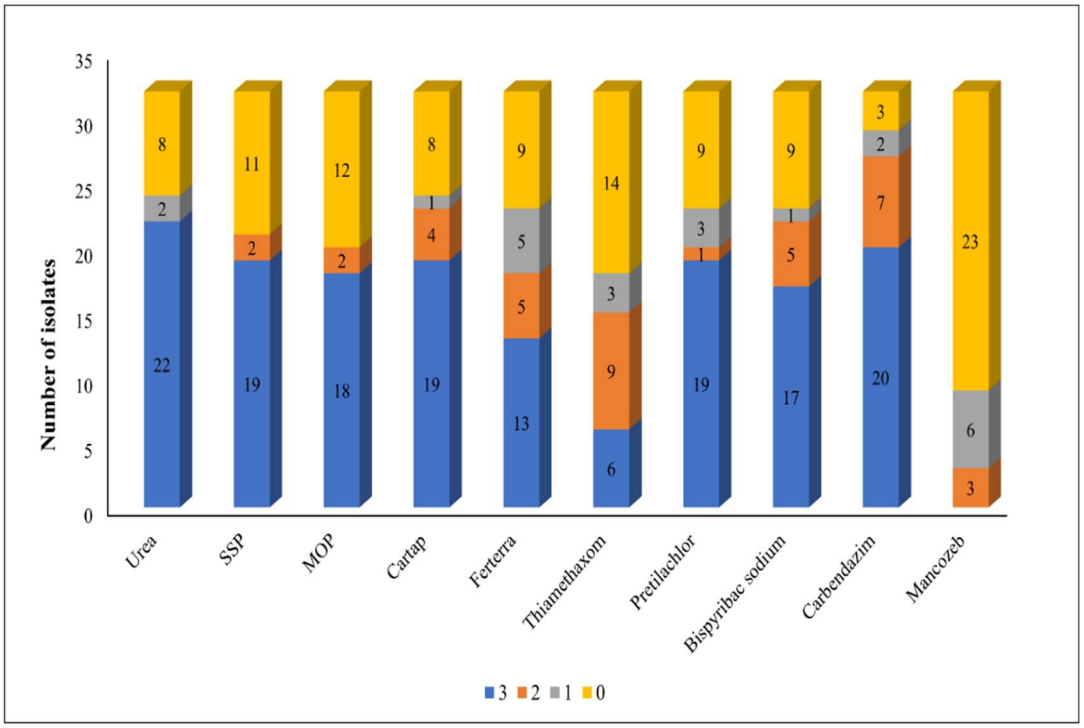
Plate 4.14 Identification of bacterial isolates sensitive and tolerant to fungicides using disc diffusion method

Table 4.6 Compatibility of bacterial isolates with agrichemicals

Isolates	Fertilizers			Insecticides			Herbicides		Fungicides	
	Urea	SSP	MOP	Cartap	Ferterra	Thiamethaxom	Pretilachlor	Bispyribac sodium	Carbendazim	Mancozeb
IIRRSS22-1	3	3	3	2	3	2	3	3	2	2
IIRRSS22-8	3	3	2	3	3	2	0	1	3	0
IIRRSS22-9	3	3	3	3	1	2	3	3	3	1
IIRRSS22-2	3	3	3	3	3	3	3	2	3	1
IIRRSS22-3	3	3	3	3	3	1	3	3	3	1
IIRRSS22-10	3	3	3	3	1	0	3	3	3	1
IIRRSS22-4	3	3	3	3	3	3	3	2	3	2
IIRRSS22-11	3	3	3	3	1	0	3	3	2	0
IIRRSS22-12	3	3	3	3	1	3	3	3	3	0
IIRRSS22-13	1	0	0	0	0	0	1	2	2	0
IIRRSS22-14	3	3	3	3	1	2	3	3	3	0
IIRRSS22-15	0	0	0	0	0	0	1	2	3	0
IIRRSS22-16	3	0	2	3	3	1	3	3	3	0
IIRRSS22-17	0	2	0	0	0	0	3	3	2	0
IIRRSS22-18	0	0	0	0	0	0	0	0	2	0
IIRRSS22-19	1	0	0	1	0	0	0	0	1	0
IIRRSS22-20	3	3	3	3	2	3	0	3	3	1
IIRRSS22-5	3	3	3	3	3	3	3	3	3	1
IIRRSS22-21	0	0	0	2	2	0	1	0	1	0
IIRRSS22-6	3	3	3	3	3	2	3	3	3	0
IIRRSS22-7	3	3	3	3	2	2	3	3	3	0
IIRRSS22-22	3	2	0	0	0	0	0	0	2	0
IIRRSS22-23	0	0	0	3	3	2	0	0	3	1
IIRRSS22-24	3	3	3	3	2	0	3	3	3	1

IIRRSS22-25	3	3	3	0	0	0	3	3	2	0
IIRRSS22-26	0	0	0	2	2	0	0	0	0	0
IIRRSS22-27	0	0	0	3	3	3	3	0	3	0
R1	3	3	3	2	3	1	3	3	3	2
P1	3	3	3	3	3	2	3	3	3	0
M1	3	0	0	0	0	0	0	0	0	0
O1	3	3	3	3	3	2	2	2	3	1
P2	0	0	0	0	0	0	0	0	0	0

3- highly compatible/tolerant, **2-** moderately compatible/ tolerant, **1-** less compatible/ tolerant, **0-** not compatible/tolerant



Legend 3, 2 1 and 0 are scores indicating high, moderate, low and no compatibility of isolates to agrichemical

Fig. 4.3 Number of isolates exhibiting compatibility with agrichemicals

4.3 SELECTION OF BACTERIAL ISOLATES BASED ON SCORING

PGP traits, abiotic stress tolerance (for high temperature tolerance, the isolates growing at 45℃ were selected for scoring) and compatibility of isolates with agrichemicals were the three categories employed for scoring of isolates (Table 4.7), following which three isolates with the highest score in each category were selected (Table 4.8). The nine selected isolates were P1, IIRRSS22-1, IIRRSS22-6, in the plant growth promotion category with scores of 11, 10 and 9; IIRRSS22-3, R1, IIRRSS22-7 all with a score of 7 in abiotic stress tolerance category and IIRRSS22-5, IIRRSS22-4, IIRRSS22-2, which showed highest compatibility with agrichemicals with scores of 28 and 27 respectively. The isolates were grouped into three consortia (consortium-1, 2 and 3) with each consortium consisting of one bacterial isolate from each category (Table 4.8). The three bacteria in each consortia were checked for compatibility followed by screening of isolates for effect on rice germination under *in vitro* conditions for selection of promising consortia group.

Hazarika *et al*. (2021) has scored the bacterial isolates similarly to select the best endophytic bacteria having high plant growth promoting potential. The 2 isolates *Stenotrophomonas sp.* and *Pseudomonas sp.* exhibiting highest score were selected for evaluation of *in vivo* plant growth promoting activity in tea plants.

4.4 COMPATIBILITY OF BACTERIAL ISOLATES WITH EACH OTHER

Compatibility between consortial isolates was tested by cross streak method for development of consortia. No inhibition zones were visible with any of the isolates (Plate 4.15). All the isolates were compatible with each other without showing any antagonistic activity.

Compatibility of bacterial isolates with each other must be done for ensuring the beneficial effects of PGP bacteria on growth of plants when applied as consortia. Daghari *et al*. (2020) has conducted a similar compatibility test between bacterial isolates by inoculating isolate in centre of NA plate and other bacterial isolates were streaked at right angles to the initial culture. The results revealed that all isolates were compatible with each other as there was merging in the growth of bacteria at the intersection.

4.7 Score of bacterial isolates under PGP traits, abiotic stress tolerance and agrichemical compatibility categories

Isolate ID	Total PGP traits score	Abiotic stress tolerance score	Compatibility with agrichemicals score
IIRRSS22-1	10	3	26
IIRRSS22-8	7	6	20
IIRRSS22-9	7	5	25
IIRRSS22-2	8	5	27
IIRRSS22-3	8	7	26
IIRRSS22-10	6	5	23
IIRRSS22-4	8	2	28
IIRRSS22-11	7	6	21
IIRRSS22-12	6	6	25
IIRRSS22-13	6	2	6
IIRRSS22-14	7	2	24
IIRRSS22-15	8	2	6
IIRRSS22-16	5	4	21
IIRRSS22-17	7	0	10
IIRRSS22-18	8	2	2
IIRRSS22-19	8	0	3
IIRRSS22-20	4	4	24
IIRRSS22-5	8	5	28
IIRRSS22-21	7	2	6
IIRRSS22-6	9	6	26
IIRRSS22-7	8	7	25
IIRRSS22-22	8	1	7
IIRRSS22-23	6	0	12
IIRRSS22-24	8	3	24
IIRRSS22-25	8	3	17
IIRRSS22-26	6	0	4
IIRRSS22-27	4	4	15
R1	8	7	26
P1	11	5	26
M1	5	0	3
O1	4	5	25
P2	1	0	0

Table 4.8 Consortia partners selected from each category of plant growth promotion, abiotic stress tolerance and compatibility with agrichemicals

Consortium group	Plant growth promoting traits	Abiotic stress tolerance	Compatibility with agrichemicals
C-1	P1 (11)	IIRRSS22-3 (7)	IIRRSS22-5 (28)
C-2	IIRRSS22-1 (10)	R1 (7)	IIRRSS22-2 (27)
C-3	IIRRSS22-6 (9)	IIRRSS22-7 (7)	IIRRSS22-4 (28)

Numbers in parenthesis indicate the score of the isolates

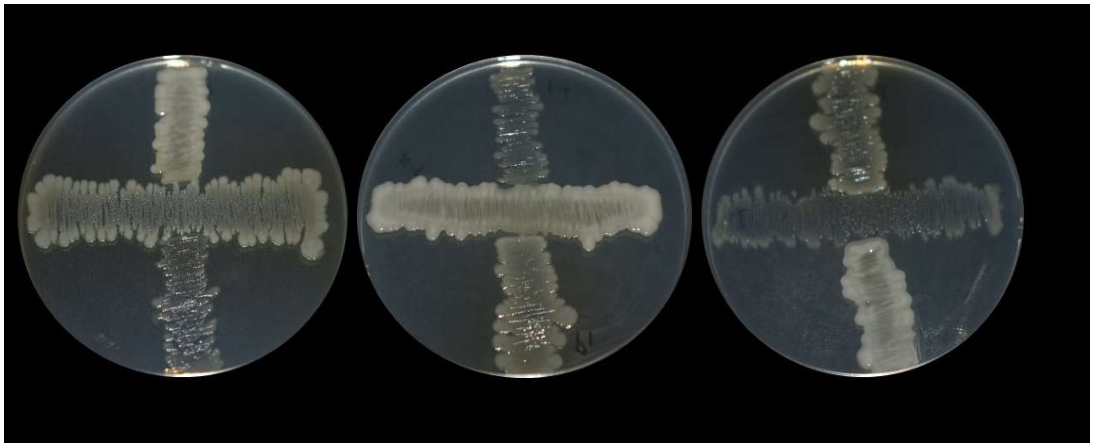


Plate 4.15 Assessing the compatibility of bacterial isolates (P1, IIRRSS22-3 and IIRRSS22-5) in the consortia by cross streak method

4.5 EVALUATION OF THE EFFECT OF RHIZOBACTERIAL CONSORTIA ON GERMINATION OF RICE UNDER *IN VITRO* GERMINATION ASSAY

The effect of 3 consortium groups and the individual bacterial isolates on germination of rice was performed under *in vitro* conditions (Plate 4.16). The results showed that highest germination percentage was found with seeds inoculated with C1, C2, IIRRSS22-6, IIRRSS22-7 and R1 i.e, 100% followed by IIRRSS22-1, IIRRSS22-2 with 99.34%, C3 with 98.67% whereas P1, IIRRSS22-3, IIRRSS22-5 with 97.34% and control showing 96.67%. IIRRSS22-4 showed the least percent germination with 93.34% (Table 4.9).

Root length was highest with C1 (9.45 cm), followed by C2 with 9.40 cm. Control treatment has lowest root length of 6.84 cm. Shoot length was found to be highest with C1 (6.70 cm), followed by IIRRSS22-2 (6.66 cm) and lowest shoot length was shown by control treatment (5.28 cm). Total seedling length was found to be highest with C1 (16.16 cm), followed by C2 (15.96 cm) and lowest seedling length was produced by control treatment (12.12 cm) (Table 4.9 and Fig 4.4)

Vigour index I of 1616 was produced by C1, followed by C2 (1596) and least percentage of vigour index was found with control (1171.46) (Table 4.9 and Fig 4.5). Vigour index II was found to be highest with C1 with value of 5.47 followed by IIRRSS22-7 with 5.15 and least percent was produced by IIRRSS22-6 with 4.05(Table 4.9 and Fig 4.6). The percentage increase in seedling length, germination percentage, vigour index I and II when C1 was compared with control were 33.3%, 3.34%, 38% and 10% respectively.

Similar results with consortium were obtained by Akintokun *et al*. (2016) in tomato plants, where there was significant difference in root length, shoot length and vigour index when the seeds were inoculated with consortium containing four organisms when compared with control treatments. Sherpa *et al*. (2021) has conducted studies on use of consortia for promotion of rice plant growth by using 3 different consortia. Greenhouse and field studies showed that the consortia-3 had the highest plant growth-promoting activity in terms of root length, number of leaflets per plant, grains per panicle, test grain weight, dry root weight per plant, and total dry biomass per plant.

Based on the germination percentage and vigour index, the consortium group-C1 performing better than the control and individual inoculations was selected for pot culture studies (Table 4.9).

Table 4.9 Effect of individual and consortial bacterial inoculations on growth parameters of rice under *in vitro* conditions

Isolate ID	Root length	Shoot length	Seedling length	Germination percentage	Vigor index I	Vigor index II
Control	6.84 ± 0.03	5.28 ± 0.23	12.12 ± 0.2	96.67 ± 1.15	1171.46 ± 10.02	4.978 ± 0.03
P1	8.36 ± 0.82	6.42 ± 0.1	14.78 ± 0.72	97.34 ± 2.31	1437.62 ± 44.1	4.99 ± 0.33
IIRRSS22-3	8.13 ± 1.2	6.29 ± 0.41	14.42 ± 0.78	97.34 ± 4.62	1406.12 ± 136.74	4.54 ± 0.14
IIRRSS22-5	9.19 ± 0.04	6.38 ± 0.02	15.58 ± 0.07	97.34 ± 2.31	1516.36 ± 30.19	5.05 ± 0.25
IIRRSS22-1	8.49 ± 0.08	6.2 ± 0.07	14.69 ± 0.15	99.34 ± 1.15	1459.6 ± 8.6	4.89 ± 0.18
R1	7.99 ± 0.70	6.40 ± 0.14	14.4 ± 0.56	100	1440 ± 56	4.52 ± 0.33
IIRRSS22-2	8.8 ± 1.44	6.66 ± 0.05	15.46 ± 1.38	99.34 ± 1.15	1536.19 ± 139.65	4.54 ± 0.28
IIRRSS22-6	7.57 ± 1.48	6.24 ± 0.08	13.81 ± 1.4	100	1381 ± 140	4.05 ± 0.1
IIRRSS22-7	7.04 ± 1.42	6.4 ± 0.3	13.44 ± 1.72	100	1344 ± 172	5.15 ± 0.2
IIRRSS22-4	9.08 ± 0.15	6.12 ± 0.16	15.21 ± 0.01	93.34 ± 2.31	1419.58 ± 34.31	4.69 ± 0.27
C1	**9.45 ± 0.06**	**6.70 ± 0.24**	**16.16 ± 0.18**	**100**	**1616 ± 18**	**5.47 ± 0.68**
C2	9.40 ± 0.36	6.55 ± 0.10	15.96 ± 0.26	100	1596 ± 26	4.8 ± 0.05
C3	8.58 ± 0.19	5.88 ± 0.34	14.46 ± 0.14	98.67 ± 1.15	1427.31 ± 30.03	5.00 ± 0.42
CD (0.05)	1.473	0.332	1.423	3.146	151.408	0.485
CV (%)	10.389	3.103	5.721	1.886	6.187	5.954

Values are the means of three replicates ± standard deviation

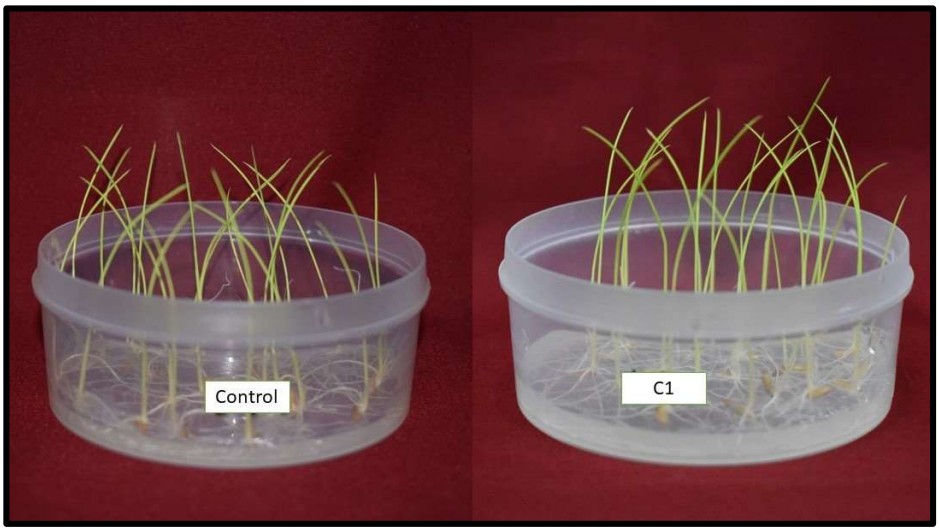


Plate 4.16 ***In vitro*** **germination assay of rice seeds with uninoculated (control) and consortia (C1) treatment**

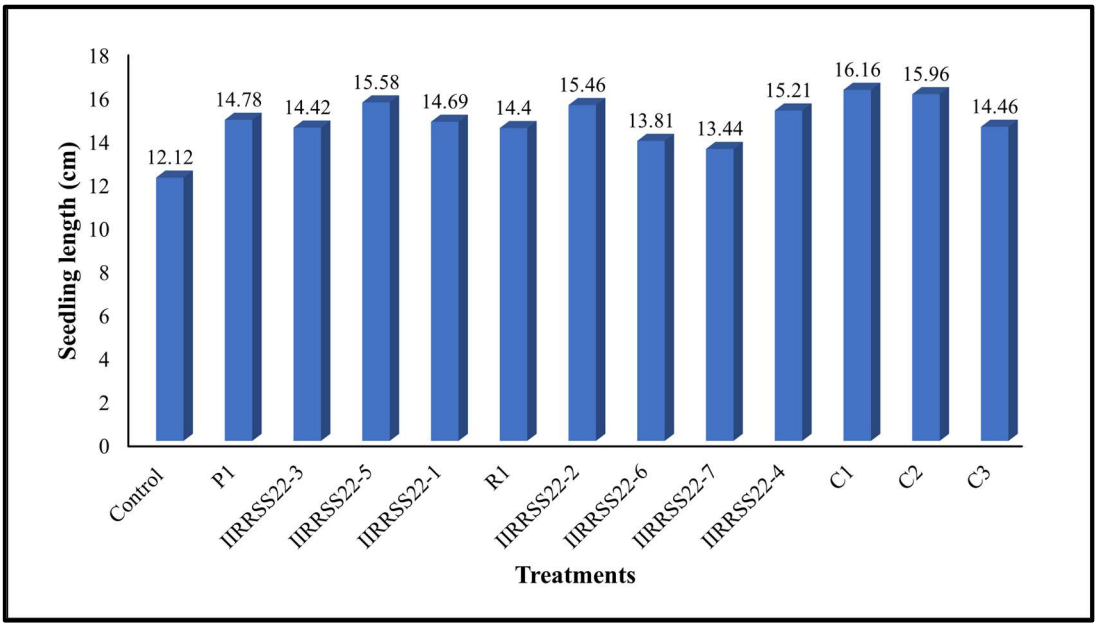


Fig. 4.4 Effect of single and consortia bacterial inoculations on rice seedling length under ***in vitro*** **conditions**

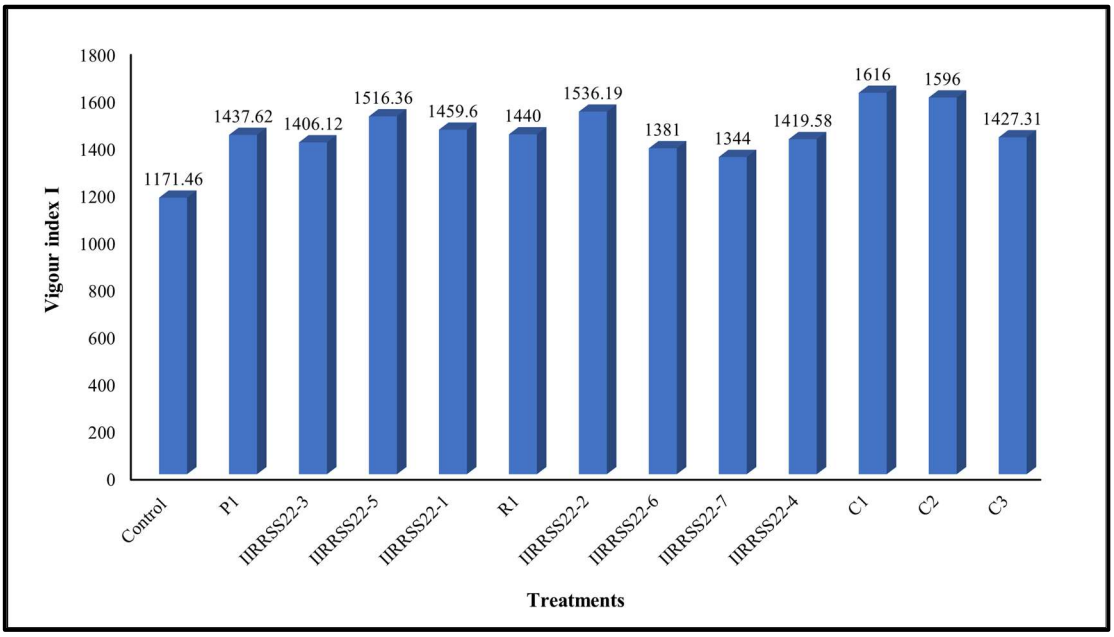


Fig. 4.5 Effect of single and consortia bacterial inoculations on Vigor index I under *in vitro* conditions

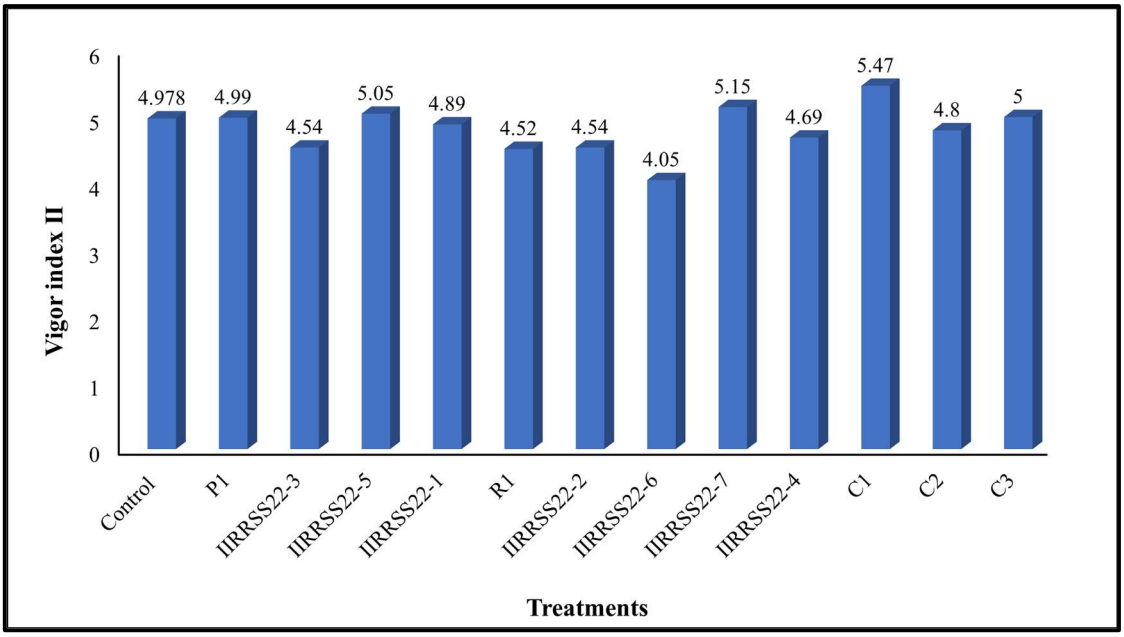


Fig. 4.6 Effect of single and consortia bacterial inoculations on Vigor index II under *in vitro* conditions

4.6 MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATES

The promising isolates were identified using 16S rRNA gene sequencing after DNA isolation and amplification of the 16S rRNA gene (Plate 4.17).

4.6.1 Sequencing and analysis

Identification of bacterial isolates based on 16S r RNA is given in (Table 4.9). In the present study the sequenced PCR products of selected isolates were matched with available sequences in genbank database. The identity of the isolates is shown (Table. 10) along with the accession numbers of 16S rRNA gene sequences that have been deposited in the National Center for Biotechnology Information database.

The results showed that IIRRSS22-5 has similarity with *Achromobacter sp*, IIRRSS22-6 has similarity with *Achromobacter insuavis*, IIRRSS22-7 has similarity with *Achromobacter xylosoxidans*, IIRRSS22-1 has similarity with *Rhizobium sp*, IIRRSS22-2 has similarity with *Stenotrophomonas maltophilia*, IIRRSS22-3 has similarity with *Stenotrophomonas sp* and IIRRSS22-4 has similarity with *Ochrobacter anthropi* (Table 4.10). The isolates R1 and P1 used in the study showed similarity with *Rhizobium* sp and *Pseudomonas stutzeri.*

The consortial partners in C-1 were identified as *Pseudomonas stutzeri, Stenotrophomonas sp and Achromobacter sp.*

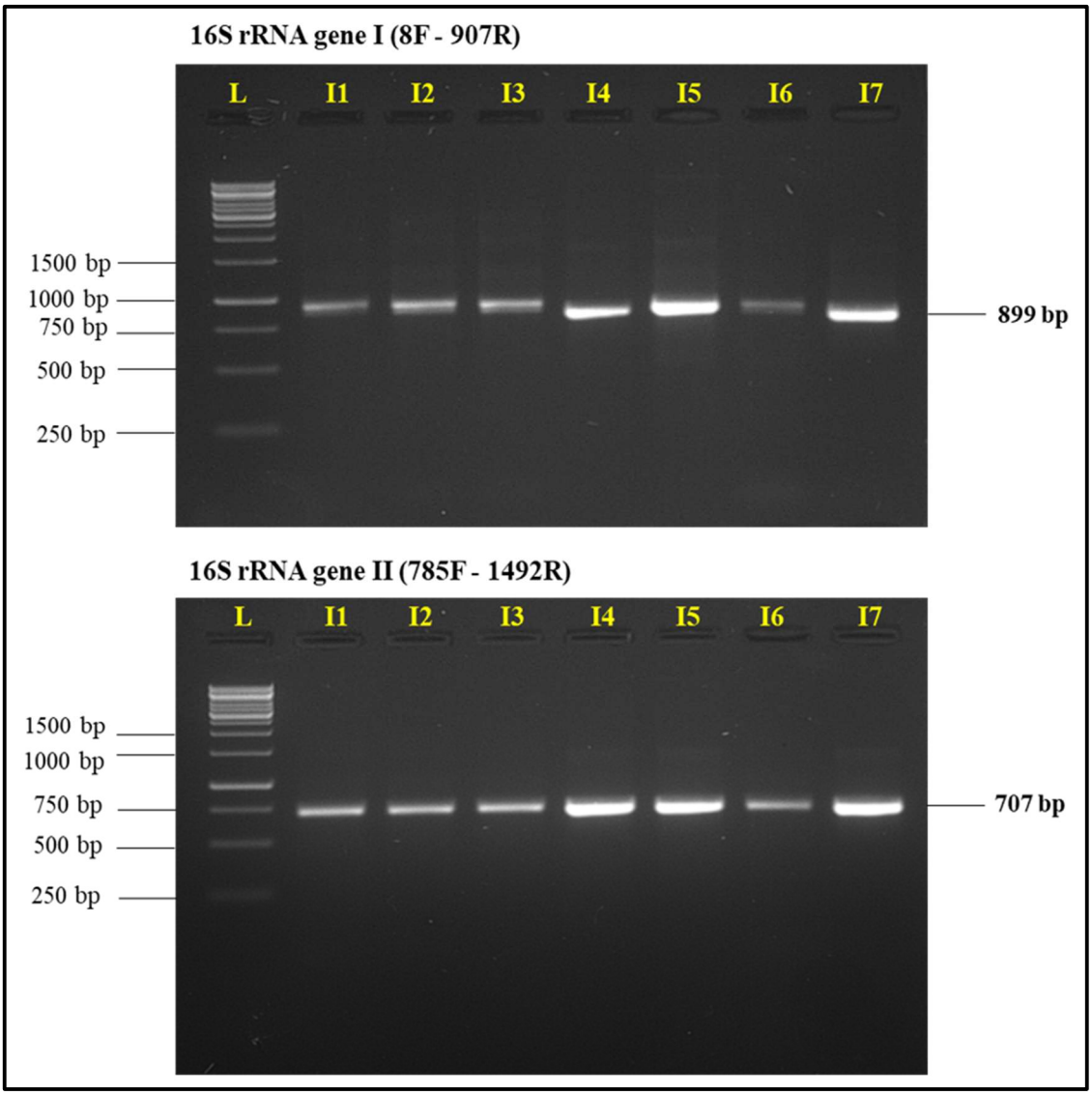
Pseudomonas stutzeri has been reported as a PGPR in several studies. *P. stutzeri* was isolated from the rhizosphere of rice and identified as diazotrophic strain by Qui *et al*. (1981). Pham *et al*. (2017) studied the effect of inoculation of *Pseudomonas stutzeri* in rice plants which showed a significant increase in shoot and root dry weight when compared with unioculated rice seedlings.

Stenotrophomonas is a common bacterial genus in many soil types and rhizospheres of different crops, and presents mechanisms for growth promotion such as biological nitrogen fixation, mineral solubilization and phytohormones production. Perez *et al*. (2020) isolated various plant growth promoting bacteria from rhizosphere of maize. Identification by sequencing of the 16S rDNA gene revealed that the isolates belong 4 different genera of *Enterobacter, Pseudomonas, Rhizobium and Stenotrophomonas.*

Shahid *et al.* (2020) studied the effect of *Achromobacter sp*. FB-14 on rice plants to alleviate the salinity effects by upregulation of the stress-responsive *CIPK* genes. The strain FB-14 was isolated by using a nutrient agar medium at 855 mM NaCl concentration and it was taxonomically identified as *Achromobacter* sp. with more than 99% 16S rRNA gene sequence similarity with many *Achromobacter* species.

Table 4.10 Identity of bacterial isolates (16S rRNA gene sequencing)

S. No	Isolate ID	Identified strain	Accession number
1	IIRR SS 22-1	*Rhizobium sp.*	OP580066
2	IIRR SS 22-2	*Stenotrophomonas maltophilia*	OP580125
3	IIRR SS 22-3	*Stenotrophomonas sp.*	OP592247
4	IIRR SS 22-4	*Ochrobactrum anthropi*	OP580149
5	IIRR SS 22-5	*Achromobacter sp.*	OP592244
6	IIRR SS 22-6	*Achromobacter insuavis*	OP580150
7	IIRR SS 22-7	*Achromobacter xylosoxidans*	OP580160
8	R1	*Rhizobium sp.*	KY348774
9	P1	*Pseudomonas stutzeri*	MW231894



L: 1 kb ladder ; I1: *Rhizobium sp.* IIRRSS22-1 ; I2: *Stenotrophomonas maltophilia* IIRRSS22-2 ; I3: *Stenotrophomonas sp.* IIRRSS22-3 ; I4: *Ochrobactrum anthropi* IIRRSS22-4 ; I5: *Achromobacter sp.* IIRRSS22-5 ; I6: *Achromobacter insuavis* IIRRSS22-6 ; I7: *Achromobacter xylosoxidans* IIRRSS22-7

Plate 4.17 PCR amplification of 16s rRNA (gene I and gene II) of promising bacterial isolates from rice rhizosphere

4.7 EVALUATION OF DEVELOPED CONSORTIA IN POT CULTURE STUDIES

The pot culture experiment was conducted during *Rabi* 2021-22 with completely randomized design, at ICAR- Indian Institute of Rice Research, Rajendranagar, Hyderabad. Experimental data were statistically analysed and were depicted under various headings and subheadings, furnished in tables and illustrated through figures wherever necessary.

4.7.1 Initial soil characteristics

Soil was analysed for N, P, K, pH and EC before sowing. Soil pH recorded was 7.75, EC of 0.65 ds m^{-1}, Nitrogen content of soil was 267.34 kg/ha, Phosphorous content of soil was 38.34 kg/ha and Potassium content recorded was 306.23 kg/ha (Table 3.5). Iron and zinc content in soil recorded was 6.89 ppm, 0.89 ppm respectively (Table 3.5). The soil dehydrogenase and fluorescein diacetate hydrolysis activity in soil before sowing was reported to be 84 µg TPF g^{-1} soil $24h^{-1}$ and 45 µg FDA g^{-1} soil $0.5h^{-1}$ respectively.

4.7.2 Influence of bacterial consortia on plant morphological traits

4.7.2.1 Root length

Root length at active tillering stage and at harvest stage was recorded and mentioned in Table 4.11. Seed treatment and seedling root dip with individual and consortia significantly enhanced root length of rice plants both at active tillering stage and at harvest stage when compared with both the control and treatment with 100% RDF.

At active tillering stage, root length was found to be highest for T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)] with 22.89 cm, followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] with 21.91. The treatments T1 [Control] has produced root length of 16.32cm (Table 4.11 and Fig 4.7) which was least among all the treatments and T2 (100% RDF) has produced a root length of 17.58 cm.

At harvest stage, highest root length among all the treatments was observed in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)] with 26.73 cm and least was found in control treatment with 21.29 cm and T2 (100% RDF) with 22.23 cm (Table 4.11 and Fig 4.8).

Similar results of bacterial consortia able to increase root length in rice thereby increasing the nutrient uptake, chlorophyll content, and plant biomass were noticed by

Purwanto *et al*. (2021). They reported that a combination of isolates *Rhizobium sp*. R11 + *Rhizobium sp.* R08 has produced the highest root length when compared with control and individual inoculations.

Similar to this study, Niveta *et al*. (2018) isolated *Stenotrophomonas maltophilia* RSD6 from rhizosphere of rice and it was observed that the treatment of rice plants with RSD6 showed significant enhancement in root length (15.66 cm) when compared with control treatment (14.33 cm).

4.7.2.2 Shoot length

Shoot length at active tillering stage and at harvest stage was recorded and mentioned in Table 4.10. Seed treatment and seedling root dip with individual and consortia significantly enhanced shoot length of rice plants when compared with control and T2 with 100% RDF.

Shoot length at active tillering was maximum in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)] by 75.20 cm among all the treatments and followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] with 74.56 cm. T1 (Control) exhibited the lowest shoot length of 66.60 cm which is followed by treatment T2 (100% RDF) 68.54 cm (Table 4.11 and Fig 4.7).

At harvest stage T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (85.87 cm) exhibited the highest shoot length of 94.23 cm when compared with all the treatments and followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] with shoot length of 92.34 cm. T1(Control) recorded the lowest shoot length of 85.87 cm among all the treatments followed by T2 (100% RDF) with 87.12 cm (Table 4.11 and Fig 4.8).

Similar results of bacterial consortia on plant height in rice were observed by Pratiwi *et al*. (2021) in which the consortium of bacteria, isolated from the rhizosphere of rice has shown a significant increase in plant height at 95 days after transplanting with bacterial consortium-2 + 100% of NPK fertilizers when compared with treatment without bacterial inoculation + 100% NPK fertilizers. Consortium -2 consisted of 4 strains *Mycobacterium senegalense* LM1, *Providencia stuartii* LM18, *Rhizobium rhizoryzae* BMU and *Bacillus methylotrophicus* N2P4.

The ability of *Achromobacter sp*. to improve shoot length was reported by Joe *et al.* (2012) who studied the effect of *Achromobacter xylosoxidans* AUM54 on improving

the growth of rice under greenhouse conditions. Inoculation of *Achromobacter sp* by seed priming significantly enhanced the rice plant height (73.5 cm) when compared with the control (65.2 cm).

4.7.2.3 Leaf area

Leaf area at the active tillering stage varied between 315.44- 328.56 cm^2 (Table 4.11). The highest leaf area was observed in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (328.56 cm^2) followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] with leaf area of 327.45 cm^2. The lowest value of leaf area was shown by T1(Control) (315.44 cm^2) followed by T2 (100% RDF) with 318.68 cm^2 among all the treatments.

At harvest stage, leaf area was highest in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (357.94 cm^2) among all the treatments and followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] with leaf area of 356.68 cm^2 (Table 4.11). With treatments T1 (control) and T2 (100% RDF) the leaf area was observed to be lowest than the treatments inoculated with individual consortial partners and as consortia.

Purwanto *et al.* (2021) in their experiment on the effect of PGPR consortia on leaf area has shown that the application of PGPR consortia increased the growth of leaf organs indicated by wider leaf area compared to the single strains and control treatment. The widest leaf area was obtained when the rice plants were treated with a consortium of R11 isolate + R08 isolate + *Rhizobium sp.* LM-5.

Table 4.11 Effect of single and consortia bacterial inoculations on plant morphological traits at active tillering and harvest

Treatments	Root length(cm)		Shoot length(cm)		Leaf area(cm^2)	
	At active tillering	At harvest	At active tillering	At harvest	At active tillering	At harvest
T1	16.32	21.29	66.60	85.87	315.44	342.18
T2	17.58	22.23	68.54	87.12	318.68	345.91
T3	19.9	24.18	70.34	89.23	323.19	350.93
T4	19.63	23.27	69.13	88.86	319.43	348.82
T5	18.8	23.48	72.56	87.94	320.48	347.85
T6	19.47	23.63	71.34	89.1	322.87	350.62
T7	18.85	23.58	71.78	88.42	321.9	349.28
T8	19.34	22.60	72.89	88.39	322.15	348.94
T9	20.87	25.03	72.72	90.78	325.53	353.6
T10	20.12	24.69	71.45	89.87	324.58	352.19
T11	20.56	24.85	74.56	90.2	323.29	352.59
T12	21.91	25.57	73.70	92.34	327.45	356.68
T13	21.73	25.19	73.23	92.04	326.97	355.23
T14	22.89	26.73	75.20	94.23	328.56	357.94
CD (0.05)	2.11	2.52	3.29	4.34	NS	NS
CV%	6.37	6.30	2.74	2.90	2.98	2.37

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

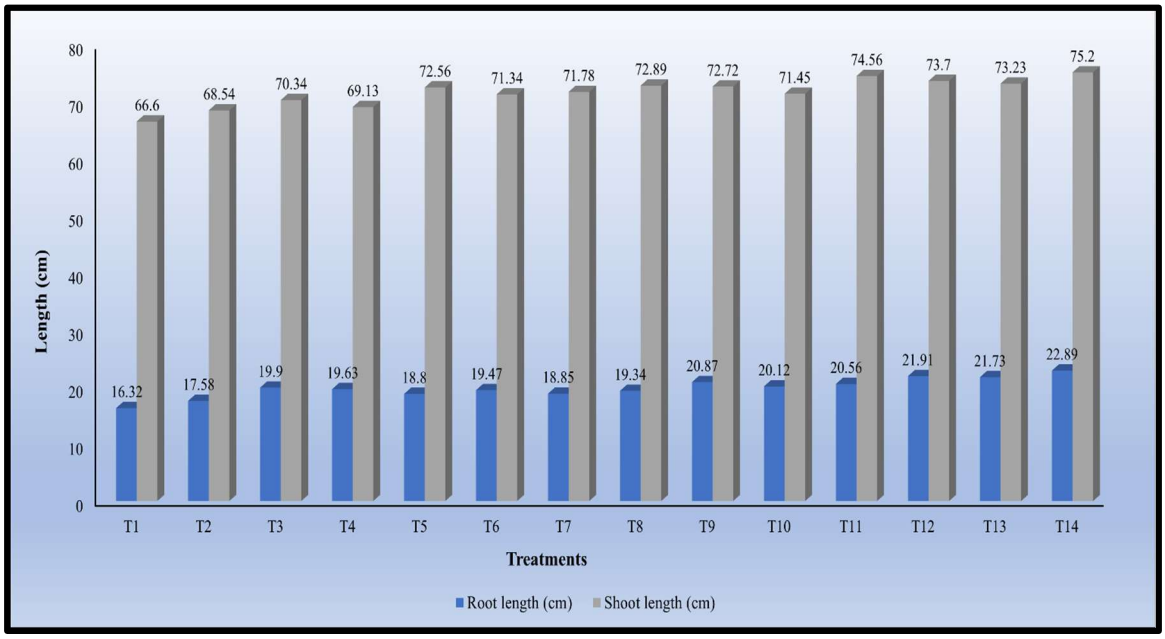


Fig. 4.7 Effect of single and consortia bacterial inoculations on root and shoot length at active tillering

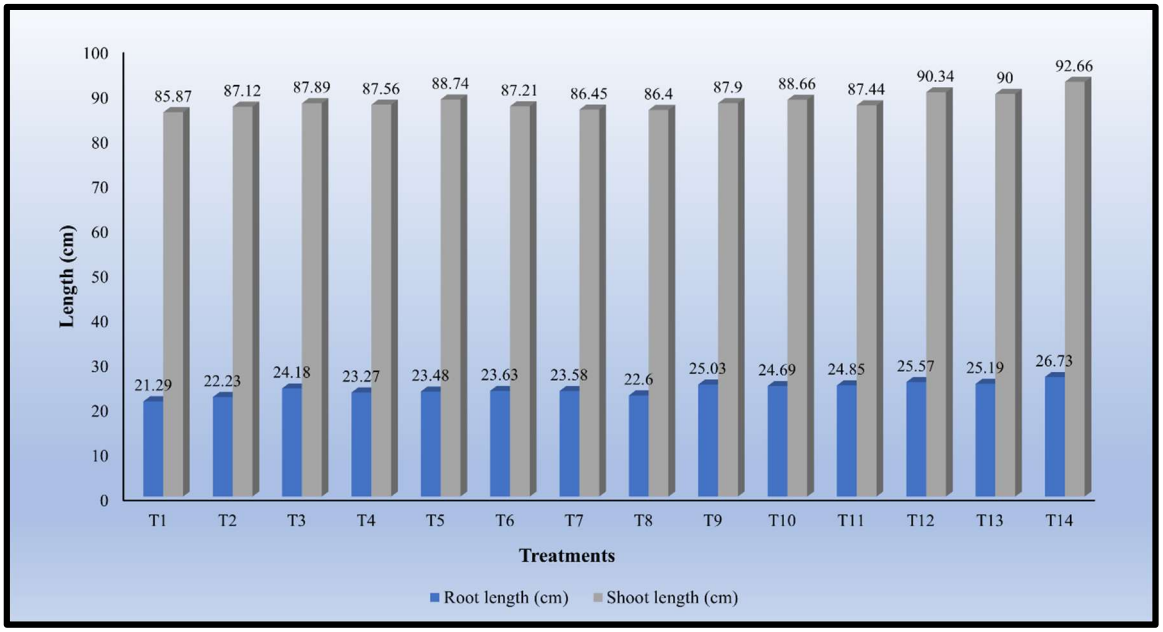


Fig. 4.8 Effect of single and consortia bacterial inoculations on root and shoot length at harvest

4.7.2.4 Plant biomass

4.7.2.4.1 Shoot fresh and dry weight

The shoot fresh weights and dry weights of the plants varied at different stages in different treatments and the results are presented in Table 4.12 and Table 4.13. Fresh weights and dry weights of the shoot were increased significantly when the plants were inoculated with consortia and individual partners than the control and T2 (100% RDF) treatments.

At active tillering stage the shoot fresh weights ranged from 30.82 g to 38.89 g (Table 4.12 and Fig. 4.9) with highest fresh weight exhibited by T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] with 38.89 g followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] (36.96 g). The shoot dry weights of different treatments ranged from 6.58 g to 13.9 g (Table 4.13 and Fig 4.9). Highest dry weight was recorded in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (13.9 g) followed by treatment T12 [100% RDF + Bacterial consortia (Seed treatment)] (13.89 g).

At harvest stage the shoot fresh weights ranged from 68.89 g to 84.5 g (Table 4.12 and Fig 4.10) with highest fresh weight exhibited by T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] with 84.5 g followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] (82.98 g). The shoot dry weights of different treatments ranged from 18.67 g to 25.99 g (Table 4.13 and Fig 4.10). Highest dry weight was recorded in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (25.99 g) followed by treatment T12 with 25.98 g [100% RDF + Bacterial consortia (Seed treatment)].

4.7.2.4.2 Root fresh weight and dry weight

The root fresh weights and dry weights of the plants at active tillering and harvest stage of different treatments and the results are presented in Table 4. 12, Table 4.13. and Fig. 4.8. There was a significant increase in weights of roots when the plants were inoculated with consortia and individual partners than the control and T2 (100% RDF) treatments.

At active tillering stage the root fresh weights ranged from 7.81 g to 8.58 g (Table 4.12 and Fig 4.11) with highest fresh weight exhibited by T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] with 8.58 g followed by T12 [100% RDF

+ Bacterial consortia (Seed treatment)] (8.43 g). The root dry weights of different treatments ranged from 1.5 g to 1.64 g (Table 4.13 and Fig 4.11). Highest dry weight was recorded in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (1.64 g) followed by treatment T12 [100% RDF + Bacterial consortia (Seed treatment)] and T13 100% RDF + Bacterial consortia (Seedling root dip) (1.62 g).

At harvest stage the root fresh weights ranged from 18.89 g to 34.5 g (Table 4.12 and Fig 4.12) with highest fresh weight exhibited by T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] with 34.5 g followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] (32.98 g). The root dry weights of different treatments ranged from 2.94 g to 5.84 g (Table 4.13 and Fig 4.12). Highest dry weight was recorded in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] (5.84 g) followed by treatment T12 (5.75 g) [100% RDF + Bacterial consortia (Seed treatment)].

A similar study was conducted by Pas *et al*. (2015) to evaluate the performance of individual and consortia treatment on shoot fresh weight of rice at 60 days after transplanting. The consortia R15 (*Stenotrophomonas* sp + *Stenotrophomonas* sp + *Bacillus* sp + *S. acidaminiphila*) has produced the highest fresh weights of shoots when compared with individual inoculations.

Comparable results of plant biomass of rice were obtained by Joshi *et al*. (2020) when the bacterial isolates were inoculated singly and as consortia. This study has reported that the plants inoculated with consortia (EN 121+ EN 108+ EN 43) has produced the highest plant biomass when it was compared with control and individual inoculations. The 16s rRNA gene sequencing identified all the organisms in consortia as *Bacillus* sp.

Table 4.12 Effect of single and consortia bacterial inoculations on shoot, root fresh weights at active tillering and harvest

Treatments	Shoot Fresh weight (g/plant)		Root fresh weight (g/plant)	
	At active tillering	At harvest	At active tillering	At harvest
T1	30.82	68.89	7.81	18.89
T2	31.13	71.23	7.97	21.23
T3	32.8	75.9	8.2	25.9
T4	32.64	75.45	8.04	25.45
T5	32.39	74.39	8.14	24.39
T6	32.24	75.78	8.18	25.78
T7	32.11	74.56	8.12	24.56
T8	31.25	74.89	8.1	24.89
T9	35.34	79.59	8.34	29.59
T10	34.76	78.7	8.23	28.7
T11	34.89	79.52	8.29	29.52
T12	36.96	82.98	8.43	32.98
T13	36.48	81.34	8.39	31.34
T14	38.89	84.5	8.58	34.5
CD (0.05)	3.57	8.16	0.32	2.97
CV%	6.33	6.3	2.36	6.55

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

Table 4.13 Effect of single and consortia bacterial inoculations on shoot, root dry weights at active tillering and harvest

Treatments	Shoot dry weight (g/plant)		Root Dry weight (g/plant)	
	At active tillering	At harvest	At active tillering	At harvest
T1	6.58	18.67	1.5	2.94
T2	7.55	19.64	1.52	3.46
T3	11.44	23.53	1.57	3.96
T4	11.03	23.12	1.56	4.36
T5	10.93	23.02	1.56	4.42
T6	11.8	23.89	1.57	4.35
T7	10.03	22.12	1.55	4.4
T8	10.7	22.79	1.54	4.52
T9	13.69	25.78	1.53	5.35
T10	13.04	25.13	1.6	5.26
T11	12.56	24.65	1.59	5.43
T12	13.89	25.98	1.62	5.75
T13	13.78	25.87	1.62	5.71
T14	13.9	25.99	1.64	5.84
CD (0.05)	1.27	2.54	0.06	0.52
CV%	6.64	6.4	2.3	6.6

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

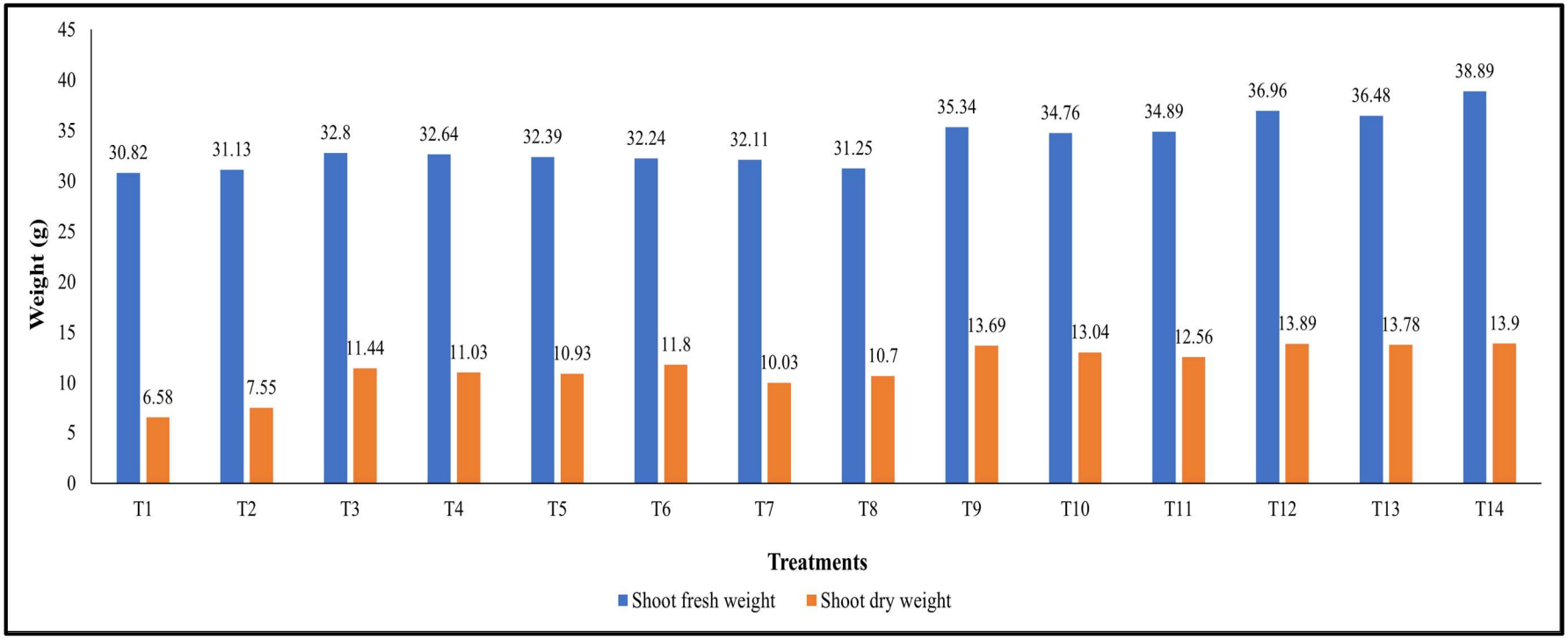


Fig. 4.9 Effect of single and consortia bacterial inoculations on shoot fresh and dry weight at active tillering

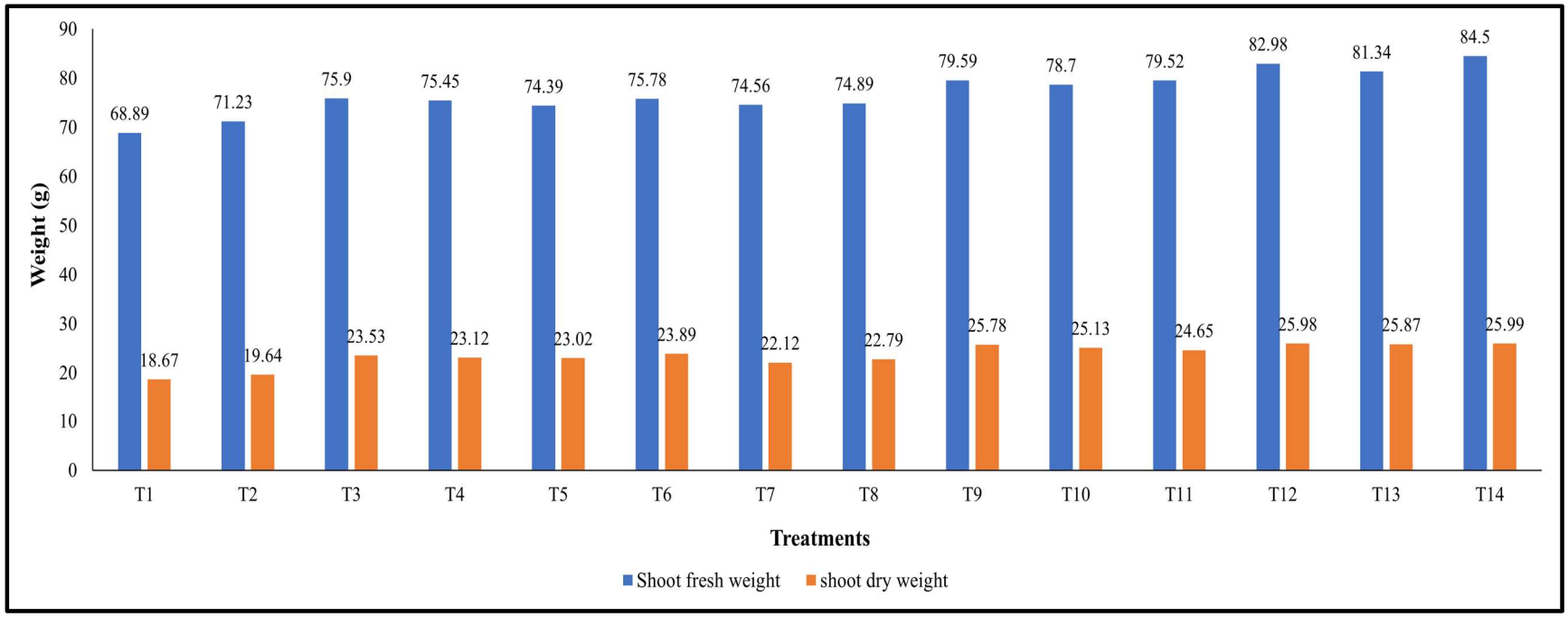


Fig. 4.10 Effect of single and consortia bacterial inoculations on shoot fresh and dry weight at harvest

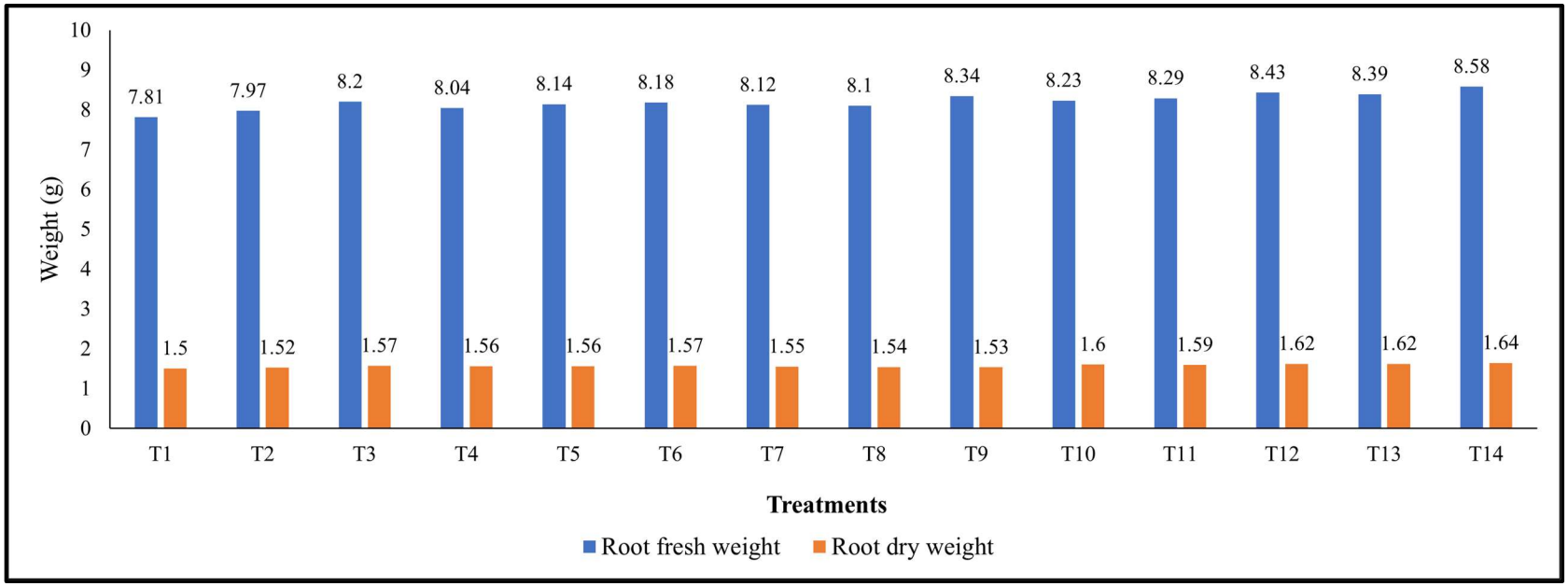


Fig. 4.11 Effect of single and consortia bacterial inoculations on root fresh and dry weight at active tillering

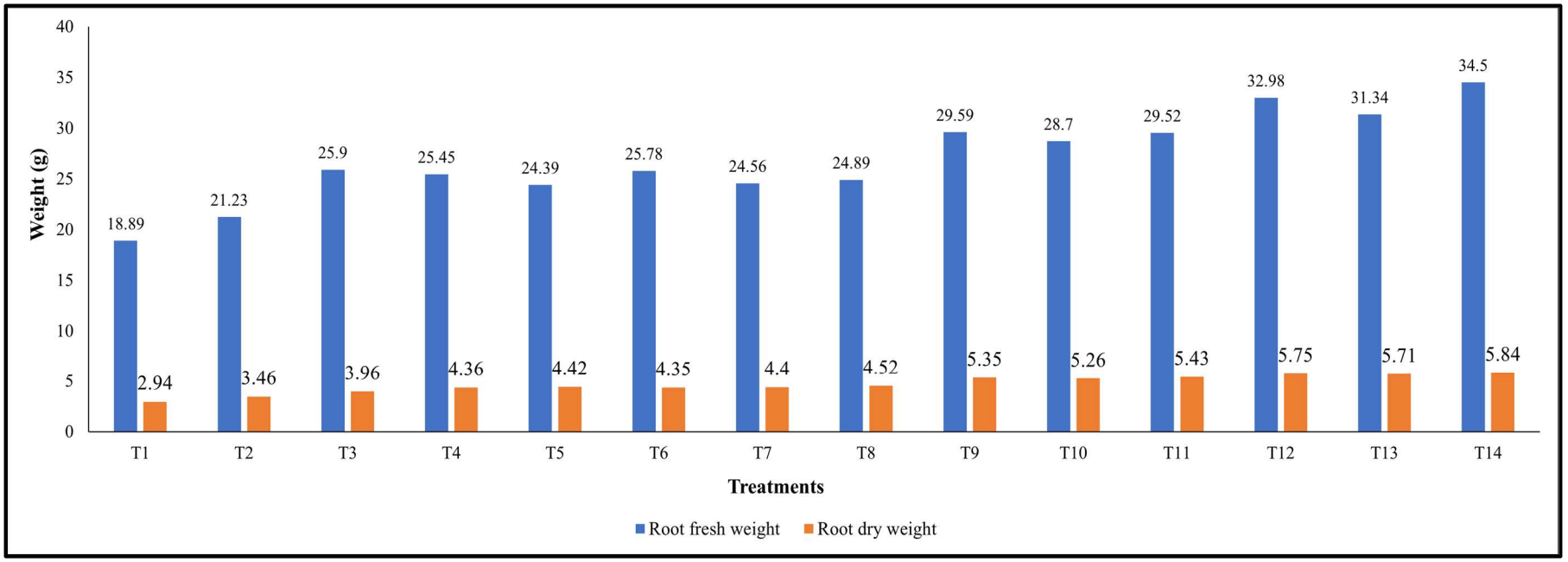


Fig. 4.12 Effect of single and consortia bacterial inoculations on root fresh and dry weight at harvest

4.7.3 Effect of single and consortia bacterial inoculations on yield parameters and yield of rice

4.7.3.1 Tiller number per hill

Number of tillers per hill of different treatment ranged from 7.67 to 11.87 (Table 4.14). The treatment T14 has significantly produced highest tiller number of 11.87 per hill when compared with all the treatments. It is followed by 10.76 tillers per hill being produced by T12. The treatments T1 and T2 has recorded lowest tiller number per hill i.e, 7.67 and 8.34 per hill respectively.

A similar study to evaluate the performance of PGPR on growth and yield characteristics in rice was conducted by Yadav *et al*. (2014). The treatment combination of PGPR [Combined *Psuedomonas* Culture (CPC) with *A. chroococcum*, *A. brasilense* and 60 kg ha^{-1} P2O5] showed maximum significant plant growth attribute as compared to controls. The number of tiller $hill^{-1}$ showed significantly greater 11.67 in treatment combination of CPC with *Azotobacter chroococcum*, *A. brasilense* and 60 kg ha^{-1} P2O5 when compared with control.

4.7.3.2 Panicle length

Panicle length of various treatments ranged from 13.16 cm to 18.76 cm (Table 4.14 and Fig. 4.13). Panicle length was maximum in treatment T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] with a length of 18.76 cm followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] with panicle length of 18.06 cm. The treatments control and T2 (100% RDF) recorded the panicle length of 13.16 cm and 14.53 cm respectively, which were lower than the treatments receiving bacterial inoculations.

Similar results of bacterial consortia increasing panicle length in rice was reported by Rois-Ruiz *et al*. (2020) in which bacterial consortia containing a combination of 3 isolates was applied to rice plants along with 100 % N. Highest panicle length was produced with consortia + 100% N when compared with individual inoculations and control treatment. The consortia consisted of *B. ubonensis* la3c3*, C. bitternis* p9a3m, *B. vietnamiensis* la1a4.

4.7.3.3 Number of grains per panicle

Number of grains per panicle among different treatments ranged from 106.92 to 131.28 (Table 4.14 and Fig. 4.14). No. of grains were found to be maximum in T14 100%

RDF + Bacterial consortia (Seed treatment + Seedling root dip] (131.28) when compared with all treatments followed by T12 [100% RDF + Bacterial consortia (Seed treatment)] (129.36). Control treatment has produced the least number of filled grains (106.92) which is followed by T2 (100% RDF) with 110.05.

Related results on number of grains per panicle were obtained by Rois-Ruiz *et al*. (2020) when the rice plants were inoculated with consortia containing three different strains of bacteria *Burkholderia ubonensis*, *Citrobacter bitternis* and *Burkholderia vietnamiensis* along with 100% N. The combination of three bacteria has produced a greater number of grains per panicle, when compared with individual inoculations and treatment with no bacterial inoculation.

4.7.3.4 Test weight (1000 seed weight)

Weight of 1000 seeds at harvest stage differed significantly as influenced by bacterial inoculations. The test weight in different treatments ranged from 16.23 g to 18.97 g (Table 4.14). The treatment T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] showed maximum test weight of 18.97g followed by treatment T13 [100% RDF + Bacterial consortia (Seedling root dip)] with 18.43 g. Test weight was lowest in control treatment (16.23g) which is followed by T2 (100% RDF) 17.12 g.

Related results of test weight in rice were reported by Chandra *et al*. (2021) when the crop was inoculated with consortia (*Ochrobactrum anthropi* + *Pseudomonas palleroniana* + *P. fluorescens* + *P. palleroniana*) followed by consortia containing (*Pseudomonas sp* + *Achromobacter marplatensis* + *Achromobacter sp* + *Varovorax sp*) when compared with uninoculated treatments and other combination of bacterial strains.

4.7.3.5 Grain yield

Grain yield of 35.60 g/plant was found to be highest with treatment T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip] followed by treatment T12 [100% RDF + Bacterial consortia (Seed treatment)] with yield of 35.35 g/plant (Fig.15). Grain yield was found to be lowest with T1 (30.70 g/plant) and followed by T2 (100% RDF) with 31.83 g/plant (Table 4.14). In this study the grain yield was found to be highest with plants treated with consortia both by seed treatment and seedling root dip method when compared with control and T2 (100% RDF) treatments.

Comparable results of grain yield per plant were obtained by Joshi *et al*. (2020) in rice when the consortia of EN 121+ EN 108+ EN 43 was inoculated with rice plants. This consortium has produced a grain yield of 34.14 g/plant which was higher than the uninoculated treatment and other single inoculations. Similar results of grain yield were obtained when the PGPR consortia containing combined pseudomonas cultures + *A. chroococcum* + *A. brasilense* and 60 kg ha^{-1} P2O5 were applied to rice plants by Lavakush *et al*. (2014) when compared with control and individual treatments receiving various fertilizer doses.

Table 4.14 Effect of single and consortia bacterial inoculations on yield parameters and yield of rice

Treatments	Tiller number per hill	Panicle length(cm)	Filled grain per panicle	Test weight(g)	Grain yield(g/plant)
T1	7.67	13.16	106.92	16.23	30.70
T2	8.34	14.53	110.05	17.12	31.83
T3	9.23	16.56	118.15	17.89	33.84
T4	8.67	15.53	115.43	17.48	32.92
T5	9.12	16.23	113.38	17.3	32.81
T6	9.18	16.34	118.23	17.78	33.65
T7	8.95	15.72	117.28	17.49	33.21
T8	8.79	15.56	115.96	17.29	33.38
T9	9.34	17.48	124.54	17.97	34.56
T10	9.67	17.24	121.63	17.93	34.24
T11	9.34	16.92	120.87	17.89	34.21
T12	10.76	18.06	129.36	18.07	35.35
T13	10.66	17.87	127.96	18.43	34.96
T14	11.87	18.76	131.28	18.97	35.6
CD (0.05)	0.39	1.74	6.27	0.68	1.47
CV%	2.48	6.35	3.12	2.31	2.62

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

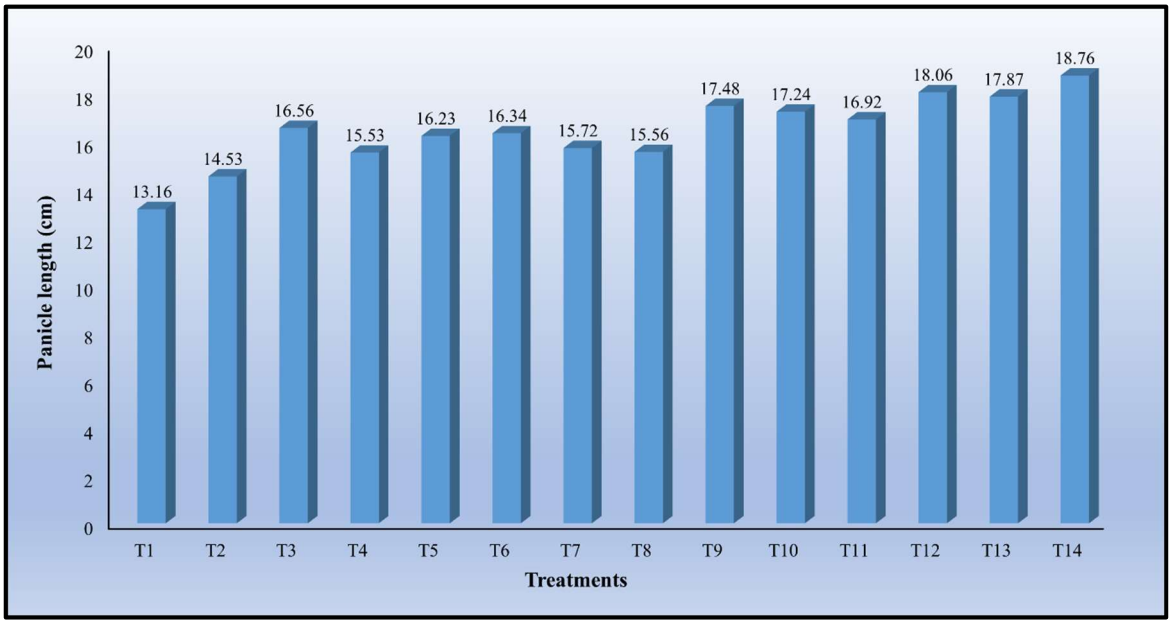


Fig. 4.13 Effect of single and consortia bacterial inoculations on panicle length

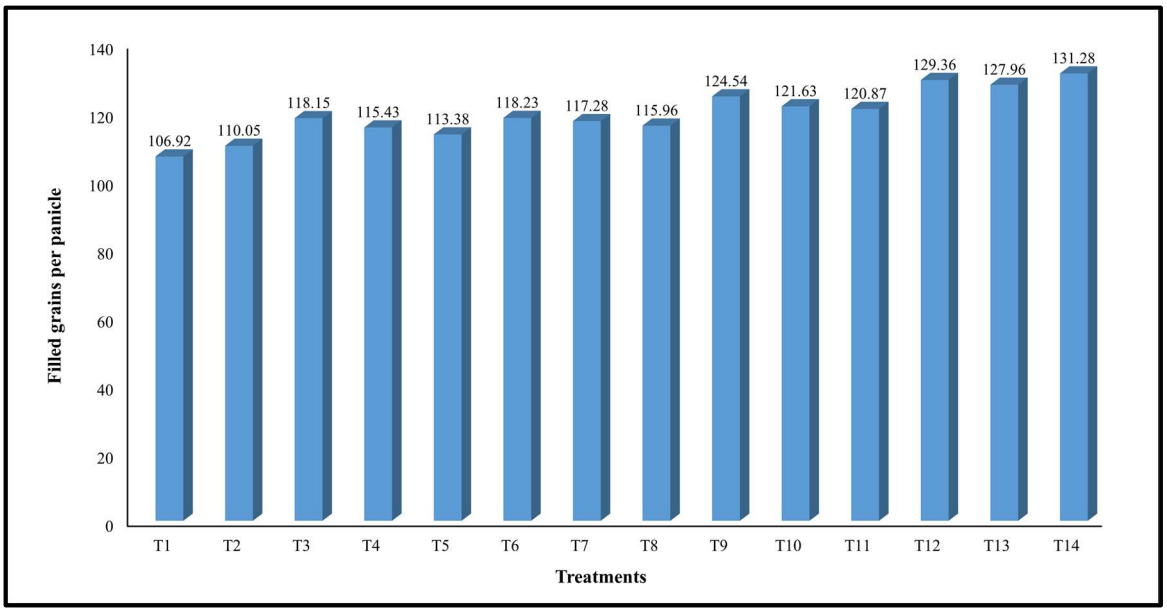


Fig. 4.14 Effect of single and consortia bacterial inoculations on filled grains

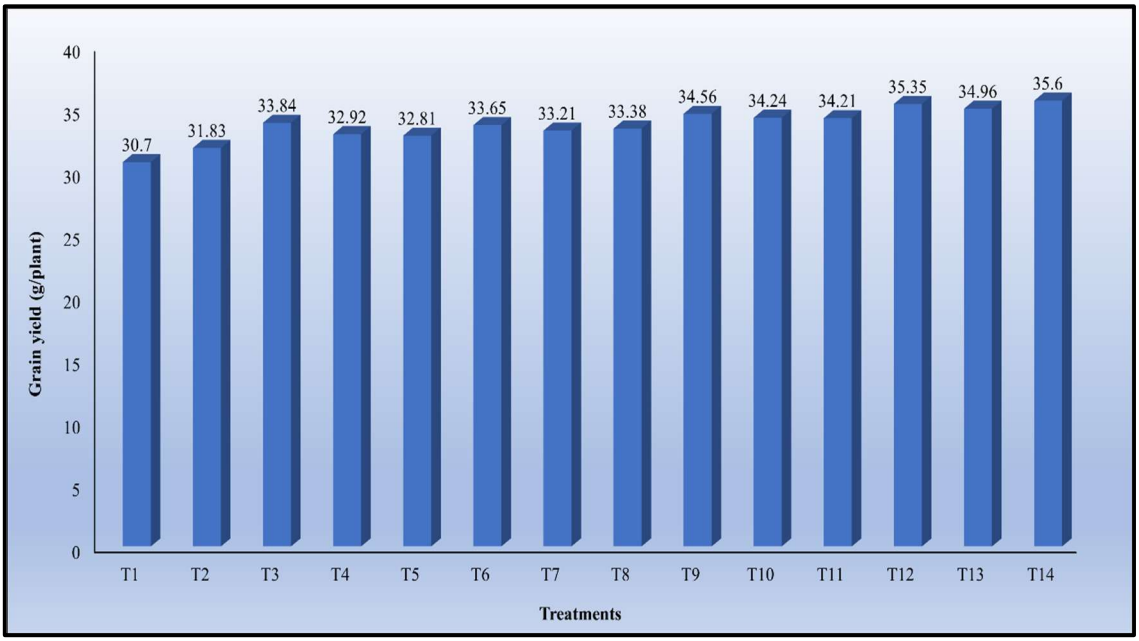
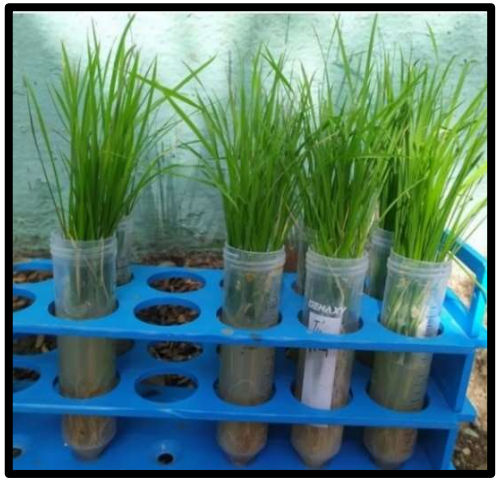


Fig. 4.15 Effect of single and consortia bacterial inoculations on grain yield

Seed treatment of rice

Raising seedlings in trays



Seedling root dip of rice

Effect of single and consortia bacterial inoculations on growth of rice

Plate 4.18 Overview of pot culture experiment

4.7.4 Nutrient uptake

4.7.4.1 N, P and K content in grain

The N, P and K content in grain was observed to be increased significantly on application of bacterial isolates with chemical fertilizers. The nutrient content of the grain ranged from 1.8-2.1% N, 0.2-0.31% P and 0.47-0.61% K (Table 4.15). Maximum N content was observed in T14[100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)] followed by T13 [100% RDF + Bacterial consortia (Seedling root dip)]. Maximum P and K content was observed in T14 followed by T12. When compared with other treatments, the least nutrient uptake by grain was observed with T1 (Control) and T2 (100% RDF).

4.7.4.2 Fe and Zn content in grain

The Fe and Zn content in grain was also observed to be enhanced significantly on application of rhizobacterial isolates. The Fe and Zn content in grain ranged from 22.34 - 31.98 ppm and 1.04 – 1.21 ppm respectively (Table 4.15). Highest Fe content in grain was observed in T14 followed by T12. Highest Zn content of grain was observed in T14 followed by T13. And the least Fe and Zn content was observed in treatments T1 (control) and T2 (100% RDF).

4.7.4.3 N, P and K content in straw

The N, P and K content of straw was observed to be increased significantly on application of bacterial isolates along with chemical fertilizers. The nutrient content of the straw ranged from 0.68 - 0.8% N, 0.11 - 0.2 % P and 1.39 - 1.5 % K (Table 4.15). Maximum N content in straw was observed in T14 followed T12 and T13. Maximum content of P was recorded in T14 followed by T13. Highest K content in straw was observed in T14 followed by T12. And the lowest N, P and K content of straw was recorded with T1 (control) and T2 (100% RDF).

4.7.4.4 Fe and Zn content in straw

The Fe and Zn content in straw was also observed to be enhanced on application of bacterial isolates with chemical fertilizers. The Fe and Zn content in the straw ranged from 41.06 - 50.34 ppm and 1.37 - 1.57 ppm respectively (Table 4.15). Fe and Zn content in the straw was maximum in T14 followed by T12. The treatments T1 (control) and T2 (100% RDF) observed the least Fe and Zn content in straw when compared with treatments inoculated by individual partners and bacterial consortia.

Table 4.15 Effect of single and consortia bacterial inoculations on nutrient uptake at harvest

Treatments	N %		P %		K %		Fe (ppm)		Zn (ppm)	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T1	1.8	0.68	0.2	0.11	0.47	1.39	22.34	41.06	1.04	1.37
T2	1.83	0.7	0.23	0.12	0.5	1.41	24.01	42.2	1.11	1.4
T3	1.88	0.73	0.26	0.13	0.53	1.45	25.64	44.83	1.14	1.45
T4	1.86	0.72	0.24	0.12	0.52	1.43	24.15	44.28	1.15	1.43
T5	1.87	0.72	0.25	0.13	0.53	1.44	24.27	43.18	1.13	1.44
T6	1.89	0.74	0.25	0.14	0.54	1.44	25.14	44.51	1.15	1.49
T7	1.87	0.73	0.24	0.13	0.52	1.43	25.39	43.29	1.14	1.46
T8	1.89	0.74	0.25	0.13	0.53	1.44	24.19	43.67	1.16	1.45
T9	1.93	0.76	0.28	0.16	0.55	1.47	27.76	46.34	1.16	1.48
T10	1.92	0.77	0.28	0.15	0.56	1.46	27.24	46.23	1.17	1.45
T11	1.92	0.76	0.27	0.15	0.54	1.46	26.29	45.56	1.15	1.47
T12	1.96	0.79	0.3	0.18	0.59	1.49	29.17	48.12	1.18	1.51
T13	1.98	0.79	0.29	0.19	0.57	1.48	29.1	48.01	1.19	1.5
T14	2.1	0.8	0.31	0.2	0.61	1.5	31.98	50.34	1.21	1.57
CD (0.05)	0.08	0.03	0.028	0.016	0.057	0.06	2.811	4.784	0.04	0.05
CV %	2.71	3.02	6.424	6.626	6.31	2.47	6.385	6.308	2.26	2.42

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

4.7.5 Soil characteristics after harvest

The available nutrients i.e, nitrogen, phosphorus, potassium, iron and zinc contents in soil as influenced by application of bacterial isolates were presented in the Table 4.16.

4.7.5.1 pH and EC of soil after harvest stage

The pH and EC of soil at harvest stage ranged from 7.7 to 7.74 and 0.62 to 0.67 respectively. No major change in pH and EC was observed across the treatments. The results of pH and EC are shown in Table 4.16.

4.7.5.2 Available N, P, K content in soil after harvest

The soils were collected for the estimation of available N, P, K in soil before sowing and after harvest of the crop. From the data given in the Table 4.16 it was shown that there was a significant influence of the treatments on the available soil nitrogen, phosphorus and potassium with maximum availability recorded in the treatment T14 which include 100% RDF+ bacterial consortia (seed treatment + seedling root dip) with the values of 204.78 kg/ha, 31.07 kg/ha and 272.25 kg/ha respectively (Table 4.16). The least availability of N, P, K was found in control followed by T2 (100% RDF).

4.7.5.3 Available iron (ppm) and Available zinc (ppm) after harvest

The values of Fe and Zn contents in soil ranged from 3.22- 4.46 ppm and 0.31 – 0.52 ppm (Table 4.16) and were significantly influenced by treatments. Fe and Zn content in soil after harvest was found to be maximum in T14[100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)] followed by T12 [100% RDF + Bacterial consortia (Seed treatment)]. The treatment T1 (control) and T2 (100% RDF) have reported the least availability of Fe and Zn in soil after the harvest, when compared with other treatments.

Similar study on the effect of bio-NPK consortium on growth, yield and nutrient uptake by rice was performed by Gohil *et al*. (2021). This study revealed that application of RDF + 75% Zn + 75% Fe + bio-NPK consortia significantly produced higher grain yield, straw yield and uptake of N, P, S, Fe and Zn by rice grain and straw. The Fe content in straw and Zn content in grain increased significantly with application of RDF + 100% Zn + 100% Fe + bio-NPK consortium. But the available N, P, K, Zn content in soil after harvest of rice failed to show any significant influence with different treatments. Fe content soil was significantly found higher in the treatment with RDF+ 75% Fe.

Table 4.16 Effect of single and consortia bacterial inoculations on pH, EC and soil available nutrients at harvest

Treatments	pH	EC (ds m^{-1})	Available N(kg/ha)	Available P(kg/ha)	Available K(kg/ha)	Fe(ppm)	Zn(ppm)
T1	7.7	0.62	186.45	23.75	259.15	3.22	0.31
T2	7.7	0.63	193.98	25.67	262.17	3.46	0.37
T3	7.72	0.65	198.86	27.93	264.87	3.75	0.42
T4	7.71	0.66	199.23	25.45	264.73	3.59	0.39
T5	7.7	0.64	195.01	25.87	263.15	3.6	0.4
T6	7.72	0.65	196.45	26.78	265.46	3.82	0.38
T7	7.71	0.64	194.49	25.15	266.49	3.54	0.39
T8	7.71	0.65	198.54	26.34	265.23	3.72	0.42
T9	7.72	0.66	200.45	28.1	267.94	4.08	0.47
T10	7.7	0.65	199.67	28.56	268.54	3.89	0.45
T11	7.73	0.65	199.12	27.95	267.78	3.71	0.44
T12	7.74	0.67	202.12	29.73	270.87	4.28	0.49
T13	7.73	0.66	200.89	29.28	270.56	4.23	0.48
T14	7.74	0.67	204.78	31.07	272.25	4.46	0.52
CD (0.05)	NS	NS	8.88	2.9	6.60	0.409	0.016
CV %	2.12	6.226	2.68	6.328	6.219	6.384	6.472
Initial	7.75	0.65	267.34	38.34	306.23	6.89	0.89

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

4.7.6 Soil enzyme activity

4.7.6.1 Soil dehydrogenase activity

Dehydrogenase activity differed significantly among the different treatments of bacterial inoculations. The dehydrogenase activity in soil ranged from 89.9 – 106.28 µg TPF g^{-1} soil $24h^{-1}$ (Table 4.17). The highest activity was found in treatment T14 with a value 106.28 µg TPF g^{-1} soil $24h^{-1}$ of followed by T13 with 104.53 µg TPF g^{-1} soil $24h^{-1}$. The dehydrogenase activity of soil was found to be low in T1 (89.9 µg TPF g^{-1} soil $24h^{-1}$) and T2 (100% RDF) 92.72 µg TPF g^{-1} soil $24h^{-1}$with which has no bacterial inoculation.

The dehydrogenase activity is microbiological in origin and represents the oxidoreduction reaction in the respiration process which is a good indicator of soil microbiological activity (Singh and Singh., 2005).

Stephen *et al*. (2015) studied the effect of phosphate solubilising bacterial inoculation among various treatment on soil dehydrogenase activity in rice cultivated under pot culture conditions. In this study the treatment with *Gluconacetobacter sp* + *Burkholderia sp* + RP60 has significantly produced highest dehydrogenase activity (40.20 µg TPF g^{-1} soil) when compared with other treatments. This study revealed that effect of dehydrogenase activity was more prominent in mixed culture inoculums when compared with single culture inoculums.

4.7.6.2 Fluorescein diacetate hydrolysis

There was a significant increase in fluorescein diacetate hydrolysis activity among the different treatments. The values of FDA hydrolysis ranged from 50.93 µg FDA g^{-1} soil $0.5h^{-1}$ – 64 µg FDA g^{-1} soil $0.5h^{-1}$. (Table 4.17). The treatment T14 has produced a highest FDA activity 64.23 µg FDA g^{-1} soil $0.5h^{-1}$ among all the treatments which is followed by T13 with 63.58 µg FDA g^{-1} soil $0.5h^{-1}$. The treatment T1 (control) and T2 (100% RDF) has produced the least FDA activity with value of 50.93 µg FDA g^{-1} soil $0.5h^{-1}$ and 57.09 µg FDA g^{-1} soil $0.5h^{-1}$.

FDA hydrolysis has been found to be correlated with microbial biomass in pure and mixed microbial cultures in pastures and cultivated soils (Vekemans *et al*., 1989) and could be used as an alternative estimate of the content of soil microflora.

Prasanna *et al*. (2012) studied the performance of 3 bacterial strains (PR3, PR7 and PR10), 3 cyanobacterial isolates (CR1, CR2 and CR3) and their combinations were evaluated in pot experiment with rice. At harvest stage of crop there was a significant

enhancement in soil biological properties such as flourescien diacetate hydrolysis in the treatment with PR3+ PR10 + CR3 when compared with control and other treatments.

In this study, it was observed that consortia-1 performs better than the control, treatment T2 with 100% RDF, and also treatments with individual bacterial inoculations in improving growth and yield of rice under pot culture conditions.

Table 4.17 Effect of single and consortia bacterial inoculations on dehydrogenase activity and fluorescein diacetate hydrolysis properties of soil

Treatments	Soil Dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$)	Fluorescein diacetate assay (µg FDA g^{-1} soil $0.5h^{-1}$)
T1	89.9	50.93
T2	92.72	57.09
T3	96.87	61.6
T4	96.28	57.32
T5	95.37	58.25
T6	98.1	61.19
T7	98.04	58.3
T8	97.2	57.34
T9	102.31	61.89
T10	101.4	59.49
T11	100.97	59.84
T12	102.79	62.28
T13	104.53	63.58
T14	106.28	64.23
CD (0.05)	4.77	1.88
CV %	2.88	1.90

Treatments

T1: Control

T2: 100% RDF

T3: 100% RDF + *Pseudomonas stutzeri* (Seed treatment)

T4: 100 % RDF + *Stenotrophomonas sp.* (Seed treatment)

T5: 100% RDF + *Achromobacter sp.* (Seed treatment)

T6: 100% RDF + *Pseudomonas stutzeri* (Seedling root dip)

T7: 100% RDF + *Stenotrophomonas sp.* (Seedling root dip)

T8: 100% RDF + *Achromobacter sp.* (Seedling root dip)

T9: 100% RDF + *Pseudomonas stutzeri* (Seed treatment + Seedling root dip)

T10: 100% RDF + *Stenotrophomonas sp* (Seed treatment + seedling root dip)

T11: 100% RDF + *Achromobacter sp.* (Seed treatment + Seedling root dip)

T12: 100% RDF + Bacterial consortia (Seed treatment)

T13: 100% RDF + Bacterial consortia (Seedling root dip)

T14: 100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)

SUMMARY AND CONCLUSIONS

Chapter V

SUMMARY AND CONCLUSIONS

In this study, the bacteria isolated from the rhizosphere of rice were collected from the ICAR- Indian Institute of Rice Research, Rajendranagar, Hyderabad. A total of 32 bacterial isolates were purified and evaluated for their plant growth-promoting traits, eco-physiological stress tolerance and compatibility of bacterial isolates with various agrichemicals which are commonly used in rice cultivation. The promising bacterial isolates selected were evaluated under *in vitro* conditions as single strains and as consortia. The best-performing consortia under *in vitro* conditions were selected for further pot culture experiments.

Among the 32 rhizobacterial isolates, the plant growth-promoting traits such as phosphate, potassium and zinc solubilizations were found to be highest in terms of solubilization index (S.I) in IIRRSS22-18 (3.1), P1 (2.1) and P1 (2.5). And highest siderophore producing activity was found in IIRRSS22- 7 with the index of 4.3. IAA production was highest in 3 isolates (IIRRSS22-1, 17 and 19), ammonia production was found to be highest in 6 isolates (IIRRSS22-1, 9, 2, 24, 25 and 26). And 17 isolates have produced HCN in less quantity. Based on the scores assigned to isolates regarding PGPR traits, the highest scoring bacterial isolates were P1, IIRRSS22-1 and IIRRSS22-6.

The isolates were screened for abiotic stress tolerance such as salinity, temperature and drought under *in vitro* conditions. In the case of salinity tolerance, the isolates were grown under NaCl concentrations of 4%, 6%, and 8%. Out of 32 isolates, 20, 15 and 8 isolates have tolerated 4%, 6% and 8% NaCl concentrations respectively. The isolates which have grown at 8% salinity and showed highest tolerance to NaCl were IIRRSS22-8, 3, 10, 12, 6, 7 27 and R1.

For temperature tolerance, the isolates were incubated at various temperatures such as at 4℃, 15℃, 25℃ and 45℃. And the isolates which have shown growth at 45℃ were considered high temperature tolerant. Among the 32 isolates, 12 isolates (IIRRSS22-8, 10, 11, 6, 9, 3, 4, 7, 24, 27, 1 and O1) were selected as high-temperature tolerant.

Drought tolerance of bacterial isolates was tested by inoculating the bacteria in a media containing Polyethylene glycol (PEG). The highly tolerant isolates were IIRRSS22-9, 2, 3, 12, 16, 20, 5, 7, R1 and P1 with O.D values (>0.5) at -0.73 MPa water

potential which was created using PEG. Based on the scores assigned to isolates with regard to abiotic stress tolerance traits, the highest scoring bacterial isolates were IIRRSS22-3, IIRRSS22-7 and R1.

The isolates were then screened for agrichemical compatibility with fertilizers and pesticides commonly used in rice cultivation. A total of 22, 19 and 18 isolates have shown high tolerance or compatibility with Urea, Single super phosphate and Muriate of potash. The isolates, when tested for compatibility with insecticides such as Cartap, Ferterra and Thiamethaxom about 19, 13 and 6 isolates, have shown high tolerance/compatibility with the insecticides tested.

With herbicides such as pretilachlor and bispyribac sodium, 19 and 17 isolates have shown high tolerance/compatibility. And a total of 20 isolates have shown high tolerance/ compatibility with carbendazim tested. Three isolates have exhibited moderate tolerance for mancozeb tested in this study. Based on the scores assigned to isolates based on their agrichemical compatibility, the highest scoring bacterial isolates were IIRRSS22-5, IIRRSS22-2 and IIRRSS22-4.

Nine isolates, 3 from each category with the highest score were selected and grouped as three consortia combinations (Consortium-1, 2 and 3) containing one isolate from each category and compatibility among the three isolates was checked by cross streak method.

An *in vitro* germination test was conducted with rice seeds Telangana sona (RNR-15048) by treating the seeds with bacterial isolates both individually and as consortia. The highest germination percentage (100%), seedling length (16.16cm), vigor index I (1616) and II (5.47) were observed after seed treatment with consortium -1 (IIRRSS22-5, IIRRSS 22-3 and P1) when compared with uninoculated treatment, individual bacterial treatments and other consortia groups (2 and 3). Therefore consortium-1 (C-1) was selected to be evaluated under pot culture conditions.

A pot culture experiment was taken up during *Rabi* 2021-22 using consortium-1 with 14 different treatments involving control, individual consortia partners applied as seed treatment, seedling root dip and seed treatment + seedling root dip. The plant morphological and yield parameters were recorded at the active tillering stage and at the harvest stage. The root length, shoot length, leaf area, plant biomass and tiller number per hill at active tillering and at the harvest stage were found to be highest with treatment T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)].

The yield parameters of rice such as panicle length, filled grains per panicle, test weight, and grain yield as affected by different treatments were found to be highest in treatment T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)]. The soil available nutrients and the nutrient uptake by the plants were also highest in the treatment T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)]. The soil enzyme activities such as dehydrogenase and fluorescein diacetate activity were also highest in T14 [100% RDF + Bacterial consortia (Seed treatment + Seedling root dip)] when compared with control and treatment receiving 100% RDF.

Based on the results obtained, the present study showed that the consortia (C-1) of rhizobacterial isolates with multiple plant beneficial traits along with tolerance to ecophysiological stresses has enhanced rice plant growth and yield. This study also suggests that use of plant-beneficial bacteria as a consortium results in better plant performance rather than as a single strain. Therefore, this study has identified a combination of rhizobacteria that can be used as consortia for improving the growth and yield of rice under pot culture conditions.

The molecular identification of selected bacterial isolates was done by 16S rRNA gene sequencing and the sequences have been deposited in the NCBI (National Centre for Biotechnology Information database. The isolates were identified as *Achromobacter sp* OP592244 (IIRRSS22-5), *Achromobacter insuavis* OP580150 (IIRRSS22-6), *Rhizobium sp*, OP580066 (IIRRSS22-1), *Stenotrophomonas maltophilia* OP580125 (IIRRSS22-2), *Stenotrophomonas sp* OP592247 (IIRRSS22-3), *Ochrobacter anthropi* OP580149 (IIRRSS22-4). *Achromobacter xylosoxidans* OP580160 (IIRRSS22-7).

Future line of work:

- Evaluating the potential of bacterial consortia (C-1) under pot culture conditions by inducing drought and salinity stress followed by assessing the performance of the consortia under field conditions along with different IPM practices.
- Screening of bacterial consortia (C-1) under different agroclimatic zones experiencing high temperatures/moisture stress and under stressed soil conditions such as salinity, sodicity and soils with agrichemical contamination.

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APPENDICES

APPENDIX I

Composition of different growth media/reagents used

ALEKSANDROV AGAR	
Magnesium sulphate	0.50 g
Calcium carbonate	0.10 g
Potassium aluminium silicate	2.00 g
Glucose	5.00 g
Ferric chloride	0.005g
Calcium phosphate	2.00 g
Agar	20.00 g
Final pH	7.2 ± 0.2
NUTRIENT AGAR	
Peptone	5.00 g
Sodium chioride	5.00 g
Yeast extract	1.50 g
Distilled water	1000 ml
pH	7.4 ± 0.2
NUTRIENT BROTH	
Peptone	5.00 g
Sodium chloride	5.00 g
Yeast extract	1.50 g
Distilled water	1000 ml
pH	7.4 ± 0.2
PIKOVSKAYA AGAR	
Yeast extract	0.05 g
Dextrose	10.00 g
Tri calcium phosphate	5.00 g
Ammonium sulphate	0.50 g
Potassium chloride	0.20 g
Manganese sulphate	0.0001g
Magnesium sulphate	0.10 g
Ferrous sulphate	0.0001 g
Agar	20.00 g
Distilled water	100 ml
Final pH	7.0

TRIS MINIMAL MEDIA

Glucose	10.00 g
Zinc oxide	1.00 g
Ammonium sulphate	0.50 g
Potassium chloride	0.20 g
Yeast extract	0.50 g
Ferrous sulphate	0.01 g
Manganese sulphate	0.01g
Dipotassium hydrogen phosphate	0.25 g
Agar	20.00 g
Distilled water	1000 ml

CAS MEDIA

Solution 1:

Chrome azurol S	60.5 mg
Distilled water	50.00 ml

Solution 2:

1mM FeCl	10.00 ml

Solution 3:

HDTMA	72.9 mg
Distilled water	40.00 ml

Solution 4:

Solution 1, 2 mixed, then added slowly to solution 3 under continuous stirring. Resultant dark blue solution (autoclaved).

Solution 5:

Dextrose	10.00 g
K2HPO4	4.00 g
KH2PO4	1.00 g
Ammonium sulphate	1.00 g
Magnesium sulphate (1g in 5 ml)	0.5 ml
Trace elements	0.1 ml
Distilled water	250 ml

Trace elements

Zinc sulphate	44 mg
Copper sulphate	40 mg
MnSO4	41 mg
Potassium iodide	42 mg

Distilled water	100 ml

Solution 6:

8- hydroxy quinolone	250 mg
Chloroform	50 ml

Solution 7:

Add solution 6 to 5 in a separatory funnel, mix well, remove the iron chloroform mixture in the bottom layer. Repeat the procedure till the light green colour in the chloroform precipitate disappears. Make up the media to 750 ml with distilled water.

Solution 8:

Add pipes solution to solution 7

Pipes	30.24 g

Adjust the pH to 6.8 using autoclaved NaOH (50%)

Solution 9:

Casmino acids	3.0 g
Distilled water	30.00 ml

All the mixed solutions autoclaved seperately

Media:

Mix solutions 4, 8 and 9 thoroughly and pour to plates (blue colour)

Reagents

Salkowski reagent

35% perchloric acid	50.00 ml
0.5 M FeCl3	1.00 ml

Picric acid

Picric acid	0.5 g
Distilled water	100 ml

Nessler s reagent

Mercuric chloride	10.00 g
Potassium iodide	7.00 g
Sodium hydroxide	16.00 g
Water (Ammonia free)	100.00 ml
Final Ph (at 25°C)	13 ± 0.05