EVALUATION OF *Gluconacetobacter diazotrophicus* AND *Bacillus subtilis* FOR ENHANCING WATER DEFICIT STRESS TOLERANCE IN RICE (*Oryza sativa* L.)

M. Sc. (Ag.) Thesis

by

MAHAPATRA SMRUTHI SAGARIKA

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
COLLEGE OF AGRICULTURE RAIPUR
FACULTY OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
RAIPUR (Chhattisgarh)

2017

EVALUATION OF *Gluconacetobacter diazotrophicus* AND *Bacillus subtilis* FOR ENHANCING WATER DEFICIT STRESS TOLERANCE IN RICE (*Oryza sativa* L.)

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

MAHAPATRA SMRUTHI SAGARIKA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Master of Science

in

Agriculture
(Microbiology)

Roll No. 120115036 U.ID. 20151622450

JUNE, 2017

CERTIFICATE - I

This is to certify that the thesis entitled **"Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L.)"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **M. Smruthi Sagarika** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate, award etc.) or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by her.

Co-Chairman

21.7.17
Chairman

Date:

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman	(Dr. S.B. Gupta)	21.7.17
Co-Chairman	(Dr. P. C. Latha)	
Member	(Smt. D. Dash)	
Member	(Dr. K.P. Verma)	
Member	(Dr. Gayatri Chandrakar)	
Member	(Dr.Tapas Chowdhury)	

CERTIFICATE - II

This is to certify that the thesis entitled **"Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L.)"** submitted by **M. Smruthi Sagarika** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the Department of Agril. Microbiology has been approved by the external examiner and Student's Advisory Committee after oral examination.

Date: 30/8/2017

Signature External Examiner
(Name) S.R.S. Raghuwanshi

Major Advisor _____ 30.8.17

Head of the Department /Section _____ 8.9.17

Dean Faculty _____

Approved/Not approved

Director of Instructions _____

ACKNOWLEDGEMENT

Research needs the close co-operation of the friends and colleagues and the guidance of experts in the field to achieve something worthwhile with light patience, vigour and dedication of the person.

I thank a lot to Almighty "GOD" for giving me the chance becoming a important part of a wonderful journey of research in which he keeps candle lights of hopes ever burning before my vagrant steps and always showered blessing on me without whose endless benevolence and blessing this tedious task could not have been accomplished.

With a sense of high determination and reverence, I would like to give my sincerest thanks to my major advisor ***Dr. S.B. Gupta****, Professor and Head of the department, Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), for his precious instructions, extra hard work, broad and philosophical knowledge, unique supervision. His patient instructions and valuable time sparing has given me a great inspiration and helped at every step during my research duration and it was certainly my pleasure to accomplish this thesis under his supervision. . I am equally indebted and with a great reverence I express my heartfelt sense of gratitude to Co-chairman* ***Dr. P.C. Latha,*** *Senior Scientist (Department of Soil Science), ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad (T.S.) for suggesting the problem, providing necessary laboratory and screen house facilities and for her healthy criticism in preparing the present manuscript of this thesis to make this task a success. She has been a constant source of inspiration and her love and affection to me will ever be remembered. I express my sincere thanks to co-major advisor* ***Dr. P.C. Latha*** *Senior Scientist, Major advisor* ***Dr. S. B. Gupta****, Professor and Head of the department, Department of Agricultural Microbiology, and respected members of my advisory committee* ***Dr. D. Dash****, Department of Agricultural Microbiology,* ***Dr. K.P. Verma****, Department of Plant Pathology****, Dr. Gayatri Chandrakar****, Department of Agricultural Statistics,* ***Dr. Tapas Chowdhury****, Department of Agricultural Microbiology, Raipur, for their critical suggestions, keen co-operation and kind help rendered as and when needed.*

I wish to record my sincere thanks to ***Dr. S.K. Patil****, Hon'ble Vice Chancellor,* ***Dr. S.S. Sha****w, Director of Instructions and* ***Dr. S.S. Rao****, Director Research, IGKV, Raipur for providing me the necessary facilities for research work. Most humbly and respectfully, I wish to express my profound sense of gratitude to* ***Dr. O.P. Kashyap****, Dean, College of Agriculture, IGKV, Raipur for his excellent guidance, valuable suggestion, memorable advices and encouragement which is the vital source of inspiration in my life.*

I take this opportunity to express sincere thanks to ***Dr. Ravindra babu****, Director ICAR-IIRR, Hyderabad who permitted me to work at IIRR. I wish to record my grateful thanks to* ***Dr. P.C. Latha*** *(Senior Scientist),* ***Dr. Bandeppa***

(Scientist,) ***Dr. K. Surekha*** *(Principal Scientist and Head of Soil Science Department) ICAR-IIRR Hyderabad providing critical comments and valuable suggestions rendered as and when needed.*

I would like to express my sincerest thanks to all the scientists and other members of the Department of Agricultural Microbiology, COA, IGKV, Raipur for their academic technical and logistic help.

I can't avoid express my sincere thanks to, Mr. Murali Krishna (TO), Dr. ***C. Chandrakala (SRF), Mr. Md. Imran Mir (SRF), Mr. K.V. Prasad (TA), G. Rajani (TA)*** *for their valuable suggestion and support. I thank other staff members of Department of Soil science microbiology, IIRR, Hyderabad for their kind help during the course of investigation.*

Words can hardly express the heartfelt gratitude to my beloved father ***Shri. Rabindranath Mahapatra****, Mother* ***Smt. Bimala Mahapatra****, whose selfless love, filial affection, obstinate sacrifices and blessing made my path easier. My most cordial thanks goes to my Sister,* ***Preethi Parimitha Mahapatra****,* ***Uppendera Rath****,* ***Biswajit Mahapatra****,* ***Susmita Mahapatra*** *and my all family members relatives for their love affection, blessing and constant inspiration to continue my study.*

Beside them, I would like to convey my greatest thanks to ***Naga Bhushan Naidu, R.K. Sharma, M. Padmavathi,*** *and all others who were always there to help me during the bad phase of my life.*

I wish to express thanks to my seniors, Prahlad sir, Chandrakala mam, Prasad sir, Imran sir, Laxman sir, Rajani mam, Praneet sir, Faisal bhaiya, Naresh sir, Ramesh sir, Supriya mam, Nagendra sir, Venu reddy sir,Madhuri mam, Humera Didi, Mahesh sir, Sandhya mam, Chandu Lal Thakur sir, Lekh Ram Sahu sir and member of friends, Ramya, , Sathish, Anil, Supriya, Srinath, Mahipal, Vijaya Lakshmi, Maithra, Joshna, Swarnalisha, Monalisha, Aparna, Mamata, Bhagyasree Biswajit, Biswaranjan, Dheeraj and my Junior Kavyasree and well-wishers who helped me in various ways towards the present study.

Last but not the least; I would like to convey my cordial thanks to all those unmentioned people who helped me directly or indirectly to fulfil my dream come true.

(Mahapatra Smruthi Sagarika)

Department of Agricultural Microbiology
College of Agriculture, IGKV, Raipur (Chhattisgarh)
Date : 21.7.17

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF NOTATIONS/SYMBOLS

Symbol/notations	Detail
%	Percent
^{0}C	Degree Celsius
C.D	Critical difference
cm	Centimeter
kg	Kilo gram
gm or g	Gram
hill^{-1}	Per hill
L	Liter
m	Meter
ml	Milliliter
mg	Milligram
Mg/ml^{-1}	Microgram per millilitre

LIST OF ABBREVIATIONS

Abbreviations	Detail
et al.	And co-workers / and others
i.e.	that is
viz.	Namely
Fig.	Figure
IAA	Indole acetic acid
VCEAC	Vitamin C Equivalent Antioxidant Capacity
ACC	1-Aminocyclopropane -1-Carboxylate deaminase
MYP	Mannitol Yeast Peptone broth
EPS	Exopolysaccharide
DF	Dworkin and Foster's minimal media
PEG	Poly ethylene glycol
DAS	Days after sowing
DAT	Days after transplanting
RWC	Relative water content
FW	Fresh weight
DW	Dry weight
TW	Turgid weight
IIRR	Indian Institute of Rice Research
IGKV	Indira Gandhi Krishi Vishwavidyalaya
pH	Potentiality of hydrogen
ROS	Reactive Oxygen Species

THESIS ABSTRACT

a)	Title of the Thesis:	"Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L.)"
b)	Full Name of the Student:	Mahapatra Smruthi Sagarika
c)	Major Subject:	Agricultural Microbiology
d)	Name and address of the : Major Advisor	Dr. S. B. Gupta (Professor & Head-Agril. Microbiology) Department of Agricultural Microbiology, IGKV, Raipur (C.G.) 492012
e)	Degree to be awarded:	"Master of Science in Agriculture" (Agricultural Microbiology)

Signature of the Student

21.7.17

Signature of the Major Advisor

Date: 21.7.17

21.7.17

Signature of the Head of the Department

ABSTRACT

The present investigation entitled "Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L.)" was conducted during the year 2016-2017 at ICAR- Indian Institute of Rice Research, Rajendranagar, Hyderabad. Initially the targeted microbes were analysed for different bio-chemical parameters then after these microbes were tested with rice variety- Swarna grown under hydroponic and pot conditions. Vertisols was used for pot grown rice. The pot experiment was laid out in Completely Randomized Block Design with 8 treatments and 3 replications. The treatments comprised of T_1: Control plants without inoculation; T_2: Seed and seedling inoculation with *Bacillus subtilis*; T_3: Seed and seedling inoculation with

Completely Randomized Block Design with 8 treatments and 3 replications. The treatments comprised of T_1: Control plants without inoculation; T_2: Seed and seedling inoculation with *Bacillus subtilis*; T_3: Seed and seedling inoculation with *Gluconacetobacter diazotrophicus*; T_4: Seed and seedling inoculation with *Gluconacetobacter diazotrophicus and Bacillus subtilis* consortia under water stressed condition and similarly under non-stressed condition.

Finding of *in vitro* study clearly showed ability of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for IAA, ACC deaminase and exopolysaccharide production. The highest IAA production (748.04µg / ml), ACC deaminase activity (4.10 µg α-ketobutrate /µg cell protein) and exopolysaccharides production (2.4g/L) was associated with *G. diazotrophicus*. Further, hydroponically grown rice root colonization ability of both the bacteria was conformed under Scanning Electron Microscope (SEM). Then after these bacteria were tested with pot grown rice for different parameters including grain yield.

Data of biomass accumulation by pot grown rice revealed that the highest shoot biomass was observed in T_4 (11.98 gm) under water stress condition and T_3 (17.93gm) under without water stress condition. However, the highest root biomass was associated with T_2 (13.83gm) under water stressed condition and T_2 (14.97gm) also under without stress condition. The maximum number of tillers hill^{-1} and panicles hill^{-1} were observed in T_2 both under water stress and without stress condition. The highest number of grains hill^{-1} was observed in T_4 both under water stress (107.22 hill-^{1}) and without stress condition (108.8hill-^{1}). The total soluble sugar with highest value was quantified in T_4 under both stressed condition (3.82 mg/g FW) and without stress condition (4.63 mg/g FW). Similarly the total antioxidant values of the leaf extract was quantified higher in T_4 under both stressed condition (194.46 mg/g extract) and without stress condition (255.33 mg/g extract). Treatment T_3 showed highest Chlorophyll a, Chlorophyll b, Carotenoid and total chlorophyll content among the treatments of water stressed. The highest percentage of relative water content was recorded in T_2 (94.29%) under without stress condition, while in water stressed condition T_1 (94.08%) showed superiority. The highest percentage of electrolyte leakage was associated with T_1 (32.19%) under stressed

condition. However, under non-stressed condition highest percentage of electrolyte leakage was recorded in T_4 (20.21%). The highest percentage of soil carbohydrate was associated with T_4 in both the situations under water stress condition (7.14%) and non-stress condition (7.75%). Culture inoculated plants showed best results as compared to un-inoculated plants.

The findings of present investigation led to a conclusion that dual inoculation of *G. diazotrophicus* and *B.subtilis* found superior over single inoculation. However, in single inoculation *B.subtilis* showed more effectiveness over *G. diazotrophicus* to improves plant growth parameters of rice under both water stressed and non-stressed conditions.

शोधग्रंथ सारांश

अ) शोधग्रंथ का शीर्षक	:	''धान (*ओराइजा सटाइवा एल.*) में पानी की कमी तनाव सहनशीलता बढ़ाने के लिए ग्लूकोनोएिसिटोबैक्टर डाआजोट्रोफिकस और बैसिलस सब्टिलिस का मूल्यांकन''
ब) छात्रा का नाम	:	महापात्रा स्मृति सागारिका
स) प्रमुख विषय	:	कृषि सूक्ष्म जीव विज्ञान
द) मुख्य सलाहकार का नाम एवं पता	:	डॉ. एस.बी. गुप्ता प्राध्यापक एवं विभागाध्यक्ष कृषि सूक्ष्म जीव विज्ञान विभाग, कृषि महाविद्यालय, इंदिरा गांधी कृषि विश्वविद्यालय, रायपुर–492012 (छ.ग.)
ई) प्रदान की जाने वाली उपाधी	:	मास्टर आफ साइन्स इन एग्रीकल्चर (कषि सूक्ष्म जीव विज्ञान)

छात्रा के हस्ताक्षर

मुख्य सलाहकार के हस्ताक्षर 21.7.17

दिनांकः 21.7.17

विभागाध्यक्ष के हस्ताक्षर 21.7.17

सारांश

इंडियन इंस्टीट्यूट ऑफ राईस रिसर्च, राजेन्द्रनगर, हैदराबाद में वर्ष 2016–17 के दौरान धान (*ओराइजा सटाइवा एल.*) को मृदा में पानी की कमी तनाव सहनशीलता बढ़ाने के लिए ग्लुकोनोएिसिटोबैक्टर डायजोट्रोपिकस और बैसिलस सब्टिलिस जीवाणुओं का मूल्यांकन किया गया।

प्रारंभिक रुप में विभिन्न मापदंडो के लिए उपरोक्त सूक्ष्म जीवों का विश्लेषण किया गया था। इसके बाद इन सूक्ष्म जीवों को धान के विभिन्न प्रकार के मापदण्ड में परीक्षण किया गया। यह प्रयोग धान की किस्म स्वर्णा को लेकर वर्टिसाल में किया गया । उक्त प्रयोग गमले में 8 उपचार और 3 प्रतिकृति के साथ कम्पलीटली रैण्डामाइज्ड ब्लॉक डिजाइन में किया गया । उपचारः टी1–कन्ट्रोल अनिवेशित पौधं, टी2–बीज और अंकुरों को बेसिलस सबटिलिस के साथ उपचार, टी3–बीज और अंकुरों को ग्लुकोनोएिसिटोबैक्टर डायजोट्रोपिकस के साथ उपचार, टी4–बीज और अंकुरों को ग्लुकोनोएिसिटोबैक्टर डायजोट्रोपिकस और बैसिलस सब्टिलिस के साथ उपचार पानी के तनावग्रस्थ स्थति में किया गया उसी तरह अगले चार उपचार (ट्रीटमेंट) में पानी के उपलब्धता स्थिती में किया गया।

प्रयोगशाला के अध्ययन में स्पष्ट रुप में पता चला है कि ग्लूकोनोएिसिटोबैक्टर डायजोट्रोपिकस और बैसिलस सबटिलिस में इंडोल एसिटिक एसिड, ए.सी.सी डिऐमीनेज और एक्सपालेसेकेराइड उत्पादन करने की क्षमता है। उच्चतम इंडोल एसिटिक एसिड उत्पादन (748.04 माइकोग्राम /मि.लि.),एसीसी डीएमीनेज (4.10 माइकोग्राम किटोब्यूट्रेट/ माइकोग्राम सेल प्रोटीन) और एक्सोपालीसेकेराइड उत्पादन

ikVhu½ vkj ,DlkikyhlSdjkbM mRiknu ¼2-4 xzke@fyVj½ Xyqdkukf,flVkCkSDVj MkbtkVªkfQDl }kjk fd;k x;k blds vykok LdzhfuZx byDVªkWu ekbdzksLdksih ds rgr nkukZ cSDVhfj;k dks gkbMªksQksfud #i ls tM+ dh कोलानाइजेशन क्षमता की पुष्टि की गई। फिर इन cSDVhfj;k ds lkFk ikS/k o`f} ds fofHkUUk ekin.M RkFkk vukt dh mit ds fy, xeys esa ijh{k.k fd;k x;kA

पौधे वृद्वि पेरामीटर जैसे उच्चतम शूट बायोमास टी4 में पानी के तनावग्रस्थ स्थिति में ¼11-983 xzke$^{\&1}$½ vkj ikuh ds fcuk rukoxzLFk LFkfr esa 17-933 xzke ik;k x;kA gkykfd] mPpre tM+ ck;ksekl Vh2 ikuh ds rukoxzLFk fLFkfr esa¼13-833 xzke$^{\&1}$½ vkj ikuh ds fcuk rukoxzLFk fLFkfr esa ¼14-973 xzke$^{\&1}$½ ik;k x;kA vf/kdre lq[;k dk Vhyj fgy$^{\&1}$ vkj ifudy fgy$^{\&1}$ mipkj Vh2 esa ikuh ds rukoxzLFk fLFkfr esa vkj ikuh ds fcuk rukoxzLFk fLFkfr esa ik;k x;kA vf/kdre vukt dh mit mipkj Vh4 ¼107-220 fgy$^{\&1}$ ½ ikuh ds rukoxzLFk fLFkfr esa vkj ds ikuh ds fcuk rukoxzLFk fLFkfr esa ¼108-88 fgy$^{\&1}$½ ik;k x;k A

ikuh dh deh dh fLFkrh esa mPPkre dqल घुलनशील शक्कर उपचार टी4(3-8276 मिलीग्राम/ ग्राम पत्ती फ्रेश वेट) में ,उच्चतम कुल एंटीऑक्सीडेंट उपचार टी4 (194-46 feyhxzke@xzke ,DlVªDV½esa]mPPkre DyksjksfQy ,] DyksjksfQy ch] dSjksVhukbM vkj dqy क्लोरोफिल कन्टेंट उपचार टी3 में, उच्चतम प्रतिशत रिलेटिव वkWVj dUVsUV mipkj Vh1 ¼94-083 प्रतिशत)में, उच्चतम प्रतिशत इलेक्ट्रोलाइट लीकेज उपचार टी1 (32-199 प्रतिशत)में, उच्चतम प्रतिशत मृदा कार्बोहाइड्रेड उपचार टी4(7-1433 प्रतिशत) में, पाया गया।

जबकि पानी की उपलब्धता की स्थिती में उच्चतम कुल घुलनशील शक्कर उपचार Vh4¼4-6361 मिलीग्राम/ग्राम पत्ती फ्रेश वेट) में ,उच्चतम कुल एंटीऑक्सीडेंट उपचार टी1 (255-33 मिलीग्राम/ग्राम एक्सट्रेक्ट) उच्चतम प्रतिशत रिलेटिव वाटर कन्टेंट उपचार टी2 (94-29 प्रतिशत)में, उच्चतम प्रतिशत इलेक्ट्रोलाइट लीकेज उपचार टी4 (20-218 प्रतिशत)में,इसी तरह mPPkre iतिशत मृदा कार्बोहाइड्रेड उपचार टी4(7-55 प्रतिशत) में, पाया गया।

वर्तमान अनुसंधान से यह निष्कर्षित किया गया कि ग्लुकोनोएिसिटोबैक्टर Mk;tkVªkfidl vkj cSflyl lkfCyfyl dh nksgjh mipkj] ,dy mipkj ls csgrj gSA cSflyl lcfVfyl ikuh ds rukoxzLFk fLFkfr esa vkj fcuk rukoxzLFk fLFkfr;ksa esa /kku ds mRiknu c<+kus ds fy;s Xyqdkukf,flVkscSDVj Mk;tkVªkfidl ls vf/kd izHkkoh ik;k x;kA

CHAPTER - I
INTRODUCTION

Rice, the staple food crop for more than half of the world population is traditionally grown under flooded conditions and uses about 2500- 3000 liters of water throughout its life period to produce 1 kg of rice. Rice thus requires a larger amount of water throughout its life cycle as compared to other crop and is hence highly susceptible to water deficit stress. Water stress is frequently encountered by rice crop at different stages of its life cycle viz., germination, seedling, tillering, flowering and grain filling, resulting in huge loss of productivity as reported by Serraj *et al.* (2011). Drought/ deficit water stress limits rice production by affecting the crop at morphological (reduced germination, plant height, plant biomass, number of tillers, various root and leaf traits), physiological (reduced photosynthesis, transpiration, stomata conductance, water use efficiency , relative water content, chlorophyll content, photosystem II activity, membrane stability, and abscisic acid content), biochemical (accumulation of osmoprotectant like proline, sugars, polyamines and antioxidants) and molecular (altered expression of genes which encode transcription factors and defense related proteins) levels, thereby drastically limiting rice yield as reported by Pandey and Shukla, (2015).

In India, rice is cultivated on 44 million hectares, mostly in *kharif* season (June to September). Approximately 42% of the rice area in the country is rainfed, characterized by erratic and low rainfall with poor crop yields, and close to one-third of the rice area i.e. 6 million ha of uplands and 7 million ha of lowlands is prone to drought as reported by Birthal *et al.* (2015). In Chhattisgarh, rainfed agriculture is the dominant production system and about 74% of the Chhattisgarh plains, 97% of the Bastar plateau and 95% of the Northern Hills are cultivated under rainfed conditions. The most common drought stress period of rainfed rice in the region occurs either at the early growth stage of the crop or at the terminal stage (reproductive phase). Drought is a recurring phenomenon in the rainfed lowland ecosystems in the state and, consequently, the average productivity is low as reported by Pandey *et al.* (2009). The most popular Indian rice varieties such as

Swarna, Samba Mahsuri, IR 64 and MTU 1010 which are highly productive in the irrigated areas are also grown on large acreage in rainfed environments of Chhattisgarh due to their high yield potential and preferred grain quality. However, as these varieties are susceptible to drought, imparting drought tolerance trait to these varieties will be highly useful to the farming community as reported by Singh *et al.,* (2015).

Development of drought-tolerant rice genotypes has been the standard approach used to mitigate the problem of drought stress on rice, and conventional plant breeding techniques have indeed resulted in the development of high-yielding, drought- tolerant varieties. The disadvantages of this approach are that it is time consuming and labour intensive, and could lead to the loss of other desirable traits from the plant gene pool. Due to the genetic complexity of water stress responses in rice, efforts to improve plant tolerance by conventional breeding has been slower than expected. Genetic engineering for improved tolerance to drought are faster, but it comes with its own set of challenges (Ngumbi and Kloepper, 2016). Another drawback of breeding and genetic engineering approaches is that plants are considered as independent entities that are solely regulated by their genetic code and cellular physiology without taking into consideration the ecological aspects of the plant growth and its interaction with the environment and other organisms, especially the micro biota. The plant is currently regarded not as a stand-alone organism but is perceived as a holobiont comprising the host plant and its micro biota where the plant fitness is attributed to both the plant and its associated microorganisms as reported by Vandenkoornhuyse *et al.,* (2015).

Consequently, a microbial based approach to mitigate drought stress can serve as a novel solution for improving rice plant tolerance to restricted water availability. Although the roles of plant growth promoting rhizobacteria in plant growth promotion, nutrient management, and disease control are well known, their roles in the management of abiotic stress such as drought has only recently gained importance stated by Ngumbi and Kloepper, (2016). Relatively few reports have been published on the microbial ability to induce drought stress tolerance

(Timmusk *et al.,* 2013). Most of the research work report the use of plant growth promoting rhizobacteria and fungi that colonize the rhizosphere/endo-rhizosphere of plants and impart drought tolerance. These rhizospheric microbes either directly affect plant growth under stress by producing metabolites like exopolysaccharides, phytohormones, 1-aminocyclopropane- 1carboxylate deaminase and volatile compounds, or indirectly influence the plant processes by induced systemic tolerance mechanism which causes plants to accumulate osmolytes, antioxidants and bring about up regulation or down regulation of stress responsive genes, leading to acquisition of drought tolerance. However, there is still very less research on the effect of endophytic bacteria on water stress tolerance. Alleviation of drought stress tolerance by PGPR has been reported for cereal crops like sorghum, wheat, maize, legumes like pea, green gram, mungbean and vegetables like pepper, tomato and potato (Ngumbi and Kloepper, 2016). Studies on microbially mediated drought stress tolerance in rice reported inoculation of *Trichoderma* (Gussain *et al.,* 2015) and *Pseudomonas* sp., *Arthrobacter* sp. and *Bacillus cereus* (Pandey *et al.,* 2016) for amelioration of drought stress. Microorganisms of rhizospheric and endophytic origin belonging to both bacteria and fungi have been reported to improve the health of rice plants subject to drought stress. One of the earliest reported studies involved the use of osmolyte and indole acetic acid producing rhizobacteria which improved shoot dry weight, root dry weight and number of tillers in inoculated rice plants under severe drought as stated by Yuwono *et al.*, (2005) by improving root proliferation thereby enhancing water uptake. Gussain *et al.* (2015) demonstrated that *Pseudomonas fluorescences* strain P2, *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, *Bacillus cereus* BSB 38 (14B), *Arthrobacter nitroguajacolicus* strainYB3 and strain YB5 alleviated oxidative damage in rice plants grown under drought conditions, thereby enhancing drought tolerance of rice under water deficit conditions. A consortium of two rhizobacteria, *Bacillus amyloliquefaciens* Bk7 and *Brevibacillus laterosporus* B4, along with biochemical elicitors' like salicylic acid and b-aminobutyric acid was observed to improve the health of rice plants and to confer induced systematic tolerance to drought stress as reported by Kakar *et al.,* (2016). Endophytic bacteria *Pseudomonas pseudoalcaligenes* was shown to induce

accumulation of higher concentrations of glycine betain-like compounds leading to improved salinity stress tolerance in rice (Jha *et al.*, 2011) which could also be useful for drought stress tolerance.

Redman *et al.* (2011) reported that fungal endophytes belonging to the genera *Fusarium, Colletotrichum, and Curvularia* confer plant resistance to salt, heat and drought to rice plants. Pandey *et al.* (2016) demonstrated a dose-dependent response of *Trichoderma harzianum* Th-56 in improving drought tolerance in rice by modulating proline, SOD, lipid peroxidation product and DHN / AQU transcript level, and the growth attributes.

Gluconacetobacter diazotrophicus is nitrogen -fixing endophytic bacterium originally associated with sugarcane but considered as plant growth promoting bacteria for diverse crops. It has been found in natural endophytic association with other host plants such as coffee, sweet potato, Cameron grass, rice, finger millet, pine apple and also colonizes and enhances growth in plants like wheat, maize, sorghum and tomato (Luna *et al.*, 2012). Plant growth stimulation by this bacterium has been ascribed not only to N -fixation but also to Phytohormones production (Indole acetic acid and gibberellins), bio control of phytopathogens, mineral nutrient solubilization (phosphorus and zinc) and disease resistance (Eskin *et al.*, 2014).

Recently, Vargas *et al.* (2014), demonstrated, for the first time, that *G. diazotrophicus* can improve drought tolerance in sugarcane cv. SP70-1143, prolonging its survival even after 40 days of withholding water. Abscisic acid and ethylene are stress responsive hormones and their signal transduction responses to drought, was mainly down regulated in inoculated plants and up regulated without the bacteria's presence indicating that partial inhibition of early drought responses. *G. diazotrophicus* is also known to colonize rice and can be hypothesized to impart similar tolerance to water stress in rice, an aspect not investigated so far.

Bacillus subtilis is commonly occurring plant growth promoting bacteria found in association with roots of diversified plants including rice. The direct beneficial multifarious effects of *B. subtilis* strains include induction of induced

systemic resistance, plant growth promotion and disease suppression as reported by Singh *et al.,* (2016). Although considerable progress has been made in understanding the mechanisms underlying Bacillus-mediated tolerance to biotic stress, information on Bacillus strains mitigating abiotic stress symptoms, is limited (Gagné-Bourque, 2015). Role of Rice- *Bacillus subtilis* interaction in ameliorating the effect of drought stress is also less studied and needs to focus upon.

Hence, this investigation was planned with the following objectives to study the effect of inoculation of an endophytic *Gluconacetobacter diazotrophicus* and rhizospheric *Bacillus subtilis* on drought susceptible Swarna variety of rice:

Objectives:

1. Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for plant growth promoting activities, exopolysaccharide production and rice plant colonization.
2. *In vitro* studies on the effect of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* on rice seed germination and seedling growth under PEG induced water stress.
3. Pot culture studies on the effect of the single and combined inoculation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* on vegetative stage water stress tolerance and yield of rice.

CHAPTER- II
REVIEW OF LITERATURE

Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense. Water stress is primarily caused by the water deficit, *i.e.* drought or high soil salinity. In case of high soil salinity and also in other conditions like flooding and low soil temperature, water exists in soil solution but plants cannot uptake it a situation commonly known as 'physiological drought'. Drought occurs in many parts of the world every year, frequently experienced in the field grown plants under arid and semi-arid climates. Regions with adequate but non-uniform precipitation also experience water limiting environments.

Drought stress is among the most destructive abiotic stresses that increased in intensity over the past decades affecting world's food security. Drought stress may range from moderate and short of extremely severe and prolonged duration, restricting the crop yields as reported by these authors (Austin, 1989; Pereira and Chaves, 1993; 1995; Bottner *et al.*, 1995)

(Ashraf, 1994; Vinocur and Altman, 2005; Kasim *et al.*, 2013) these authors stated that drought is expected to cause serious plant growth problems for more than 50% of the arable lands by 2050.

2.1 Effect of water stress on plant growth and development

Water stress in plants reduces the plant cells water potential and turgor, which elevate the solutes concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases leading to growth inhibition and reproductive failure. This is followed by accumulation of abscisic acid (ABA) and compatible osmolytes like proline, which cause wilting. At this stage, overproduction of reactive oxygen species (ROS) and formation of radical scavenging compounds such as ascorbate and glutathione further aggravate the adverse influence. Drought not only affects plant water relations through the reduction of water content, turgor and total water, it also affects stomatal closure,

limits gaseous exchange, reduces transpiration and arrests carbon assimilation (photosynthesis) rates. Negative effects on mineral nutrition (uptake and transport of nutrients) and metabolism leads to a decrease in the leaf area and alteration in assimilate partitioning among the organs. However, water stress influences cell enlargement more than cell division. Plant growth under drought is influenced by altered photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones. Decreased leaf growth, total leaf area and leaf-area plasticity were observed under the drought conditions in many plant species, such as peanut and *Oryza sativa*.

Rice crop affected by drought or water stress have:

1) stunted plants
2) rolled leaves
3) delayed flowering
4) burned tip
5) naturally drying (senescence) leaves
6) whitehead (but the tiller will still be attached to the stem)

Drought affects plant–water potential and turgor, enough to interfere with normal functions changing physiological and morphological traits in plants.

(Hendry, 2005; Smirnoff, 1993; Sgherri *et al.,* 2000) these authors reported that drought also induces free radicals affecting antioxidant defenses and Reactive Oxygen Species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals resulting in oxidative stress. At high concentrations ROS can cause damage to various levels of organization like initiate lipid peroxidation, membrane deterioration and degrade proteins, lipids and nucleic acids in plants.

(Crane *et al.,* 2011; Ahmad *et al.,* 2015; Jiang *et al.,* 2016) these authors reported that plants need light, water, carbon and mineral nutrients for their optimal growth, development and reproduction. Extreme conditions (below or above the optimal levels) limit plant growth and development. An unfavorable environment comprising extreme high or low of temperature, salinity and drought pose a complex set of stress conditions. Plants can sense and react to stresses in

many ways that favor their sustenance. They remember past exposure to abiotic stresses and even mechanisms to overcome them in such a way that responses to repeated stresses can be modified accordingly (Hilker *et al.*, 2015)

(Osakabe *et al.* 2014 and Xu *et al.* 2016) studied that the prolonged water stress decreases leaf water potential and stomata opening, reduces leaf size, suppresses root growth, reduces seed number, size, and viability, delays flowering and fruiting and limits plant growth and productivity.

Bian and Jiang (2009) demonstrated that under severe water deficit conditions, peroxidation may be induced leading to negative impact on antioxidant metabolism. Rewatering further decreases the level of peroxidation and restores growth and development of new plant parts and stomata opening. In roots, both drought and rewatering lead to high accumulation of H_2O_2 Drought responses vary from plant to plant in terms of the activity of superoxide dismutase (SOD) enzyme that plays a central role in antioxidant metabolism as reported by Xu *et al.*, 2015).

2.2 Oxidative stress in plants

Oxidative stress, which frequently accompanies many abiotic stresses like high temperature, salinity, or drought stress, causes a serious secondary effect on cells. Oxidative stress is accompanied by the formation of ROS such as O_2-, $^1O_2$, H_2O, and OH-. ROS damage membranes and macromolecules affect cellular metabolism and play a crucial role in causing cellular damage under drought stress. Drought creates an imbalance between light capture and its utilization, which inhibits the photosynthesis in leaves. In this process imbalance between the generation and utilization of electrons is created. Dissipation of excess light energy in photosynthetic apparatus results in generation of reactive oxygen species (ROS). Denaturation of functional and structural macromolecules is the well-known results of ROS production in cells. DNA nicking, amino acids, protein and photosynthetic pigments oxidation, and lipid peroxidation are the reported effects of ROS.

McCord (2000) reported that plants under various abiotic or biotic stress conditions may lead to the overproduction of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical (O- $_2$) hydrogen peroxide (H_2O_2) and

hydroxyl radical (OH). But out of these three main type of ROS are superoxide radical, hydrogen peroxide and hydroxyl radical. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. These cytotoxic ROS are incessantly produced throughout usual metabolic procedures in the cytoplasm, peroxisomes and mitochondria and they can devastate usual metabolism during oxidative hurt of nucleic acids, proteins and lipids when they are created in overload.

a) Super Oxide: Superoxide (O_2-) is the primary ROS, which is formed when the molecular oxygen undergoes one electron reduction. In this reaction, NADPH supplies the electron and NADPH oxidase acts as the reaction catalyst.

b) Hydrogen peroxide: On further reduction of molecular oxygen hydrogen peroxide (H_2O_2) is formed.

Koji *et al*. (2009) reported that the one electron reduction of superoxide first forms peroxide (O_2^{2-}), which is neutralised by two protons to form hydrogen peroxide. The spontaneous dismutation unlikely occurs at physiological pH when superoxide is in anionic form (O^{2-}) because there is repulsion of superoxides due to negative charge. On the other hand, when the pH is acidic, the proportion of neutral form (HO_2) rises and then the spontaneous dismutation starts to largely participate in hydrogen peroxide formation.

c) Hydroxyl radical: Hydroxyl radicals are most toxic and formed on further reduction. It is an extremely potent oxidant and reacts with organic molecules at nearly diffusion rates. Hydroxyl radicals are formed by two ways.

1. Haber–Weiss reaction: Under normal conditions, this reaction proceeds at a very slower rate and resulting in the low production of OH ions.

$$H_2O_2 + O-_2 \rightarrow OH- + OH + O_2$$

2. Fenton reaction: It is common in biological systems. It occurs in the presence of transition metals like Fe^{2+}, Cu^{+}, etc

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH- + OH$$

The antioxidant systems can be divided into three groups:

1) Antioxidant enzymes
2) Lipid soluble, membrane associated antioxidants, (e.g. α-tocopherol, β-carotene)
3) Water soluble antioxidants (e.g., glutathione and ascorbate)

An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance. Antioxidant enzymes are the most active and efficient protective mechanism. The enzymatic mechanisms are designated to minimize the concentration of O_2 and H_2O_2. The enzymes overproduced so far include catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR).

2.3 PEG Induced Water Stress

Jiang *et al*. 1995 and Ranjbarfordoei *et al.* (2000) reported that the stimulation of drought stress by polyethylene glycol (PEG) induces drought stress on the plants and significant deviation from the control continues to increase with the increasing solute potential (Ψs).

(Jiang *et al.*, 1995; Chezen *et al.*, 1995; Ashraf and O'Leary 1996) these authors reported that PEG-6000 has long been utilized as a reliable marker under laboratory conditions for testing the drought tolerant genotypes. This is because polyethylene glycol acts as a non-penetrating osmotic agent resulting into increasing solute potential (Ψs) and blockage of absorption of water by the root system.

Rubinstein (1982) and Tarkow *et al.* (1996) stated that PEG does not enter the cell wall space and PEG molecules with a molecular weight greater than 3000 are apparently not absorbed. PEG induced significant water stress in plants and not having any toxic effects.

2.4 Plant Growth Promoting Bacteria as Inoculant

The rhizosphere can be defined as the soil region where processes mediated by microorganisms are specifically influenced by the root system. This area includes the soil connected to the plant roots and often extends a few millimeters off the root surface, being an important environment for the plant and microorganism interactions as reported by (Lynch, 1990; Gray and Smith, 2005)

(Dimkpa *et al.*, 2009 a; Grover *et al.*, 2011 and Glick, 2012) these authors conveyed that the plant growth-promoting bacteria (PGPB) belong to a beneficial and heterogeneous group of microorganisms that can be found in the rhizosphere, on the root surface or associated to it, and are capable of enhancing the growth of plants and protecting them from disease and abiotic stresses.

Bacon and Hinton (2006) reported that interactions between plants and bacteria occur through symbiotic, endophytic or associative processes with distinct degrees of proximity with the roots and surrounding soil. Endophytic PGPB are good inoculant candidates, because they colonize roots and create a favorable environment for development and function. Non-symbiotic endophytic relationships occur within the intercellular spaces of plant tissues, which contain high levels of carbohydrates, amino acids, and inorganic nutrients.

To achieve maximum benefits in terms of fertilizer savings and better growth, the PGPB-based inoculation technology should be utilized along with appropriate levels of fertilization. Moreover, the use of efficient inoculants can be considered an important strategy for sustainable management and for reducing environmental problems by decreasing the use of chemical fertilizers.

(Danhorn and Fuqua, 2007; Meneses *et al.*, 2011; Alquéres *et al.*, 2013 and Beauregard *et al.*, 2013) these authors reported that the success and efficiency of PGPB as inoculants for agricultural crops are influenced by various factors, among which the ability of these bacteria to colonize plant roots, the exudation by plant roots and the soil health. The root colonization efficiency of PGPB is closely associated with microbial competition and survival in the soil, as well as with the

modulation of the expression of several genes and cell-cell communication via quorum sensing.

2.5 Role and Mechanism of PGPB to Drought Stress

Grover *et al.* (2011) demonstrated that the role of microorganisms in plant growth, nutrient management and bio-control activity is very well established. These beneficial microorganisms colonize the rhizosphere/endo-rhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms.

(Yang *et al.*, 2009; Dimkpa *et al.*, 2009; Timmusk and Nevo 2011; Kim *et al.*, 2013 and Timmusk *et al.*, 2014) these authors reported that the role of microorganisms in management of biotic and abiotic stresses is gaining importance. The possible explanation for the mechanism of plant drought tolerance induced by rhizobacteria include: (1) Production of Phytohormones like abscisic acid (ABA), gibberellic acid, cytokinins, and indole-3-acetic acid (IAA); (2) ACC deaminase to reduce the level of ethylene in the roots; (3) induced systemic tolerance by bacterial compounds; (4) bacterial exopolysaccharides.

2.6 *Gluconacetobacter diazotrophicus*

Ryan *et al*. (2008) studied that the endophytic bacteria can be defined as those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host.

Khalid *et al.* (2004) reported that these bacteria significantly affect plant growth by increasing nutrient cycling, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances and or by producing biologically active substances such as auxins and other plant hormones.

Cavalcante and Debereiner (1988) reported that *G. diazotrophicus* was discovered within sugarcane (monocotyledon) plants in Alagoas, Brazil. This bacterium can actively fix atmospheric nitrogen and provide significant amounts of nitrogen to plants.

(Gillis *et al.,* 1989; Yamada *et al.,* 1997 and Cavalcante and Dobereiner 1988) these authors reported that *G. diazotrophicus* has been found in places such as Mexico and India and in crops ranging from Sugarcane, Sweet potato, Coffee, Finger millet, Tea, Pineapple, Banana, Carrot, Radish, Beetroot etc. The bacterium was initially named as *Saccharobacter nitrocaptans* and was later classified under acetic acid bacteria and named *Acetobacter diazotrophicus,* before being classified as *G. diazotrophicus* based on 16S ribosomal RNA analysis.

G. diazotrophicus is a gram-negative, non-spore forming, non-nodule producing, endophytic (bacterium colonize intercellular spaces with in roots and stem of plants) nitrogen fixing bacterium. The bacterium is an obligate aerobe, when viewed under a microscope the cells measures of about 0.7-0.9µm by 2µm and appears as single, paired, or chain like structures. The bacterium cells have peritrichous flagella used for motility. *G. diazotrophicus* is an acid-tolerant bacterium, being capable of growing at pH levels below 3.0; but its optimum pH for growth is 5.5.

Kersters *et al.* (2006) reported that this bacterium is accommodated with in the phylum Proteobacteria, the class Alpha proteobacteria, the order Rhodospirillales, the family Acetobacteraceae, and genus *Gluconacetobacter*

Cavalcante and Dobereiner (1988) studied the growth of *G. diazotrophicus* under laboratory conditions by plating it on LGIP medium because it contains high sugar levels which are very similar to those found with in sugarcane. Colonies on LGIP medium initially appear semitransparent and became dark orange in colour due to their uptake of Bromothynol blue from medium.

2.7 *Bacillus subtilis*

One of the earliest bacteria to be described was "*Vibrio subtilis*" by Ehrenberg in 1835. In 1872, Cohn renamed the organism *Bacillus subtilis,* was reported by (Gordon, 1981)

Bacillus subtilis, known also as the hay *bacillus* or grass *bacillus*, is a Gram-positive, catalase positive bacterium, found in soil and the gastrointestinal

tract of ruminants and humans. *B. subtilis* is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies.

B. subtilis cells are typically rod shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase.

Bacillus subtilis is a plant growth promoting rhizobacterium that establishes robust interactions with roots. Colonization of plant roots by *Bacillus subtilis* is mutually beneficial to plants and bacteria. Plants can secrete up to 30% of their fixed carbon via root exudates, thereby feeding the bacteria, and in return the associated *B. subtilis* bacteria provide the plant with many growth-promoting traits. Formation of a biofilm on the root by matrix-producing *B. subtilis* is a well-established requirement for long-term colonization.

2.8 Colonization of Plants by Endophyte

(James *et al*., 1994, 2001; Reis *et al.*, 1999; Rouws *et al.*, 2010) These authors proposed that endophyte first colonize the root and lower stem epidermal surfaces and then used root tips, sites of lateral root emergence, and stomata to enter the sugarcane plant internal tissues. Later, microscopy studies have also been extended to seed plants such as rice, which was also shown to be endophytically colonized. Previous studies with *G. diazotrophicus* have been conducted using micro propagated sugarcane plants. However, the micro propagation technique is highly time consuming and laborious. In fact, hydroponically grown rice seedlings have proven to be a valuable model system for *G. diazotrophicus* plant interactions, allowing rapid assays and presenting low auto fluorescence, which facilitates microscopy studies.

Compant *et al.* (2010) stated that a successful endophytic association commonly involves an initial phase of bacterial attachment to the root surface. The subsequent endophytic colonization is believed to involve bacterial cell-wall-degrading enzymes (CWDE) such as endoglucanases and pectinases.

2.9 Exopolysaccharide (EPS) production by PGPB and alleviation of drought stress

Roberson and Firestone (1992) studied that drought stress can make physico–chemical and biological properties of soil unsuitable for soil microbial activity and crop yield. Water availability controls the production and consumption of protein and polysaccharides by the bacteria and thus indirectly influences soil structure. Exopolysaccharide (EPS) production by microbes protects them from inhospitable conditions and enables their survival.

Tisdall and Oades (1982) and Sandhya *et al.* (2009) reported that the EPS released into soil as capsular and slime materials by soil microbes can be adsorbed by clay surfaces due to cation bridges, hydrogen bonding, Van der Waals forces, and anion adsorption mechanisms thus forming a protective capsule around soil aggregates.

Hepper (1975) studied that EPS provides a micro environment that holds water and dries up more slowly than the surrounding environment thus protecting the bacteria and plant roots against desiccation.

(Miller and Wood 1996; Alami *et al.*, 2000 and Selvakumar *et al.*, 2012) observed that Production of EPS by bacteria has been shown to improve impermeability by increasing soil aggregation and maintaining higher water potential around the roots, thereby increasing in the uptake of nutrients by pant with an increase in plant growth and protection from drought stress. Plants treated with EPS producing bacteria display increased resistant to water stress as reported by (Bensalim *et al.*, 1998)

Cavalcante and Dobereiner (1988) reported that *G. diazotrophicus* is perfectly able to fix nitrogen with 10% added sucrose, and it still grows well in 30% sucrose.

Reis *et al.* (2007) reported that G. *diazotrophicus* is not able to transport and respire sucrose, but it excretes the saccharolytic enzyme levan sucrose, which provides the bacterium with glucose for growth and fructose for the formation exopolysacchride levan. It is hypothesized that the exopolyscchride levan is the glue that hold micro colonies of *G. diazotrophicus* together at the colonization site inside sugarcane.

Stephan *et al*. (1991) stated that this could be of great importance for the protection of nitrogen-fixing micro-colonies against the oxygen damage of Nitrogenase. During growth, the bacterium strongly acidifies the environment resulting in pH values of 3 and below. Nevertheless, this bacterium continues to grow and fix nitrogen at this pH level for several days.

Hernandez *et al.* (1995) and Martínez-Fleites *et al.* (2005) stated that *G. diazotrophicus* is unable to transport or take up sucrose, as such it secretes an extracellular enzyme called levansucrase, a fructosyltransferase exoenzyme which hydrolyzes sucrose into fructooligosaccharides and levan. This enzyme is critical for the survival of the bacterium and can constitute over 70% of all secreted proteins by specific strains of G. diazotrophicus.

Velázquez-Hernández *et al.* (2011) and Meneses *et al.* (2011) reported that in addition to sucrose hydrolysis, levansucrase is also involved in tolerance to desiccation and NaCl and in biofilm formation. Biofilm formation begins with the gumD gene homologue, an essential step in the production of exopolysaccharides, which along with levan, a product from the hydrolysis of sucrose by levansucrase, leads to the formation of biofilm in G. diazotrophicus. Dong *et al.* (2002) stated that the removal of either of these two factors results in the bacterium being unable to form a biofilm. This leads to changes in colony morphology, tolerances, nitrogenase activity, and abilities to aggregate to abiotic and biotic surfaces, resulting in diminished colonization abilities.

2.10 Production of Phytohormonal Substances by Bacteria

2.10.1 Role of Indole Acetic Acid (IAA) produced by PGPB to drought stress tolerance

Costacurta and Vanderleyden (1995) demonstrated that the phytohormones plays an important role as signals and regulators of growth and development in plants. The capacity to produce them is often considered as a trait of the plant kingdom. However, production of Phytohormones is also widespread among soil and plant associated prokaryotes.

Lynch (1985) reported that Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L- tryptophan metabolism produced by several microorganisms including Plant Growth-Promoting Rhizobacteria (PGPR).

Arshad *et al.* (1992) reported that bacteria that colonize the rhizosphere and plant roots, to enhance plant growth by any mechanism are referred to as PGPR. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (like auxin, gibberellin, and ethylene), siderophores, HCN and antibiotics.

Shih-Yung (2010) revealed that the bacteria synthesize auxins in order to perturb host physiological processes for their own benefit.

Datta and Basu (2000) validated that the microorganisms isolated from rhizosphere region of various crop have an ability to produce Indole acetic acid as secondary metabolites due to rich supply of substrates. Indole acetic acid helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake.

Zhao (2010) studied that IAA stimulates cell elongation by modifying certain conditions like, increase in osmotic contents of the cell, increase in permeability of water into cell, decrease in wall pressure, an increase in cell wall synthesis and inducing specific RXA and protein synthesis. It promotes embial

activity, inhibit It promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting.

Bianco and Defez (2009) recently, it was also found that IAA induces an increased level of protection in plants against external stress conditions.

L-tryptophan (Trp), an amino acid serves as a physiological precursor for biosynthesis of auxins in plants and microbes. Auxins of microbial origin in the interior of plants could evoke a physiological response in the host plant. Therefore, screening of the endophytes for their *in vitro* potential of auxin production could provide a reliable base for selection of effective plant growth promoting bacteria. Literature survey indicated lots of research work on the diazotrophic nature of *Acetobacter diazotrophicus*. However, very few reports described its ability to produce IAA. Considering this, the present investigation was conducted to demonstrate the IAA production in culture of *Acetobacter diazotrophicus.*

Pattern and Glick (2002) studied that IAA is a metabolite derived from Trp by many Trpdependant and Trp-independent pathways in plants and bacteria. More than one pathway could be present in a bacterium.

Sevilla *et al.* (2001) reported that *G. diazotrophicus* is known to produce indole-3- acetic acid (IAA), with particularly high amounts produced by strain PAl5.

Costacurta *et al.* (1995) and Patten *et al.* (1996) reported that biosynthesis of IAA is not limited to higher plants. Organisms such as bacteria, fungi, and algae are able to make physiologically active IAA that may have pronounced effects on plant growth and development. Many bacteria isolated from the rhizosphere have the capacity to synthesize IAA in vitro in the presence or absence of physiological precursors, mainly tryptophan (Trp).

Barazani *et al.* (1999) and Costacurta *et al*. (1995) reported that microbial isolates from the rhizosphere of different crops appear to have a greater potential to synthesize and release IAA as secondary metabolites because of the relatively rich supply of substrates.

Sevilla *et al.* (2001) showed that *G. diazotrophicus* has two potential beneficial effects on sugarcane: one probably dependent on nitrogen fixation and the other possibly through microbial production of a plant growth-promoting substance. Since *G. diazotrophicus* is known to produce indole-3- acetic acid (IAA), with particularly high amounts produced by strain PAl5 (used in the plant inoculation experiments of Sevilla *et al.* 2001)

2.10.2 Role of ACC deaminase produced by PGPB to drought stress tolerance

Lucy *et al.* (2004) reported that plant growth-promoting rhizobacteria (PGPR), free living soil bacteria thriving in the plant rhizosphere, have been studied as plant growth promoters for increasing agricultural productivity.

Lucy *et al.* (2004) conveyed that PGPR can either directly or indirectly facilitate growth of plants. Indirect stimulation of plant growth includes mechanisms by which the bacteria prevent phytopathogens from inhibiting plant growth and development, while direct stimulation may include providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate.

Shaharoona *et al.* (2006) and Saleem *et al.* (2007) studied that ethylene is an endogenously produced gaseous phytohormone that acts at low concentrations, participating in the regulation of all processes of plant growth, development and senescence.

(Glick *et al.* 1998, 2007 a, b; Glick 2004, 2005 and Saleem *et al.* 2007) these authors conveyed that many PGPRs can also increase plant resistance to biotic and abiotic stress factors. Presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity in several rhizospheric bacteria and regulation of ACC, a precursor to plant ethylene levels, is one of the principal mechanisms by which bacteria exert beneficial effects on plants under abiotic stress.

Glick *et al.* (2007a) Earlier studies indicated that bacteria having ACC deaminase activity reduce the level of stress ethylene conferring resistance and

resulting in better growth of plants under various stresses such as salt stress, flooding stress, heavy metal stress and pathogen attack.

Honma and Shimomura (1978) stated that 1-Aminocyclopropane-1-carboxylate (ACC) deaminase catalyses the cleavage of ACC, the immediate precursor of ethylene in plants, to a-ketobutyrate and ammonia.

Glick *et al*. (1998) reported that bacteria containing ACC deaminase attach to plant cells, act as a sink for plant ACC to uptake and cleave the ACC secreted by plant cells and thus reduce plant ACC concentration, ethylene evolution and the extent of ethylene inhibition of plant growth, particularly, under a variety of abiotic and biotic stresses.

Hontzeas *et al.* (2004) revealed that bacteria containing ACC deaminase can down-regulate the plant genes involved in ethylene-induced stress responses and defence signalling pathways and up-regulate the plant genes involved in growth and protein production.

Glick *et al.* (2007) earlier studies revealed that ACC deaminase activity has long been a key marker for identifying the plant growth-promoting bacteria.

Glick *et al.* (1998) According to his hypothesis bacterial auxin activates plant ACC synthase. The produced ACC can be used by some microbes as an N-source, thereby, decreasing ethylene levels. In order to explain how ACC produced by the plant is converted by ACC deaminase from the bacterial cytoplasm, (Glick *et al.*1998) assumed that a significant portion of ACC is exuded from plant roots and seeds and then taken up by the microbe.

Jacobson *et al.* (1994) studied that ACC deaminase is present in the cytoplasm of bacteria at a low level until it is induced by ACC, and the induction of enzyme activity is a relatively slow process. ACC deaminases do not bind ACC with a high affinity; the Km values range from 1.5 to 17.4 mmol $^{-}$l.

CHAPTER- III
MATERIAL AND METHODS

The details of materials used along with geographical situation, climate and the experimental protocols followed during the course of the study entitled **"Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L.)"**are briefly described below.

3.1. General Information

3.1.1 Experimental site

The experiment was conducted at the ICAR-(IIRR) Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana state during 2016-2017 with rice crop. The experimental site is located at 17°19' N latitude and 78°23' E longitude at an altitude of 1719 ft.

3.1.2 Climatic conditions

The climate of the place is subtropical. It receives rainfall mainly from South-west monsoon (June-October) receiving an average annual rainfall of 1200-1400 mm out of which about 85 per cent is received during the rainy season (third week of June to mid September) and the rest 15 per cent during winter season (October – February).

3.1.3 Bacterial isolates used

Pure cultures of *Gluconacetobacter diazotrophicus* Pal 5 (Type strain) and *Bacillus subtilis* (GenBank accession number- MF171124) maintained in the Microbiology lab, Soil Science Section, IIRR were used for in vitro and pot culture experiments.

3.2 Evaluation of *G. diazotrophicus* and *B. subtilis* for plant growth promoting activities, exopolysaccharide production and rice plant colonization.

Laboratory studies were conducted to determine the IAA (Indole acetic acid) production, ACC (1-Aminocyclopropane-1-Carboxylate) deaminase activity, the potential to produce exopolysaccharides and the ability of the selected isolates to colonize the roots of rice plants under *in vitro* conditions.

3.2.1 IAA production

IAA production was assessed by using culture supernatant of *G. diazotrophicus* and *B. subtilis* grown for seven days at 28^0C in MYP (Mannitol Yeast Peptone broth) and NB (Nutrient broth) respectively with and without tryptophan amendment. IAA production was determined by using Salkowski reagent following the method of Gordon and Weber (1951). The formation of red color was taken to be indicative of IAA production and the amount of IAA produced was determined by comparing the absorbance at 530nm of the sample to a standard curve of IAA ranging between 10-100µg/ml.

3.2.2 ACC deaminase activity

The method of Dworkin *et al.,* 1958, was adopted for the quantification of ACC deaminase activity in the cultures, *G. diazotrophicus* and *B. subtilis* cultures were incubated for 24h in Dworkin and Foster's (DF) minimal medium containing 3mM ACC as sole nitrogen source. The toluenized cell suspension was then used to determine ACC deaminase activity according to a modified Honma and Shimomura method which measures the amount of α-ketobutyrate produced when the enzyme ACC deaminase cleaves ACC in to ammonia and α-ketobutyrate.

3.2.3 Evaluation of polysaccharide production

G. diazotrophicus was grown in LGIE broth (LGI supplemented with 1 g/L tryptone, 0.2 g/L yeast extract, 5% (w/v) sucrose, and 1% (v/v) glycerol). *Bacillus subtilis* was grown in nutrient broth containing 5% sucrose. Both the isolates were incubated at 27^oC for 15 days with constant shaking at 200 rpm in a Gyromax

787R incubator shaker (Amerex Instruments, Inc., Lafayette, CA). After fifteen days of growth the bacterial biomass was removed by centrifugation. The supernatant was filtered through a 0.22 µm membrane filter, mixed with two volumes of acetone, stirred vigorously and kept overnight at 4^{o}C.The exopolysaccharides were collected by centrifugation at 10,000 rpm for 20 min, washed twice with deionized water and re-dissolved in distilled water, followed by de-proteinization with 1/5 volume of Sevag ($CHCl_3$–BuOH, v/v) reagent (Staub, 1965). The exopolysaccharide in the de-proteinized solution was precipitated once again with two volumes of ethanol, washed with water and quantified. The exopolysaccharide obtained was re-suspended in sterile distilled water and heated at 80°C for 10 minutes for depolymerization. The suspension was hydrolyzed in dilute HCL at 80°C for 10 minutes for formation of hydroxymethyl furfural which in the acid medium reacts with 1% resorcinol reagent to give a red color product (Ashwell 1957) and was assayed spectrophotometrically at 520 nm using fructose as standard since both the cultures produce levan as exopolysaccharide in the presence of sucrose. The exopolysaccharide content was expressed in terms of fructose g L^{-1} (Viikari and Gisler 1986).

3.2.4 Evaluation of root colonization ability of the bacteria by hydroponics

Swarna rice seeds were surface sterilized and soaked overnight in bacterial inoculums (*G. diazotrophicus*, *B. subtilis*) and then kept for germination at room temperature. After 3 days of germination seedlings were transferred in to Yoshida solution. Root colonization by the bacteria was observed under Scanning Electron Microscope (SEM) after 7 days of growth in hydroponics. For SEM analysis, the root surface and transverse section of root samples were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH – 7.2) for 24 hours at 4^0 C and post fixed 2% in aqueous osmium tetroxide for 4 hours, dehydrated. The processed samples were then coated with a thin layer of gold by using an automated sputter coater for 3 minutes and they were scanned under SEM under 5 µM x 3500 magnification to observe for internal and external root colonization by the bacteria.

3.3 *In vitro* studies on the effect of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* on rice seed germination and seedling growth under poly ethylene glycol - 6000 (PEG) induced water stress

The effect of inoculation of rice seed of Swarna variety with *G. diazotrophicus* and *B. subtilis,* singly or in combination, on germination and vigor index under water deficit induced by 15% and 20% PEG was studied in this experiment under laboratory conditions.

3.3.1. Germination indices

Swarna seeds were surface sterilized with 70% ethanol, 0.1% mercuric chloride and after six times washing with sterile distilled water, was soaked overnight in bacterial inoculum (*G. diazotrophicus* , *B. subtilis* and combination of both the cultures) containing ($1x10^8$ CFU/ml), for seed bacterization and then kept for germination. To study the responses of rice plant to PEG-induced water stress, seeds were germinated under various PEG concentrations (0, 15, and 20%) and the differences in germination percentage root, shoot length were recorded and vigor index was calculated.

Germination tests in petri plates with 0, 15 and 20% PEG 6000 was used for induction of water stress. After seven days of seed germination, root and shoot lengths were measured, while germination percentage and vigor index were calculated by using the formulas given below (Shende, 1977). Germination percentage (%) = (Number of seeds germinated in petri plate/Total no of seeds in the petri plate used for test) X 100.Vigour Index= (Mean of plumule + radical length) X Germination percentage.

3.4 Pot culture studies on the effect of the single and combined inoculation of *G. diazotrophicus* and *B. subtilis* on water stress tolerance and yield of rice.

The pot culture experiment was conducted using the soil collected from Rajendranagar farm, IIRR, Hyderabad.

3.4.1 Experimental details

Crop **:** Rice (*Oriza sativa*)

Variety **:** Swarna

Soil **:** Vertisols

Replications **:** Three

Design **:** Completely Randomized Block Design

Treatments **:** Eight

Table 3.1. Treatment details of pot culture

S.No.	Treatments with water stressed conditions
T1	Control plants without inoculation
T2	Seed and seedling inoculation with *Bacillus subtilis*
T3	Seed and seedling inoculation with *Gluconacetobacter diazotrophicus*
T4	Seed and seedling inoculation with *Gluconacetobacter diazotrophicus and Bacillus subtilis* consortia
	Treatments without water stressed conditions
T1	Control plants without inoculation
T2	Seed and seedling inoculation with *Bacillus subtilis*
T3	Seed and seedling inoculation with *Gluconacetobacter diazotrophicus*
T4	Seed and seedling inoculation with *Gluconacetobacter diazotrophicus and Bacillus subtilis* consortia

3.4.2 Pot and nursery preparation

Inoculated/un-inoculated seeds were sown in separate trays and then transplanted at 20 DAS after seedling and root dip into puddled soil (non-sterilized Vertisols) in 5 kg pots (@ 4 seedlings per pot) arranged in 3 replicates in a Completely Randomized Block design. Irrigation was provided twice daily to maintain standing water of 5 cm above the soil. For drought-stress treatments, water was drained at panicle initiation stage 90 days after transplanting (90 DAT) and irrigation was withheld for 8 days after which re-watering was continued till maturity.

3.4.3 Inoculum preparation

The freshly grown culture of was inoculated to 500.0 mL of Nutrient broth and Mannitol Yeast extract Peptone broth and incubated in shaker at 28◦C for 48.0 h in shaker. The cell suspension was then centrifuged, the cell biomass was re-suspended in water to obtain 1 x 108 cells/ml and used for the experiment.

3.4.4 Plant Morphological parameters

3.4.4.1 Plant Height

Plant height at harvest was measured from randomly selected hills selecting main shoot and recording plant height from ground level to the base of the fully opened leaf. The mean plant height was worked out and expressed in cm.

3.4.4.2 Shoot Biomass

Shoot biomass were determined before harvesting and shoot dry biomass were determined by drying in an oven at 65° C until constant weight was obtained and was expressed as gm.

3.4.4.3 Root Biomass

Root biomass were determined before harvesting and root dry biomass were determined by drying in an oven at 65° C until constant weight was obtained and was expressed as gm.

3.4.4.4 Number of tillers hill^{-1}

Number of tillers was recorded from the randomly selected hills before harvest. They were pooled and average number of tillers / plant was presented.

3.4.4.5 Number of panicles hill^{-1}

Number of panicles was recorded from the randomly selected hills at the time of harvest. They were pooled and average number of panicles/ plant was presented.

3.4.4.6 Number of grains hill^{-1}

Harvesting of crop was done at maturity. The total number of grain hill^{-1} were counted were recorded.

3.4.5 Plant Biochemical Parameters

The total soluble sugar content in leaves and the antioxidant activity of leaves were determined seven days after rewatering.

3.4.5.1 Total soluble sugars in leaves

Total soluble sugars in leaves was determined by Anthrone method (Thimmaiah, 2006).

Reagents

1. Take 2.5 N HCL
2. ***Anthrone reagent:*** Dissolve 200mg anthrone in 100 ml of ice cold 95% H_2SO_4. Prepare fresh before use.

3. ***Standard Glucose:*** Stock – Dissolve 100 mg in 100 ml water. Working standard – 10 ml of stock dilution to 100 ml with distilled water. Store refrigerated after adding a few drops of toluene.

Procedure

1. 100 mg of the sample was weighed into a boiling tube and hydrolysed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5 N HCL and cooled to room temperature.

2. The sample was then neutralized with sodium carbonate until the effervescence ceases and the volume was made up to 100 ml.

3. After centrifugation, 1 ml aliquot of the supernatant was analysed for total sugars.

4. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard.'0' served as blank. The volume in all samples were made up to 1 ml by adding distilled water. Then 4 ml of anthrone reagent was added.

5. After heating for 8 minutes in a boiling water bath, the samples were cooled rapidly and the green to dark green color was read at 630nm.

6. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis and the amount of carbohydrate present in the sample tube was calculated

Calculation

Amount of carbohydrate present in the 100 mg of the sample = mg of glucose / volume of test sample x 100

3.4.5.2 Total antioxidant activity in leaves (Prieto *et al.*, 1999)

1. Leaf samples were ground with pestle and mortar using one volume of solvent (methanol 1 ml/g). The homogenized suspension was transferred to polypropylene tubes, and shaken for 1 hour at room temperature in the dark and centrifuged.

2. After centrifugation at 10,000g for 15 minutes, the supernatant was transferred to new tubes and used to determine the antioxidant activity of the samples. An aliquot of 0.1 ml of sample supernatant was combined in an eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate).

3. The tubes were capped and incubated in a thermal block at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank.

4. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples. Ascorbic acid was used as standard and antioxidant capacities were expressed as equivalents of ascorbic acid.

3.4.6 Plant Physiological Parameters

Chlorophyll and relative water content in leaves and the leaf membrane stability determined through electrolyte leakage assay were estimated seven days before and after rewatering.

3.4.6.1 Leaf chlorophyll content

1. Leaf tissue (25 mg) was cut into small pieces. 80% acetone was prepared and the cut leaves were placed into 10 ml of 80% acetone in falcon tubes

2. The tubes were incubated in the dark for 24 hours, then measure the absorbance at 663.2 nm, 646.8 nm and 470 nm for Chlorophyll a, Chlorophyll b and Carotenoid content (Lichtenthaler, 1987).

3.4.6.2 Relative water content in leaves

Relative water content (RWC) was measured according to Barrs and Weatherley (1962). Leaf were weighed and then immediately floated on distilled water in a petridish for 8 hours in the dark. Turgid weights (TW) were obtained after drying excess surface water with paper towels. Dry weights (DW) were measured after drying at 80^{o}C for 48 hours.

Formula for calculating RWC (%) = (FW − DW /TW − DW) × 100.

3.4.6.3 Leaf membrane stability through electrolyte leakage (Gayen *et al*., 2014)

Fresh leaves were weighed and washed with distilled, deionized water, patted dry with tissue paper, and placed in 15-ml Falcon tubes containing 10 ml of distilled, deionized water for 1 hour at room temperature. The electrical conductivity of these solutions was measured (EC1) using conductivity meter. Leaves were then autoclaved for 15 minutes to release all the electrolytes, after that electrical conductivity was measured again (EC2).

Formula for calculating electrolyte leakage EL% = (EC1/EC2) x 100

3.4.7 Soil analysis

Soils from the rhizosphere of plants were analyzed for soil moisture evaporation rate and soil carbohydrate content after re-initiation of irrigation.

3.4.7.1 Rhizosphere Soil Moisture Content

Soil Moisture Content was estimated from Fresh weight of soil and dry weight of soil by using the following equation Soil Moisture Content (%) = Fresh weight of the soil – Dry weight of the soil / Dry weight x 100

3.4.7.2 Soil carbohydrate content (Anonymous, 1994)

Reagents

1. Distilled water.
2. 0.5 M K_2SO_4 solution. In a 1 liter volumetric, dissolve 87.135 g K_2SO_4 (dried for 2 hours at 110°C) in distilled water. Dilute to volume and invert to mix thoroughly.
3. Stock standard glucose solution (SSGS), 20.0 mg glucose L^{-1}. In one liter of 0.5 M K_2SO_4 solution, dissolve 0.02 g glucose (dextrose). Dilute to volume and invert to thoroughly mix. Store in polyethylene containers. Make fresh weekly. Store in a refrigerator.
4. Solution 1: Add 4 g Na_2CO_3, 4 g sodium hexametaphosphate $[(NaPO_3)]_6$, and 0.2 g DLaspartic acid in 100 ml distilled water. pH solution to 11.25 with NaOH.
5. Solution 2: Dissolve 0.48 g bicinchoninic acid in 12 ml of Reverse Osmosis RODI water (0.1 M).
6. Solution 3: Dissolve 1 g $CuSO_4$ in 25 ml distilled water (0.25 M).
7. Disodium bicinchoninic (BCA) reagent: Mix 100 ml Solution 1, 12 ml Solution 2, and 1.8 ml of Solution 3 = BCA reagent. Store in polyethylene containers. Make fresh daily. Store in a refrigerator.
8. Standard glucose working solutions (SGWS), 10.0, 5.0, 2.5, 1.25, 0.75, and 0.375 mg glucose L^{-1}. In six test tubes, add 5 ml RODI water. Perform six serial dilutions. Begin as follows: add 5 ml of SSGS to Tube 1 and shake (10.0 mg glucose L^{-1}) and extract 5 ml from Tube 1, add to Tube 2, and shake (5.0 mg glucose L^{-1}). Proceed to make all six SGWS.
9. Standard glucose calibration solutions (SGCS). Add 2 ml of each SGWS to a separate test tube, followed by 2 ml BCA reagent. Blank = 2 ml distilled water and 2 ml BCA reagent.

Procedure

1. 10 g of air-dry soil was placed into a 125-ml and distilled water was added in the ratio of 1:4 and autoclaved for 1 hour at 121°C and 15 psi.

2. The samples were cooled and filter. Two ml of each sample extract, 0.5 ml K_2SO_4, and 2 ml disodium BCA reagent were added into test tubes and placed in a hot water bath for 2 h at 60°C.

3. The samples were allowed to cool and sample extract were transferred to cuvettes and absorbance was read at 562 nm.

Calculations

Formula for calculating Soil carbohydrate content:

$$C\ (mg\ kg\text{-}1) = [(A \times B \times 0.40 \times 1000)/E]$$

Where: A = Sample reading (mg L-1),

B = Extract volume (L),

0.40 = Mass fraction C in glucose,

1000 = Conversion factor to kg-basis and

E = Soil weight (g).

3.4.8 Statistical analysis

All the laboratory and pot culture experiments were arranged in Completely Randomized Block Design. All the observations recorded during research work were tabulated in a systematic manner. Values were given as means for their respective number of replications used. The data were subjected to Analysis of Variance (ANOVA) using the online statistical analysis package (OPSTAT, Computer section, CCS HAU Hisar, Hariyana).

CHAPTER- IV
RESULTS AND DISCUSSION

Sustainable rice production in India is currently confronting issues such as declining soil fertility, scarcity of water and degradation of the environment. Of these, water deficit is one of the foremost problems encountered during rice production in India, limiting productivity of the crop. Improving water deficit tolerance in rice through sustainable environment friendly strategies is the key to deliver food security to the continuously rising population of the country.

Microorganisms have the ability to improve crops ability to resist /tolerate abiotic stress. Specifically, bacteria possessing various plant growth promoting activities can used to enhance crop performance under various stresses including decreased availability of water during the growth and reproductive periods. Bacteria that produce growth hormones like Indole acetic acid and which display ACC deaminase activity can help plants tolerate water stress by increasing the root biomass and by reducing the water stress perception in plants. Microbes can also indirectly aid plant during water deficit stress by improving the soil water retention in the vicinity of the roots by production of Exopolysaccharides thereby reducing the evaporative loss of soil water and delaying soil drying.

The investigation entitled **"Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L.)"** was conducted in the Microbiology lab, Soil Science Section, Indian Institute of Rice Research, Rajendranagar, Hyderabad, during the year 2016-2017 to assess if Swarna a popular rice variety with high productivity can be imparted water deficit stress by inoculation with *G. diazotrophicus* and *B. subtilis*

4.1 Characterization of plant growth promoting bacteria (*G. diazotrophicus* and *B. subtilis)*.

Production of IAA and the potential for ACC deaminase activity are traits that can confer fitness to a plant growing under stress situations, including water deficit stress. In this experiment, the two selected cultures were assessed for these characteristics (Table.4.1; Plate.15,16,17)

Table 4.1: Plant growth promoting characteristics and exopolysaccharide production of *G. diazotrophicus* and *B. subtilis*

PGP character/bacteria	*G. diazotrophicus*	*B. subtilis*
1) IAA production : (µg / ml)		
• Without tryptophan	104.98	182.26
• With tryptophan	748.04	529.33
2) ACC deaminase activity (µg α-ketobutrate /µg cell protein)	4.10	1.67
3) Exopolysaccharide production (g / L)	2.4	1.86

4.1.1 Indole Acetic Acid Production

Both the selected cultures (*G. diazotrophicus, B. subtilis)* were IAA producers. There was a significant increase in both the cultures after adding L-Tryptophan. The highest IAA production was observed in *G. diazotrophicus* (748.04µg / ml) followed by *B.subtilis* (529.33 µg / ml) with tryptophan. Without tryptophan amendment in the media, *B.subtilis* (182.26 µg/ml) and *G. diazotrophicus* (104.98 µg / ml) were observed to produce lower amounts of IAA (Table.4.1; Plate.4.3). Production of IAA is widespread among soil and endophytic bacteria, and it has been estimated ~80% of all soil bacteria are able to synthesize IAA as reported by Patten and Glick (1996). *G. diazotrophicus* is known to produce indole-3-acetic acid (IAA), with particularly high amounts produced by strain PAl5, which is used in this study as reported by Lee *et al.* (2004) and Madhaiyan *et al*. (2004). Similarly several strains of *B. subtilis* have been reported to possess the IAA producing trait Reetha *et al.* (2014) and Bal *et al.* (2013)

4.1.2 ACC deaminase Production

Highest value of deamination was observed in *G.diazotrophicus* (4.10 µg α-ketobutrate /µg cell protein) followed by *B.subtilis* (1.67 µg α-ketobutrate /µg cell protein) is given in (Table.4.1; Plate.4.2) ACC (1-aminocyclopropane-1-carboxylate) deaminase are produced by the various bacterial strains and its production is mostly related to free-living soil bacteria and endophytic bacteria. According to Onofre-Lemus *et al.* (2009) the bacterial enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase promotes plant growth by lowering plant ethylene levels. Barnawal *et al.* (2013) has reported the isolation of a *B. subtilis sp.* that exhibited ACC deaminase activity. Gamalero and Glick (2010) suggested that bacterial ACC deaminase and IAA work synergistically to facilitate plant growth during stress conditions.

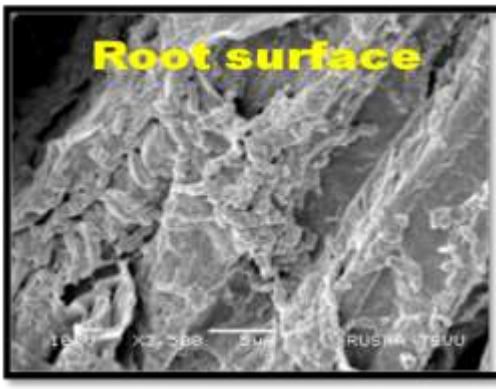
4.1.3 Exopolysaccharide Production

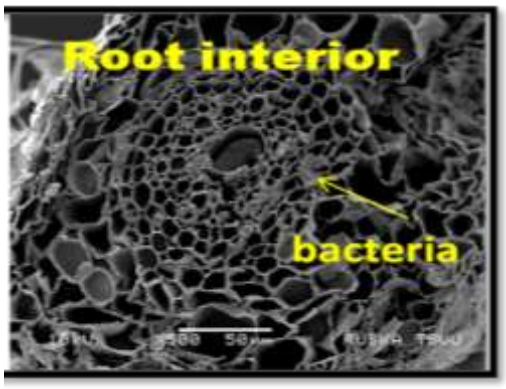
Both the selected cultures (*B.subtilis* and *G.diazotrophicus*) were exopolysaccharide producers. The EPS protect these bacteria from desiccation under drought stress by enhancing water retention. There was a significant increase in exopolysaccharide production by both the cultures with an increase in sucrose concentration.

Highest exopolysaccharides production was observed in *G.diazotrophicus* (2.4g/L) the lowest exopolysaccharide production was observed in (1.86 g/L) is given in (Table.4.1; Plate.4.4) Meneses *et al.* (2017) have reported exopolysaccharide production in *G. diazotrophicus* that is involved in cell protection. *G. diazotrophicus* synthesizes levan, a D-fructofuranosyl polymer with β-(2→6) linkages, as an exopolysaccharide and the synthesized levan improves the stress tolerance of the bacterium as reported by Idogawa *et al.* (2014). Exopolysaccharide production has been described in *B. subtilis* strains also by Aza *et al.*(2012) and Porras-Domínguez *et al.* (2015) has demonstrated the presence of levansucrase enzyme in *B. subtilis* which produces the exopolysaccharide levan.

4.1.4 Evaluation of root colonization ability of the bacteria under hydroponic conditions

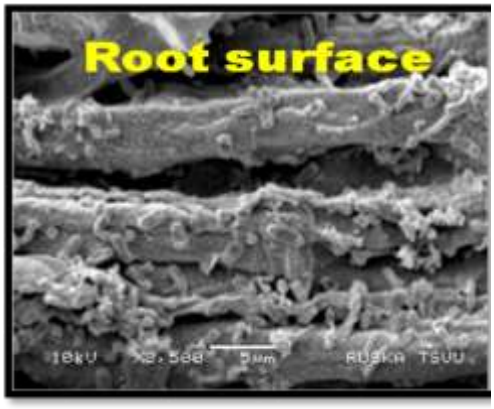
The root colonization ability of both the bacterial cultures were assessed by scanning electron microscopy. *G. diazotrophicus* were found to colonize both surface and an interior regions of the rice root while *B. subtilis* an isolate from the rhizosphere of rice was found to colonize only the surface of roots after seven days (Plate.4.5;4.6;4.7;4.8;4.9)





Colonization of the root surface and interior by *G. diazotrophicus*

Rouws *et al*. (2010) evaluated the colonization process of hydroponically grown rice (genotype IAC4440) seedlings by *G. diazotrophicus* strain PAL5 marked with the gusA and gfp reporter genes. *G. diazotrophicus* PAL5 could be isolated from the root surface and surface disinfected root of inoculated plants, suggesting that PAL5 colonized the internal plant tissues. The same strain exhibited similar colonization pattern in the Swarna rice genotype which was used in the present study.



Colonization by *B. subtilis* on root surface only

4.2 *Invitro* studies on the effect of *G.diazotrophicus* and *B.subtilis* on rice seed germination and seedling growth under poly ethylene glycol -6000 (PEG) induced water stress.

Swarna seeds were surface sterilized and inoculated with *(B.subtilis, G.diazotrophicus* and combination of *B.subtilis* and *G.diazotrophicus*) cultures by soaking in inocula containing $1x10^8$ cells/ml and allowed to germination in the presence of different concentrations of PEG (0% control or no PEG, 15% PEG, 20% PEG) (Plate.4.1)

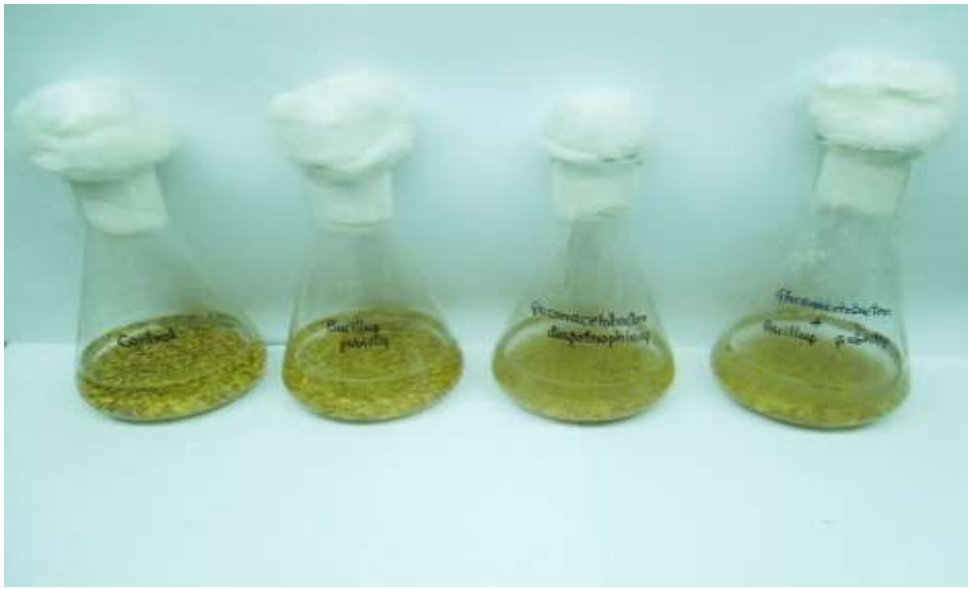


Plate. 4.1: Seed priming of Swarna seeds.

Under 0% PEG concentration the highest shoot length was observed in T_2 (5.55cm) followed by T_4 (5.42cm), T_3 (5.15cm) and the lowest value of shoot length, was recorded for the treatment 0% PEG T_1 (5.09cm). In 15% PEG and 20% PEG concentration highest shoot length was recorded in T_4 15% (5.95cm), 20% (5.82cm) followed by T_2 15% (5.74cm), 20%(5.65cm), T_3 15%(5.50cm), 20%(5.76cm) and the lowest value of shoot length, was recorded for the treatment (Control 0% PEG) T_1 15%(4.55cm), 20%(4.42cm) (Table.4.2 and Figure.4.1). Similarly under 0% PEG the highest root length was recorded in T_2 (8.59cm) followed by T_4 (8.56cm) and T_3 (8.36cm) and the lowest value of root length, was recorded for Control 0% PEG T_1 (8.03cm). Under 15% PEG concentration highest root length was recorded in treatment T_4 (9.04cm) followed by T_2 and T_3 (8.68cm), (8.45cm) lowest value of root length was observed in T_1 (7.3533). Under 20% PEG

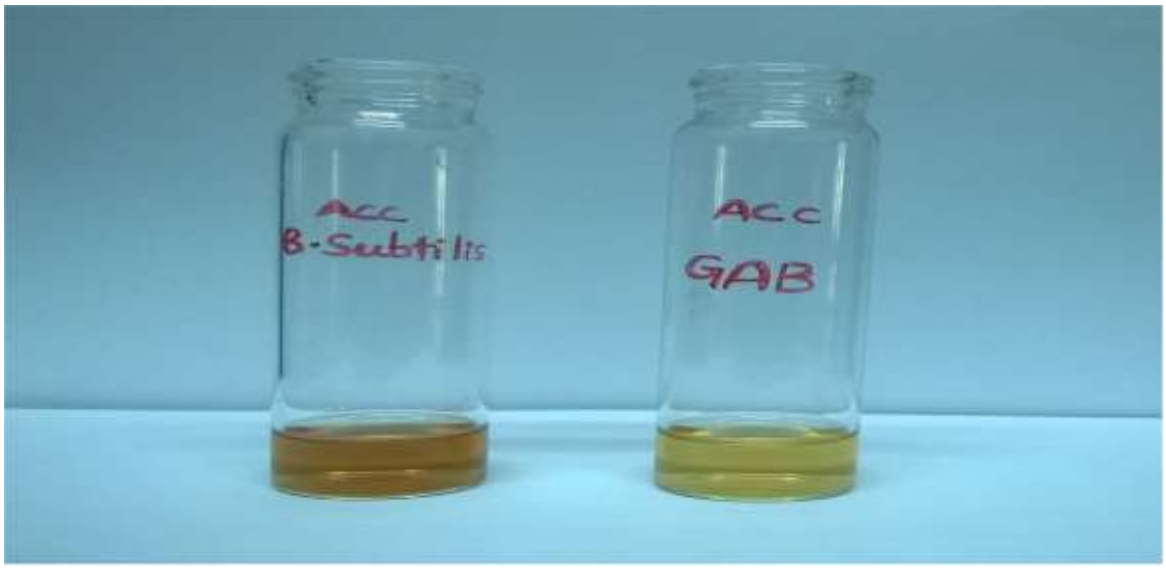


Plate.4.2: Production of ACC deaminase activity by *G.diazotrophicus and B.subtilis*

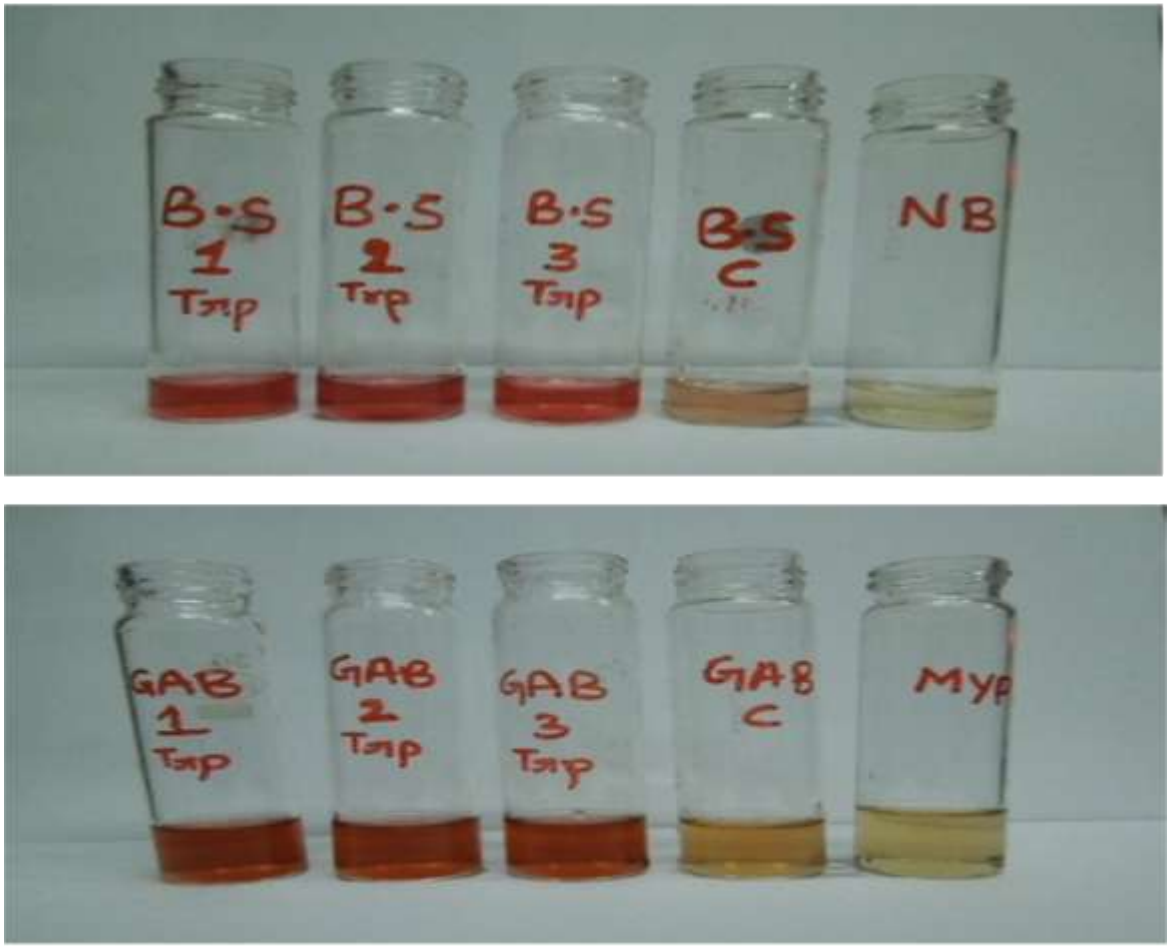
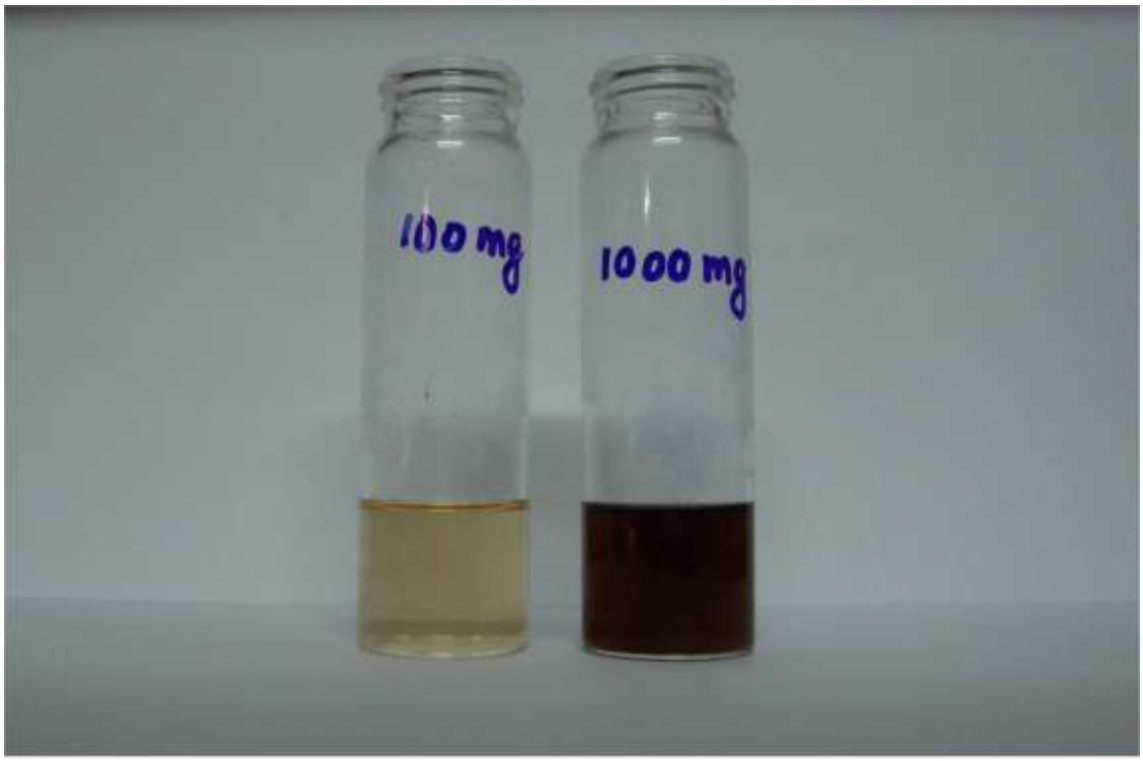


Plate.4.3: Production of Indole Acetic Acid (IAA) by *G.diazotrophicus and B.subtilis*



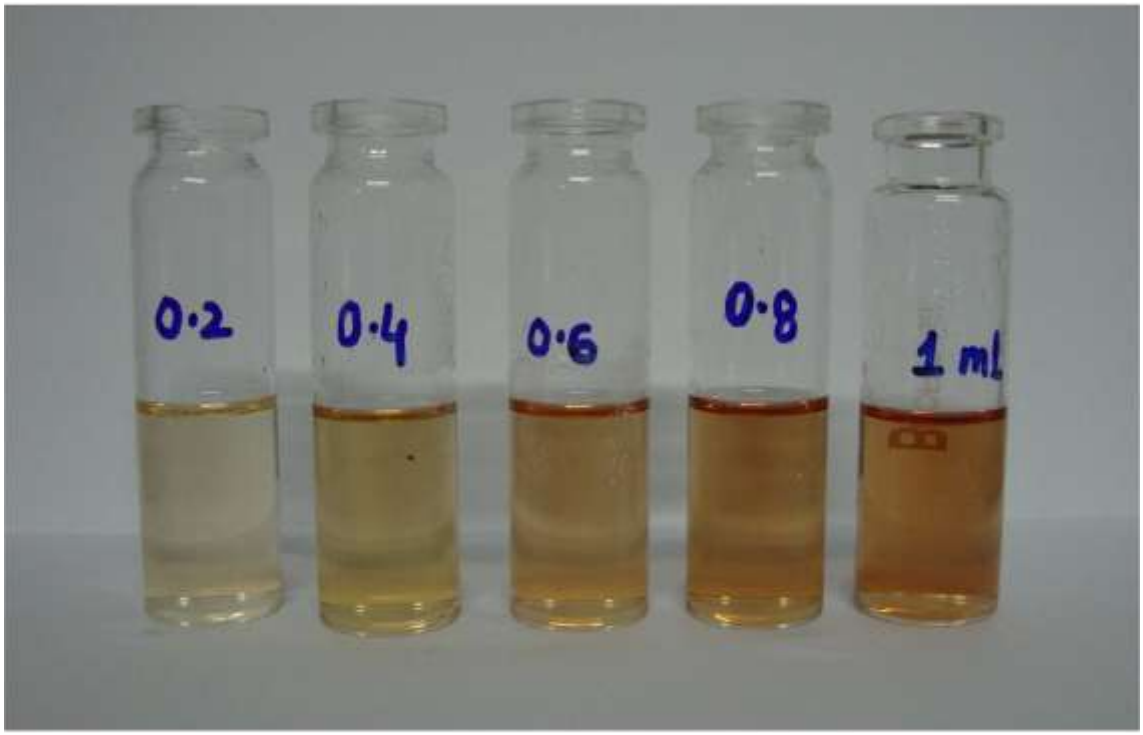


Plate.4.4: Production of Exopolysaccharide (EPS) by *G.diazotrophicus and B.subtilis*

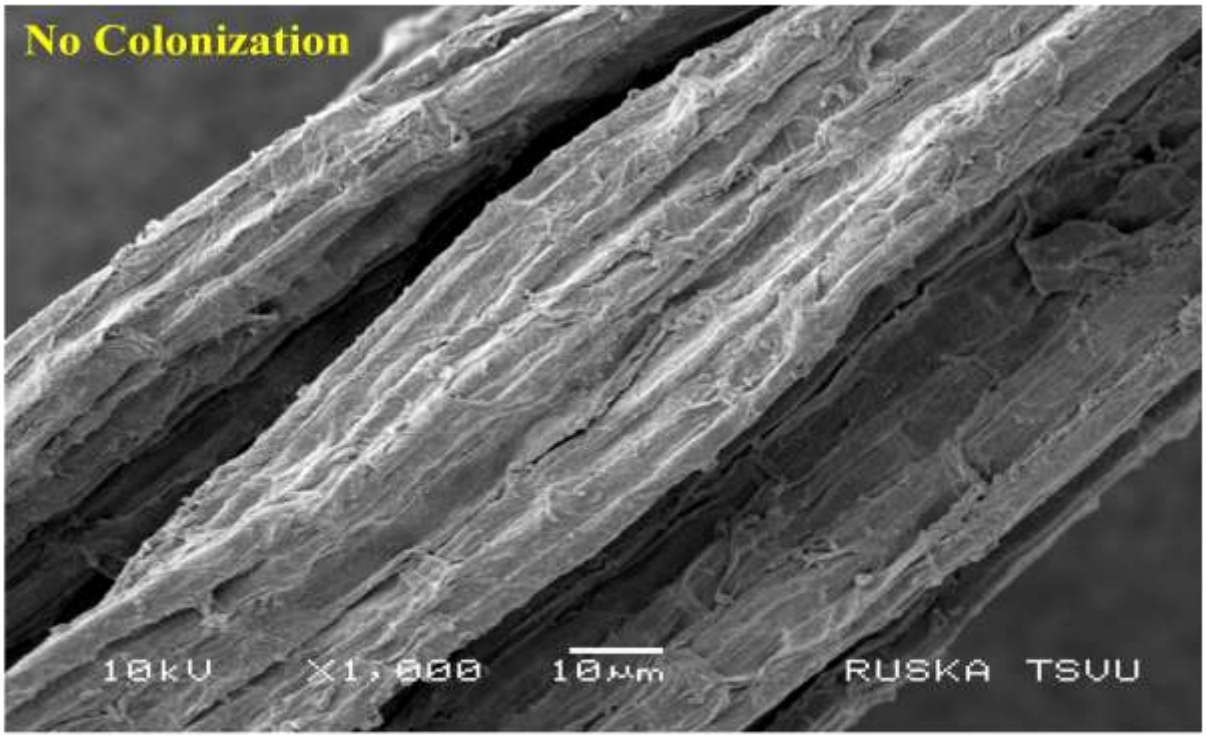


Plate.4.5: SEM picture of root surface without colonization of bacteria

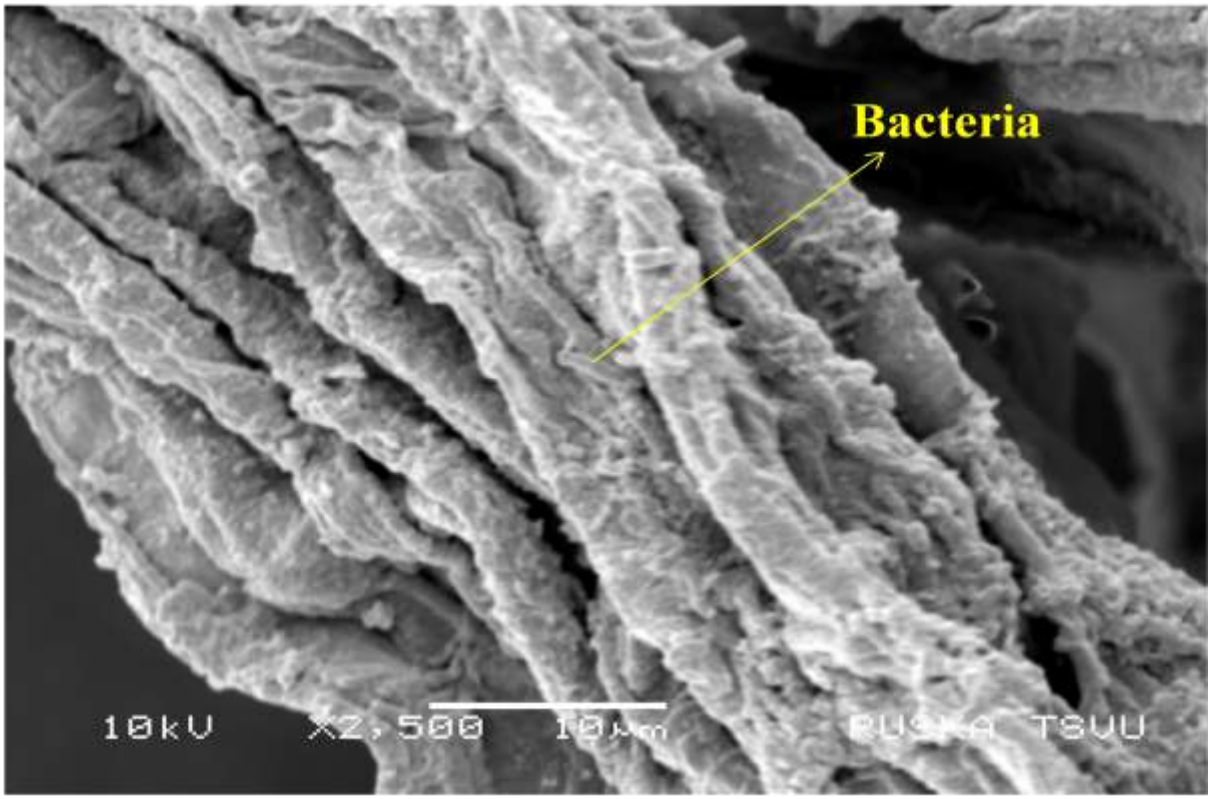


Plate.4.6: Colonization of root surface by *B.subtilis*

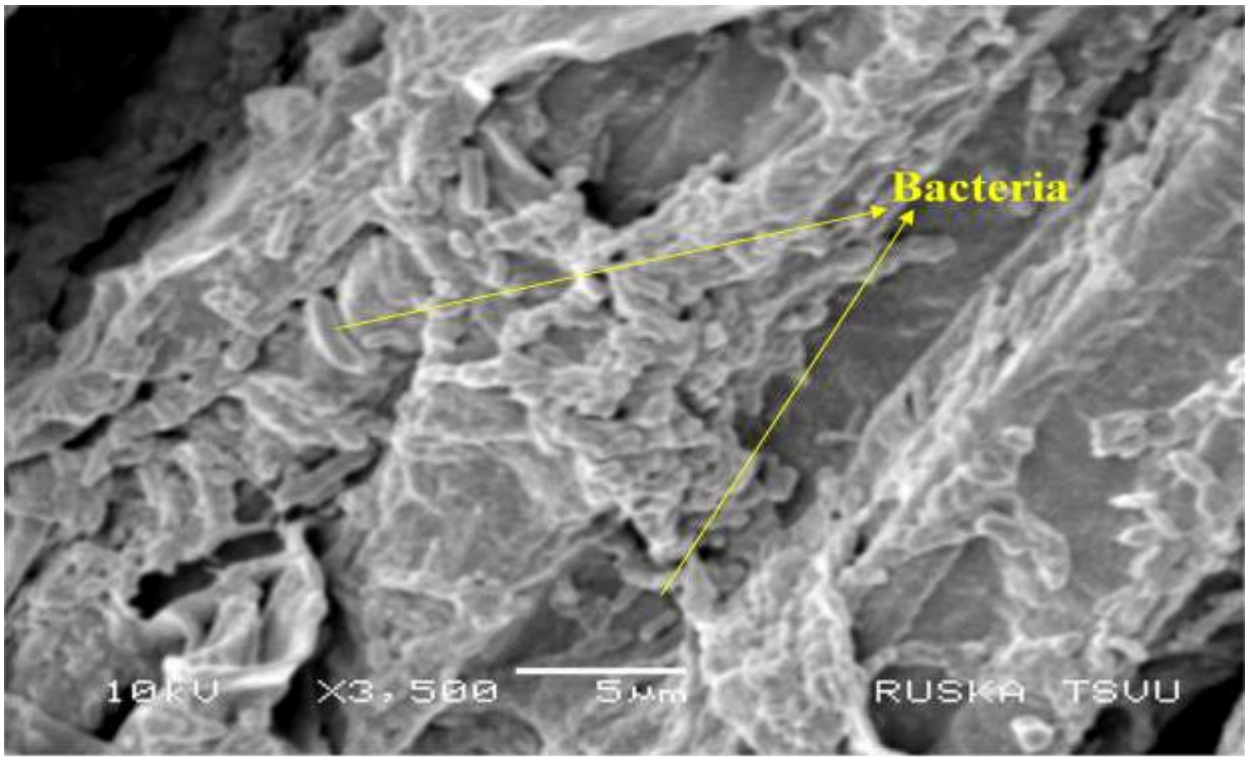


Plate.4.7: Colonization of root surface by *G. diazotrophicus*

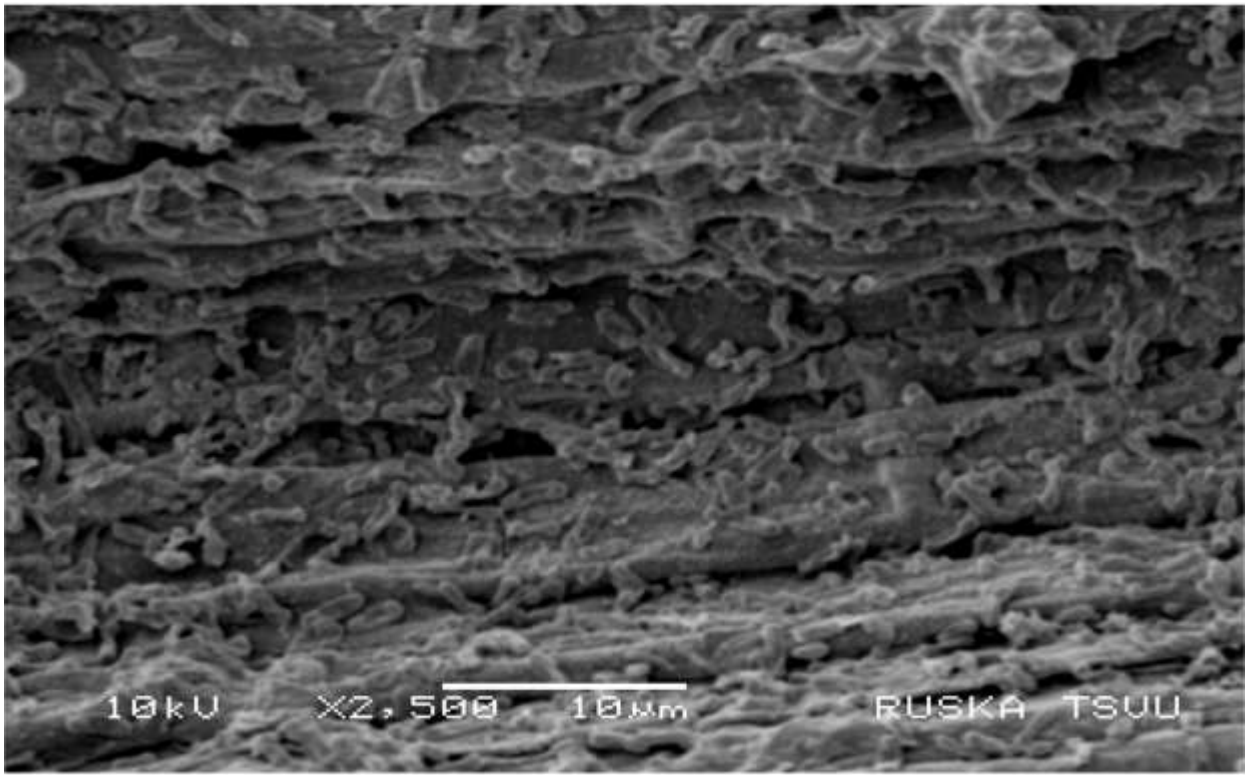


Plate.4.8: Colonization of root surface by *G.diazotrophicus and B.subtilis*

Plate.4.9: Root colonization ability of bacteria under hydroponic condition.

concentration highest root length was recorded in treatment T_3 (8.64cm) followed by T_2 and T_4 (8.55cm), (8.10cm) lowest value of root length was observed in T_1 (6.42cm) (Table.4.3 and Figure.4.2). Inoculation of plant growth promoting bacteria significantly influenced the seedling vigor index. Seed inoculated with *B.subtilis* (T_2) showed higher vigor index in all the three concentrations of PEG (0%, 15%, 20%) followed by treatment T_4 and T_3 showed comparable vigor index. However seeds inoculated without any culture showed poorest vigor index (Table.4.4 and Figure.4.3).

Table 4.2: Effect of *G.diazotrophicus* and *B.subtilis* on rice seed shoot growth under poly ethylene glycol - 6000 (PEG) induced water stress.

Treatments	Shoot Length (cm)		
	0% PEG	15% PEG	20% PEG
T_1	5.09	4.55	4.42
T_2	5.55	5.74	5.65
T_3	5.15	5.50	5.76
T_4	5.42	5.95	5.82
Grand mean	5.38		
C.V (%)	4.70		
C.D (0.05)	0.43		

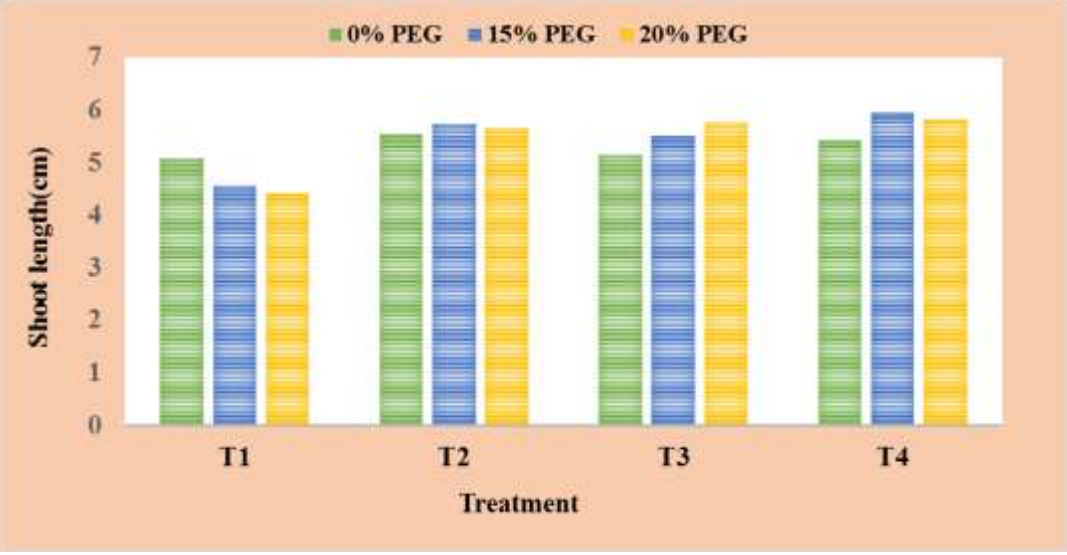


Fig. 4.1: Effect of *G. diazotrophicus and B.subtilis* on rice seed shoot growth under poly ethylene glycol-6000 (PEG) induced water stress.

Table 4.3: Effect of *G.diazotrophicus* and *B.subtilis* on rice seed root growth under poly ethylene glycol-6000 (PEG) induced water stress.

Treatments	Root Length (cm)		
	0% PEG	**15% PEG**	**20% PEG**
T_1	8.03	7.35	6.42
T_2	8.59	8.68	8.55
T_3	8.36	8.45	8.64
T_4	8.56	9.04	8.10
Grand mean	8.23		
C.V (%)	6.67		
C.D (0.05)	0.93		

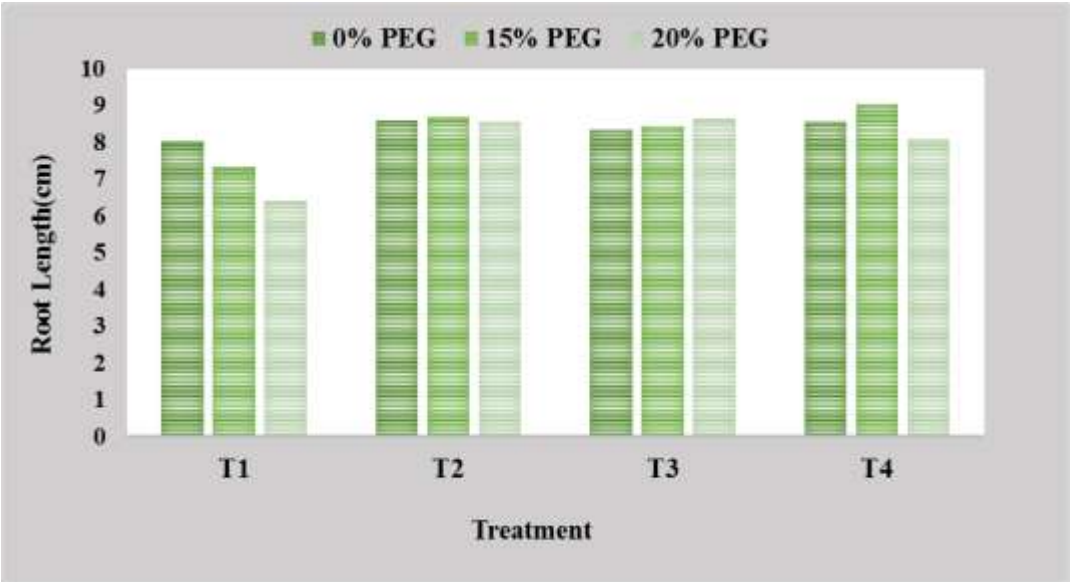


Fig. 4.2: Effect of *G. diazotrophicus* and *B.subtilis* on rice seed root growth under poly ethylene glycol-6000 (PEG) induced water stress.

Table 4.4: Effect of *G.diazotrophicus* and *B.subtilis* on rice seed vigor index under poly ethylene glycol-6000 (PEG) induced water stress.

Treatments	Vigor Index		
	0% PEG	**15% PEG**	**20% PEG**
T_1	517.37	432.98	400.81
T_2	564.26	574.92	551.21
T_3	523.70	551.37	507.85
T_4	550.89	572.96	544.12
Grand mean	524.37		
C.V (%)	5.21		
C.D (0.05)	46.04		

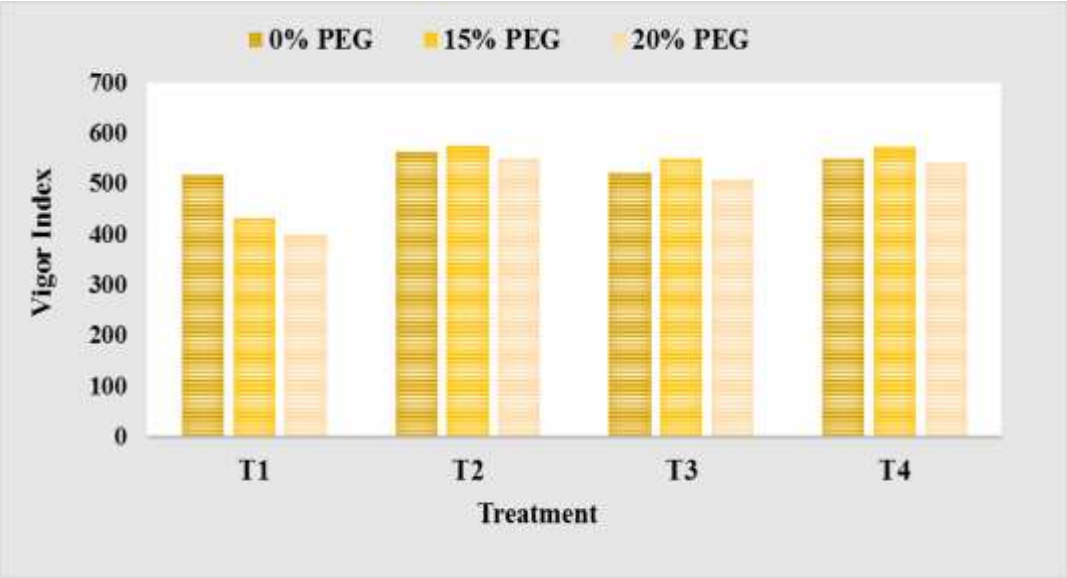


Fig. 4.3: Effect of *G.diazotrophicus* and *B.subtilis* on rice seed vigor index under poly ethylene glycol-6000 (PEG) induced water stress

4.3 Pot culture studies on the effect of the single and combined inoculation of *G. diazotrophicus* and *B. subtilis* on vegetative stage water stress tolerance and yield of rice.

4.3.1 Plant Biochemical Parameters:

4.3.1.1 Determination of Total Soluble Sugars in leaves.

The total soluble sugar with highest value under stress was obtained in T_4 (3.82 mg/g FW) followed by T_3 (3.05 mg/g FW), T_2 (2.24 mg/g FW) and the lowest value was obtained in T_1 (1.13 mg/g FW). The total soluble sugar with highest value without stress was obtained in treatment T_4 (4.63 mg/g FW) followed by T_3 (3.07 mg/g FW), T_2 (3.01 mg/g FW) and the lowest value was obtained in T_1 (1.95 mg/g FW) is represented in (Table.4.5; Figure.4.4 and Plate.4.10).

Soluble sugars occupy a central position in the cellular redox balance through their close relationships with photosynthesis, mitochondrial respiration and fatty acid b-oxidation as reported by Couée *et al*. (2006).Therefore, variations in sugar levels are able to influence the extent of ROS production in plant cells coupled to the oxidative metabolism in chloroplasts, mitochondria and peroxisomes. In addition, soluble sugars accumulate during different biotic and abiotic stress conditions related to oxidative stress as reported by Couée *et al*. (2006). Morsy *et al*. (2007) reported that rice seedlings challenged by chilling, salt and osmotic stress conditions show an enhanced lipid peroxidation and altered carbohydrate metabolism.

Lisar *et al.* (2012) and Lipiec *et al.* (2013) reported that when plants are faced by drought stress, the osmotic pressure of the plant cell regulates many process through the accumulation of non-toxic solutes inside the cell. This osmotic accumulation occurs because the cell water potential decrease thereby increasing the concentration of dissolved material to maintain turgidity of the cell. Moreover, compatible solutes prevent interaction between the ions and cellular components by replacing the water molecules around the component, thus preventing destabilization during drought. Osmotic accumulation is also due to increased biosynthesis without degradation by Lisar *et al.* (2012).

The sugar content in leaves of the plant can increase under drought conditions. Arabzadeh (2012) stated that the sugar dissolves compatible metabolites and absorption increases with increased drought stress and reduced soil water content.

Moreover, one of the mechanisms plants use to withstand drought stress is by regulating osmotic potential of the cell, especially if drought stress increase gradually from mild stress to severe one as reported by these authors Lisar *et al.* (2012), Lipiec *et al.* (2013) and Naghavi *et al.* (2013).

Sugar accumulation in drought stress conditions helps to maintain the stability of the membrane, prevent and protect membrane fusion and; keep protein so as to remain functional as reported by these authors Xonostle-Cazares *et al. (*2011); Arabzadeh (2012); Lisar *et al.* (2012) and Lipiec *et al.* (2013).

Table 4.5: Total soluble sugar content in leaf with stress and without stress condition.

Treatments	Total soluble sugars (mg / g FW)
T_1 (S)	1.13
T_2 (S)	2.24
T_3 (S)	3.05
T_4 (S)	3.82
T_1 (WS)	1.95
T_2 (WS)	3.01
T_3 (WS)	3.07
T_4 (WS)	4.63
Mean	2.86
C.V (%)	11.77
C.D (0.05)	NS

NS: Non Significant

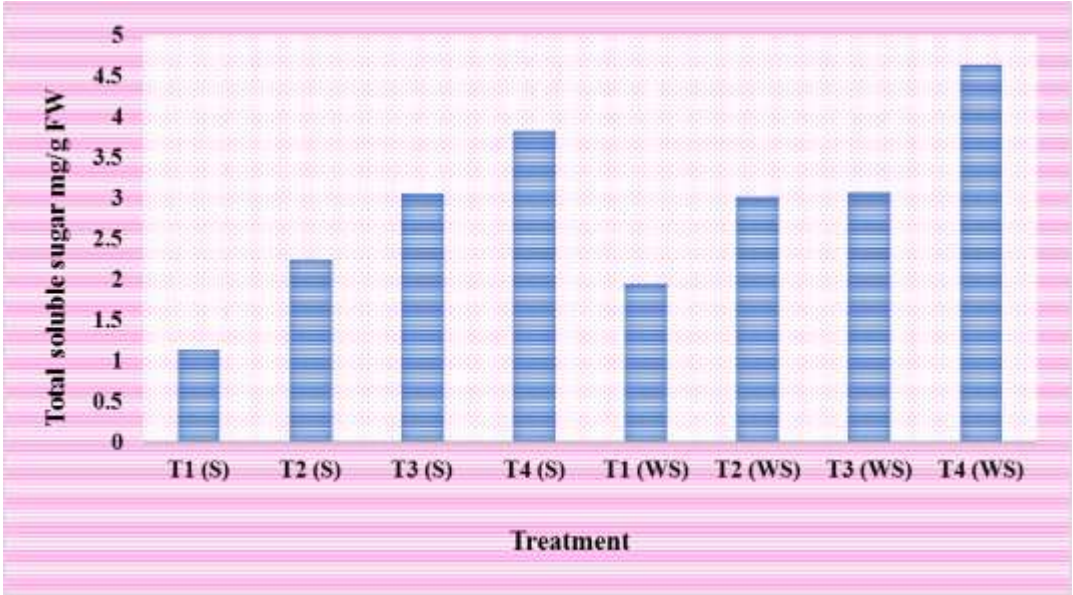


Fig. 4.4 Total soluble sugar content in leaf with stress and without stress condition.

4.3.1.2 Determination of Total Antioxidant Activity in Leaves.

It was observed that total antioxidant values of the leaf extract under stress are generally higher in T_4 (194.46 mg/g extract) followed by T_3 (140.07 mg/g extract), T_2 (96.06 mg/g extract) and the lowest value was recorded in T_1 (81.31 mg/g extract). Total antioxidant values of without stress condition shows higher value in T_4 (255.33 mg/g extract) followed by T_3 (206.36 mg/g extract), T_2 (186.92 mg/g extract) and the lowest value was recorded in T_1 (181.56 mg/g extract) is represented in (Table.4.6; Figure.4.5 and Plate.4.11) Severe water stress causes perturbations in the metabolic processes of the mitochondria and chloroplasts, leading to an overproduction of reactive oxygen species (ROS) as reported by Apel and Hirt (2004), which cause damage to macromolecules and subcellular components and are the most damaging consequence of abiotic stress reported by Berjak (2006). Plants have evolved a complex antioxidative defense system (including POD, SOD and CAT) to alleviate the damage caused by ROS as reported by Khan and Panda (2008)

Table 4.6: Total Antioxidant Activity in Leaves with stress and without stress condition.

Treatments	Total antioxidants mg VCEAC/g extract
T_1 (S)	81.31
T_2 (S)	96.06
T_3 (S)	140.07
T_4 (S)	194.46
T_1 (WS)	181.56
T_2 (WS)	186.92
T_3 (WS)	206.36
T_4 (WS)	255.33
Mean	167.76
C.V (%)	2.95
C.D (0.05)	NS

NS: Non Significant

VCEAC: Vitamin C Equivalent Antioxidant Capacity

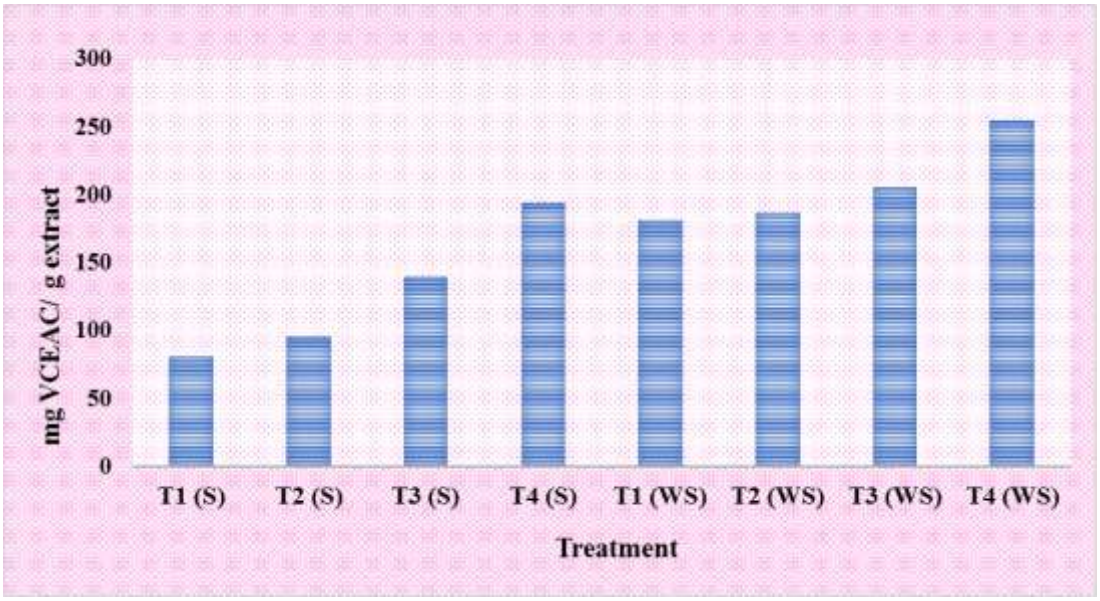


Fig.4.5 Total Antioxidant Activity in Leaves with stress and without stress condition.

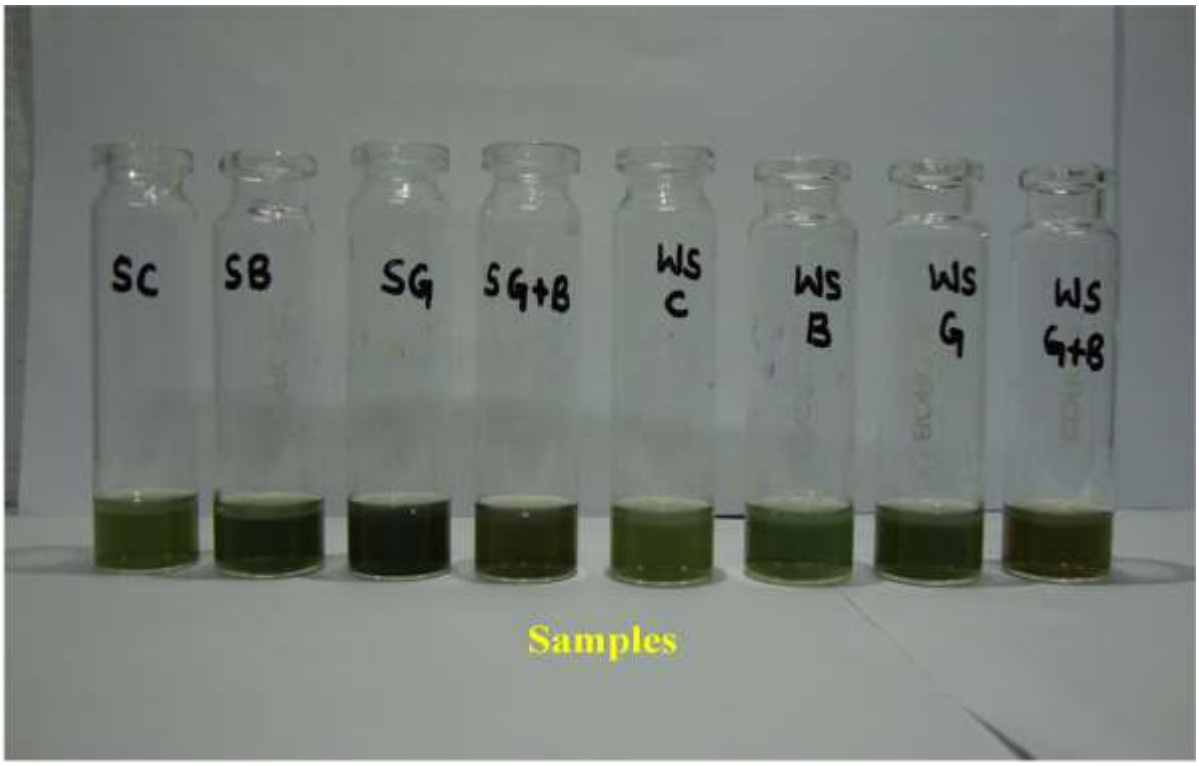


Plate.4.10: Determination of total soluble sugar in leaves

Plate.4.11: Determination of total antioxidant activity in leaves

4.3.2 Plant Physiological Parameters:

4.3.2.1 Leaf Chlorophyll Content

Chlorophyll estimation was done in the fresh green leaf samples extracted with the acetone solvent the absorbancy readings of chlorophyll extracts were measured in two different wavelengths 663.2nm, 646.8nm and 470nm respectively. Based on the absorbancy value calculations were made using Arnon's (1949) equation and the amount of chlorophyll a, chlorophyll b, carotenoid and total chlorophyll were estimated and tabulated. Chlorophyll is a green pigment consists of tetra pyrrole ring with a central magnesium ion. It has a long hydrophobic phytol chain in its structure. Two types of chlorophyll a and b are present. The ratio of chlorophyll a to chlorophyll b in higher plants is approximately 3:1. Chlorophyll absorbs light mainly in the red (650 – 700 nm) and the blue - violet (400 – 500 nm) regions of the visible spectrum. Green light (~550 nm) is not absorbed but reflected giving chlorophyll its characteristic color. Chlorophyll a possesses a green-blue color, and chlorophyll b possesses a green-yellow color (Arnon, 1949). Exposure to drought stress leads to a significant effect in Chlorophyll a and Chlorophyll b contents as stated by Ranjbarfordoei *et al*, (2000). Generally, healthy plants are expected to have higher chlorophyll content than unhealthy plants growing in the same growth period.

The highest chlorophyll a was detected under stress condition in T_3 (S) 3312.8mg/g FW followed by T_4 (S), T_2 (S) and lowest value was detected in T_1 (S) 1883.6mg/g FW. The highest chlorophyll b was detected under stress condition in T_3 (S) 973.8mg/g FW followed by T_4 (S), T_2 (S) and lowest value was detected in T_1 (S) 582.6mg/g FW. The highest Carotenoids was detected under stress condition in T_3 (S) 9936.1mg/g FW followed by T_4 (S), T_2 (S) and lowest value was detected in T_1 (S) 591.9mg/g FW. The highest total chlorophyll was detected under stress condition in T_3 (S) 4286.6mg/g FW followed by T_4 (S), T_2 (S) and lowest value was detected in T_1 (S) 2466.1mg/g FW.

Under without stress condition highest chlorophyll a was observed in T_4 (WS) 3482.6mg/g FW followed by T_2, T_3 and T_1. Highest chlorophyll b was detected in T_2 (WS) 1197.4mg/g FW followed by T_4, T_3 and T_1. Highest

carotenoids was detected in T_4 (WS) 1024mg/g FW followed by T_2, T_3 and T_1. Highest total chlorophyll content was recorded in T_4 (WS) 4442.3mg/g FW followed by T_2, T_3 and T_1 is represented in (Table.4.7 and Figure.4.6)

Table 4.7: Leaf Chlorophyll Content of stressed and without stress plants.

Treatments	Chl a mg/g FW	Chl b Mg/g FW	Carotenoids mg/g FW	Total chlorophyll mg/g FW
T_1 (S)	1883.6	582.6	591.9	2466.1
T_2 (S)	2287.9	717.6	755.7	3005.5
T_3 (S)	3312.8	973.8	936.1	4286.6
T_4 (S)	2795.7	760.8	848.6	3556.5
T_1 (WS)	2749.5	798.9	814.2	3548.3
T_2 (WS)	3096.9	1197.4	846.5	4294.3
T_3 (WS)	2762	802.6	842.1	3564.6
T_4 (WS)	3482.6	959.7	1024	4442.3
Mean	2796.4	849.16	832.31	3645.5
C.V (%)	38.34	35.07	37.87	36.27
C.D (0.05)	1877.3	521.54	551.90	2315.4

FW: Fresh Weight

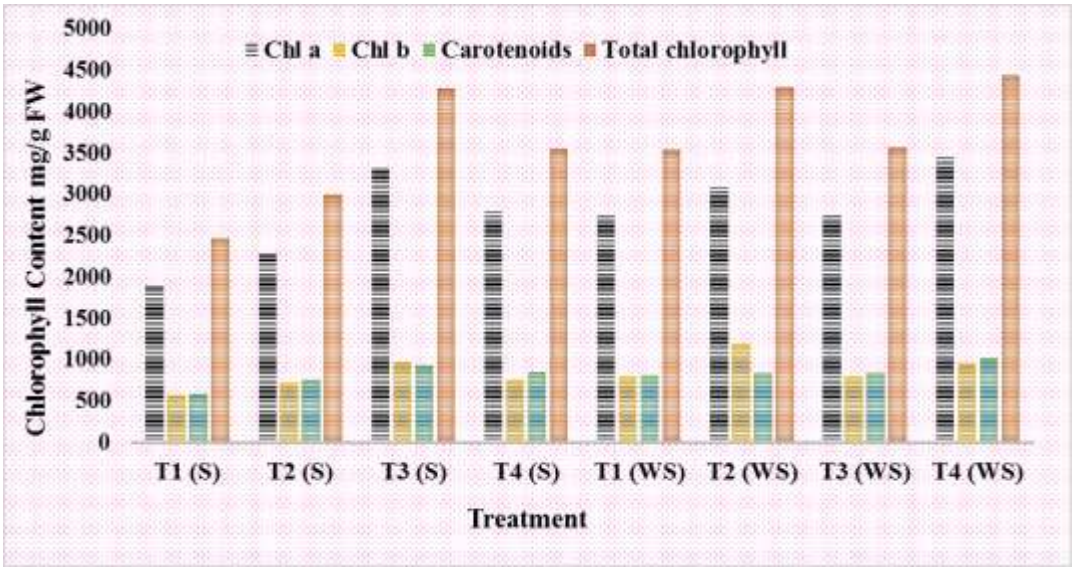


Fig.4.6 Leaf Chlorophyll Content of stressed and without stress plants.

4.3.2.2 Relative Water Content in Leaves

The relative water content (RWC; or 'relative turgidity') of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water holding capacity at full turgidity. RWC provides a measurement of the 'water deficit' of the leaf, and may indicate a degree of stress expressed under drought and heat stress. RWC integrates leaf water potential (ψ; another useful estimate of plant water status) with the effect of osmotic adjustment (a powerful mechanism of conserving cellular hydration) as a measurement of plant water status.

The highest relative water content was recorded under stressed condition is T_1 (93.135%) and lowest value was recorded in T_3 (89.734%). The highest relative water content was recorded under without stress condition is T_2 (94.290%) and lowest value was recorded in T_4 (87.394%) is represented in (Table.4.8 and Figure.4.7

Table 4.8: Calculating the leaf relative water content during stress and without stress condition.

Treatment	Relative Water Content (%)
T_1 (S)	94.083
T_2 (S)	93.135
T_3 (S)	89.734
T_4 (S)	91.659
T_1 (WS)	92.599
T_2 (WS)	94.290
T_3 (WS)	93.285
T_4 (WS)	87.394
Mean	92.022
C.V (%)	8.03
C.D (0.05)	12.947

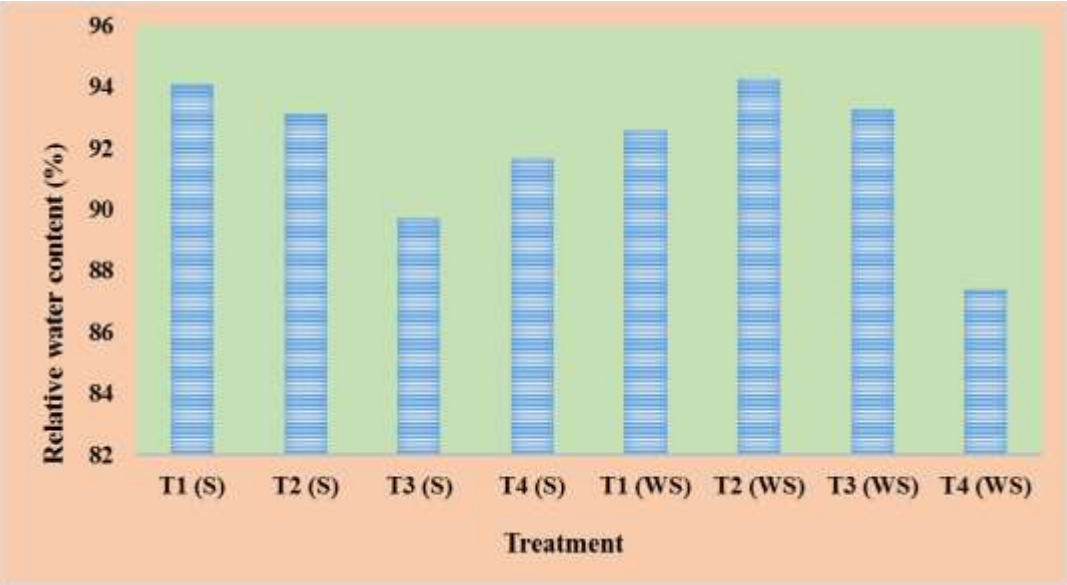


Fig.4.7 Relative Water Content in Leaves with stress and without stress.

4.3.2.3 Leaf membrane stability through electrolyte leakage

Unstressed, undamaged plant cells maintain electrolytes within the membrane. As the cells are subjected to stress, electrolytes leak into surrounding tissues. An estimation of cell damage and hardiness can be made by comparing the conductivity of the leaked contents from injured and uninjured tissues in water as reported by Mattsson, (1996); McNabb and Takahashi (2000).

The highest electrolyte leakage under stress was recorded in T_1 (32.19%) followed by T_2 (19.80%), T_4 and T_3. Highest electrolyte leakage was recorded under without stress condition is T_4 (20.21%) followed by T_3 and the lowest value was recorded in T_1 (4.05%) is represented in (Table.4.9 and Figure.4.8)

Table 4.9: Leaf membrane stability through electrolyte leakage under stress and without stress condition.

Treatment	Electrolyte Leakage (%)
T_1 (S)	32.19
T_2 (S)	19.80
T_3 (S)	10.01
T_4 (S)	16.70
T_1 (WS)	4.05
T_2 (WS)	6.36
T_3 (WS)	10.38
T_4 (WS)	20.21
Mean	14.96
C.V (%)	23.86
C.D (0.05)	6.236

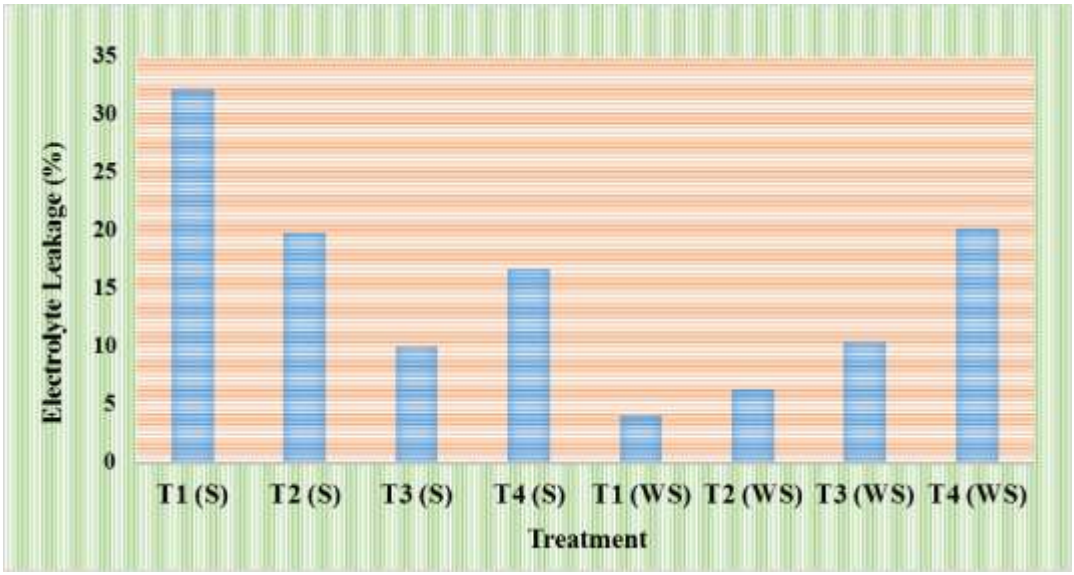


Fig.4.8 Leaf membrane stability through electrolyte leakage under stressed and without stress condition.

4.3.3 Plant Morphological Parameters:

4.3.3.1 Plant Height (cm)

The data regarding plant height was recorded at the harvesting time. The results shows that, the inoculation had a positive impact on plant height. *B.subtilis* T_2 (S) 55.66 cm at water stress condition and T_2 (WS) 57 cm at without stress condition produced highest plant height followed by T_4 and T_3. The lowest plant height was recorded in control T_1 (S) 48.33cm is given in (Table.4.10 and Figure.4.9)

4.3.3.2 Number of Tillers hill^{-1}

Total number of tillers of rice were recorded at harvesting stage, the total number of tillers hill^{-1} increased with increase in crop age but at maturity number of tillers were slightly reduced. Increase in plant height or number of leaves in successive growth stage helped in increasing the effective tillers. The data revealed that the maximum number of tillers hill^{-1} was recorded in *B.subtilis* T_2 (WS) 3.77 hill^{-1} followed by T_4 and T_3. Lowest number of tillers was recorded in Control T_1 (S) 2.77 hill^{-1} is given in (Table.4.10 and Figure.4.10)

4.3.3.3 Number of Panicles hill^{-1}

Total number of panicles hill^{-1} was recorded at harvesting stage, the number of panicles hill^{-1} is an important yield attributing character which ultimately determines the yield of rice crop. Maximum number of panicles was recorded in *B.subtilis* T_2 (WS) 3.66 hill^{-1} followed by T_3 and T_4 (Table.4.10 and Figure.4.11). Lowest number of panicles were recorded in control T_1 both in stress and without stress conditions.

4.3.3.4 Shoot Biomass (gm)

The data regarding shoot biomass was recorded after harvesting. The average shoot biomass observed was 12.66 gm. The highest shoot biomass was recorded in T_3 (WS) 17.93 gm followed by T_4 (WS) and T_2 (WS). Lowest shoot biomass was recorded in control T_1 both stress and without stress condition (Table.4.10 and Figure.4.12)

4.3.3.5 Root Biomass (gm)

The effect of inoculation of *B.subtilis* and *G.diazotrophicus* on plant shoot was determined, the inoculation had a positive effect on root growth and root biomass. The highest root biomass was recorded in T_2 (WS) 14.97gm followed by T_3 (WS) and T_2 (WS). Lowest shoot biomass was recorded in control T_1 both stress and without stress condition (Table.4.10 and Figure.4.13).

4.3.3.6 Grain yield hill^{-1}

The total number of grains hill^{-1} was recorded significantly higher under T_4 treatment both under water stress and without water stress condition, followed by T_2 and T_3. Lowest grain yield was recorded under control condition T_1 (Table.4.10 and Figure.4.14).

Table 4.10: Plant morphological parameters showing plant height, tillers hill^{-1}, panicles hill^{-1}, root biomass, shoot biomass and grain yield.

Treatments	Plant height (cm)	No. of Tillers (hill^{-1})	No. of Panicles (hill^{-1})	Root biomass (gm)	Shoot biomass (gm)	No. of Grains (hill^{-1})
T_1 (S)	48.33	2.77	2.77	9.16	7.96	88.44
T_2 (S)	55.66	3.33	3.11	13.83	11.86	100.77
T_3 (S)	54.33	3.11	3.00	12.38	11.33	99.33
T_4 (S)	55.83	3.33	3.11	6.08	11.98	107.22
T_1 (WS)	50.00	3.00	2.77	6.04	8.45	89.99
T_2 (WS)	57.00	3.77	3.66	14.97	15.59	105.77
T_3 (WS)	54.33	3.44	3.32	12.60	17.93	101.88
T_4 (WS)	55.76	3.44	3.26	8.80	16.21	108.88
Mean	53.90	3.276	3.130	10.486	12.665	101.98
C.V (%)	2.538	13.987	12.130	34.553	34.469	3.057
C.D (0.05)	2.389	NS	NS	-	NS	2.504

NS: Non Significant

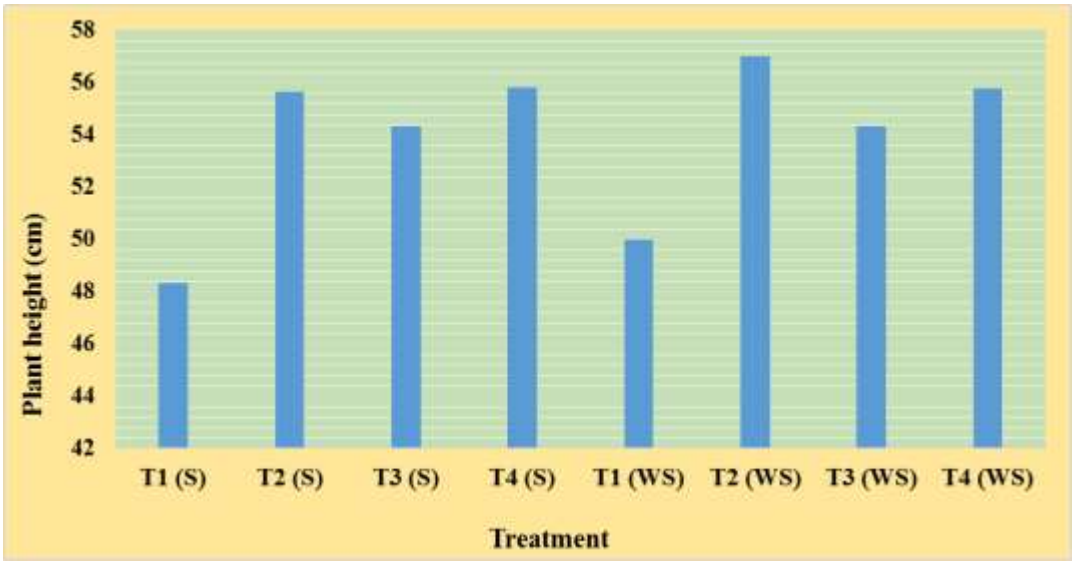


Fig.4.9 Effect of plant height under water stress and without water stress condition

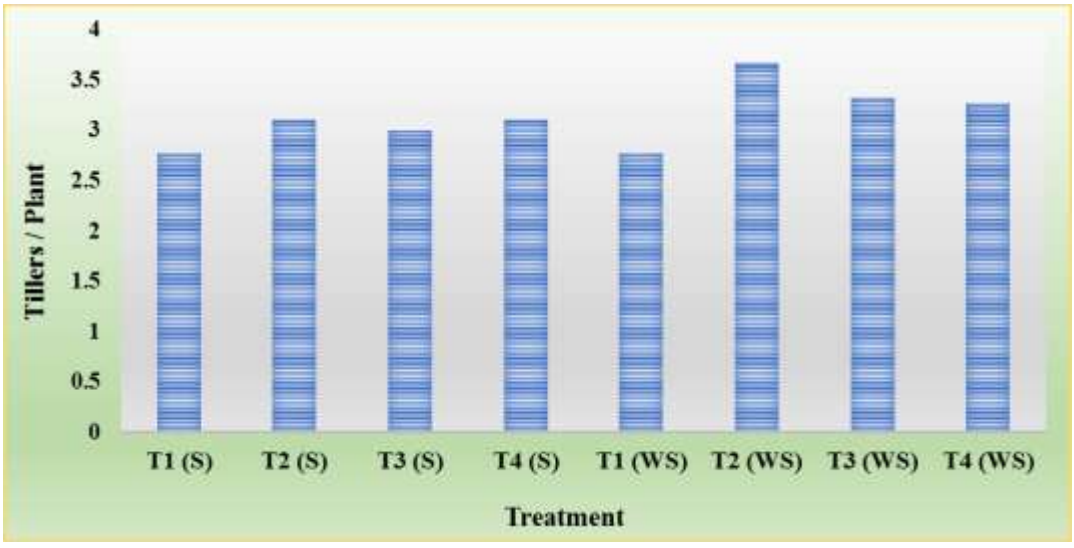


Fig.4.10 Effect of number of tillers hill^{-1} under water stress and without stress condition.

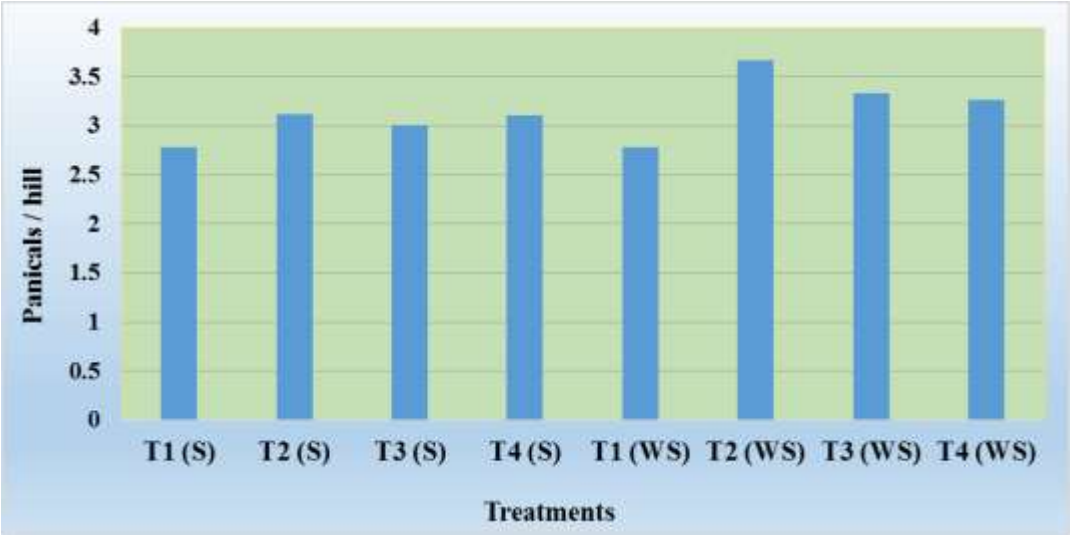


Fig.4.11 Effect of number of panicles hill^{-1}undr stress and without stress condition.

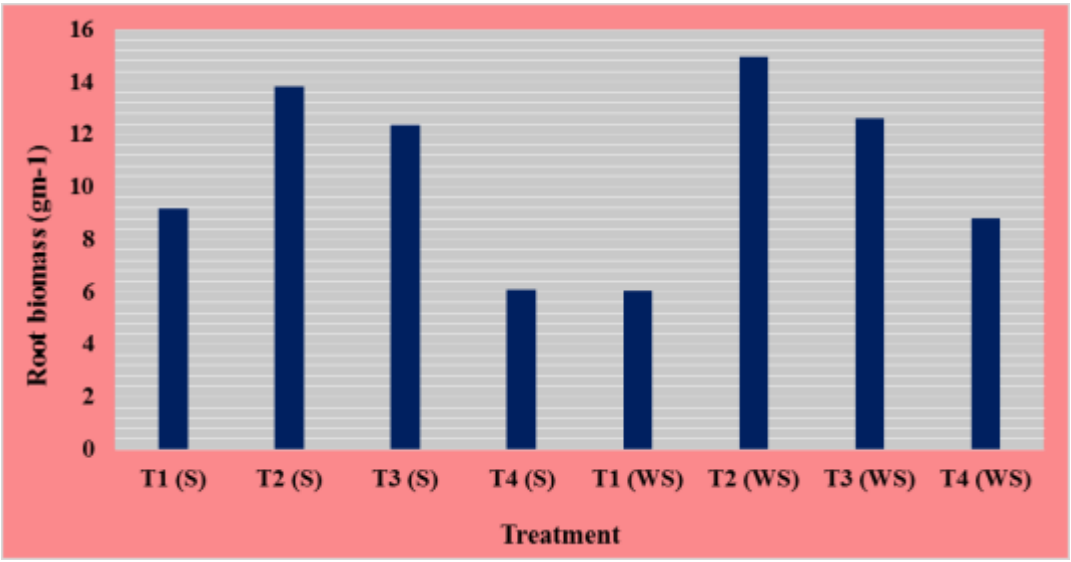


Fig.4.12 Effect of shoot biomass hill^{-1} under water stress and without stress condition.

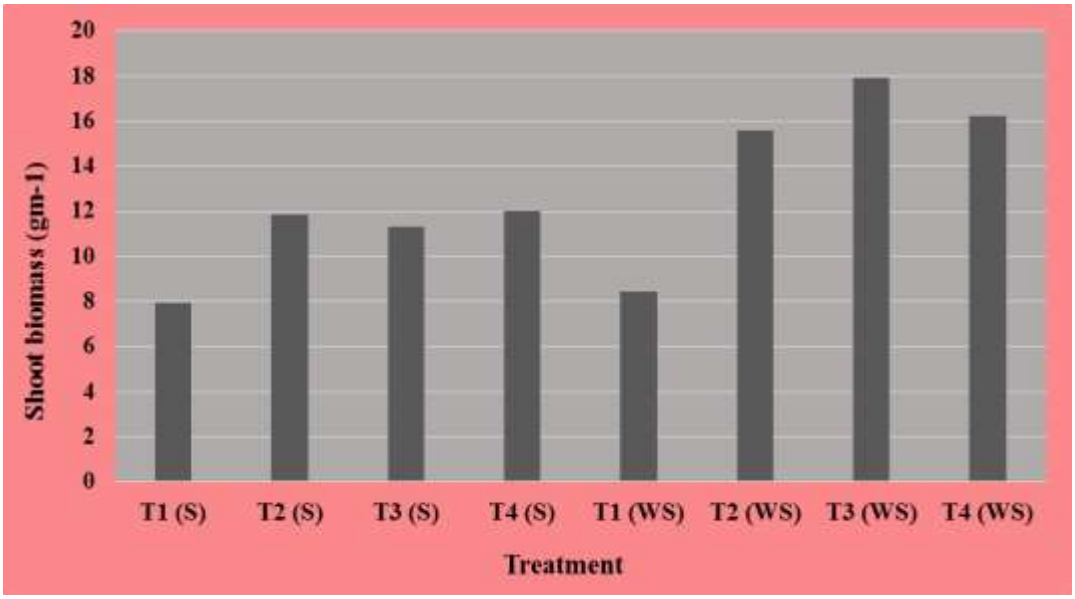


Fig.4.13 Effect of root biomass hill^{-1} under water stress and without stress condition.

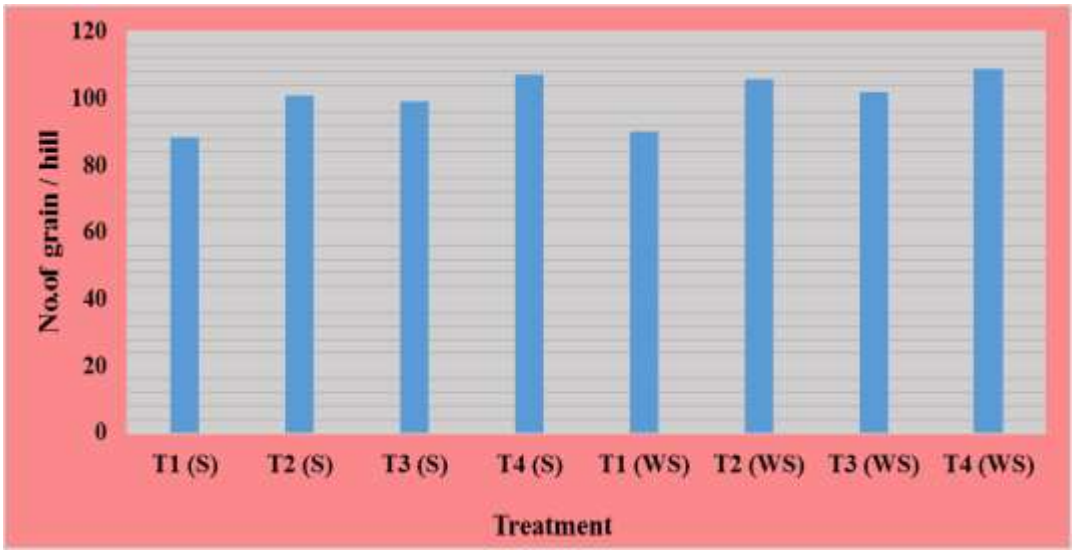


Fig.4.14 Effect of number of grains hill^{-1} under water stress and without stress condition.

4.3.4 Soil analysis:

4.3.4.1 Soil Moisture Content

The method is based on removing soil moisture by oven-drying a soil sample until the weight remains constant. The moisture content (%) is calculated from the sample weight before and after drying is represented in (Table.4.11 and Figure.4.15). Water content is an important property of soils, influencing soil solution chemistry and nutrient uptake by plants. Morphology and other specific properties of the root, nutrient concentration in the soil solution, the mobility of nutrients in the soil, and supply from solid phases, affect nutrient uptake reported by (Nye and Tinker, 1977; Barber, 1995).

Table 4.11: Effect of soil moisture content under water stress and without stress condition.

Treatment	Soil moisture content (%)
T_1 (S)	0.191
T_2 (S)	0.168
T_3 (S)	0.165
T_4 (S)	0.181
T_1 (WS)	0.192
T_2 (WS)	0.164
T_3 (WS)	0.153
T_4 (WS)	0.183
Mean	0.175
C.V (%)	0.331
C.D (0.05)	0.001

4.3.4.2 Soil Carbohydrate Content

The highest soil carbohydrate content was recorded in T_4 (S) 7.14% under stress condition followed by T_3(S) 6.65%, T_2 (S) 5.02% and lowest carbohydrate content was recorded in T_1 (S) 4.29%. The highest soil carbohydrate content was recorded in T_4 (S) 7.75% under non stress condition followed by T_3 (S) 7.28%, T_2 (S) 5.45% and lowest carbohydrate content was recorded in T_1 (S) 4.45% is represented in (Table.4.12; Figure.4.16 and Plate.4.12). It was observed that under water deficit stress conditions there was soil carbohydrate content was reduced. Inoculation with these water tolerant strains significantly enhanced soil carbohydrate content under both normal and water stress conditions. However, increase in carbohydrate content of soil was considerably higher due to inoculation under water stress conditions. The inoculation of combined strain plants showed better results as compared to singly inoculated strain and un-inoculated plants.

Table 4.12: Effect of soil carbohydrate content under water stress and without stress condition.

Treatments	Soil carbohydrate content (%)
T_1(S)	4.29
T_2(S)	5.02
T_3(S)	6.65
T_4(S)	7.14
T_1(WS)	4.45
T_2(WS)	5.45
T_3(WS)	7.28
T_4(WS)	7.75
Mean	6.01
C.V (%)	26.37
C.D (0.05)	2.77

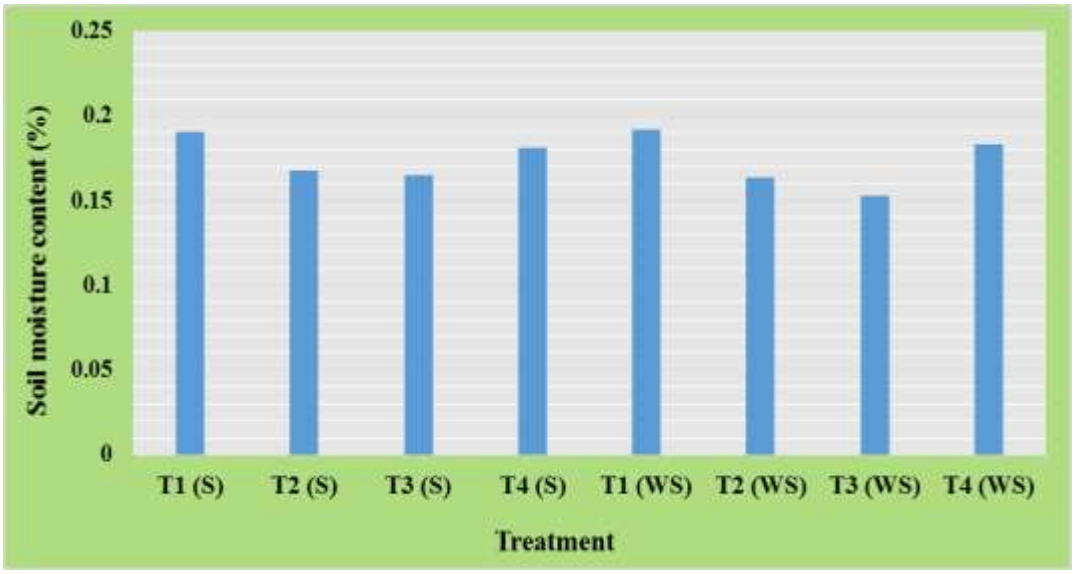


Fig.4.15 Soil moisture content under water stress and without stress condition.

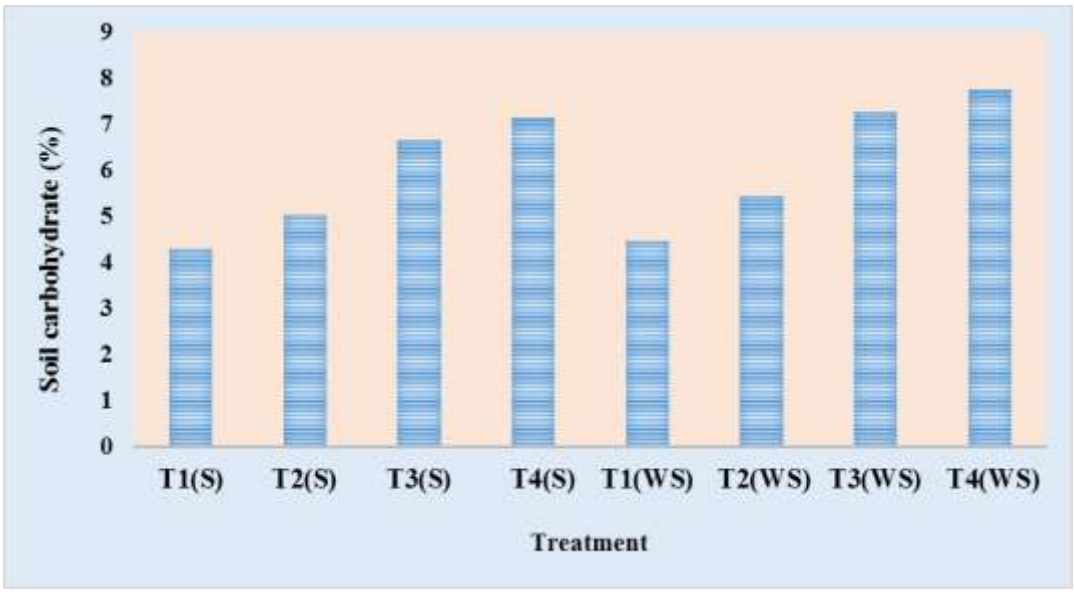


Fig. 4.16 Soil carbohydrate content under water stress and without stress condition.

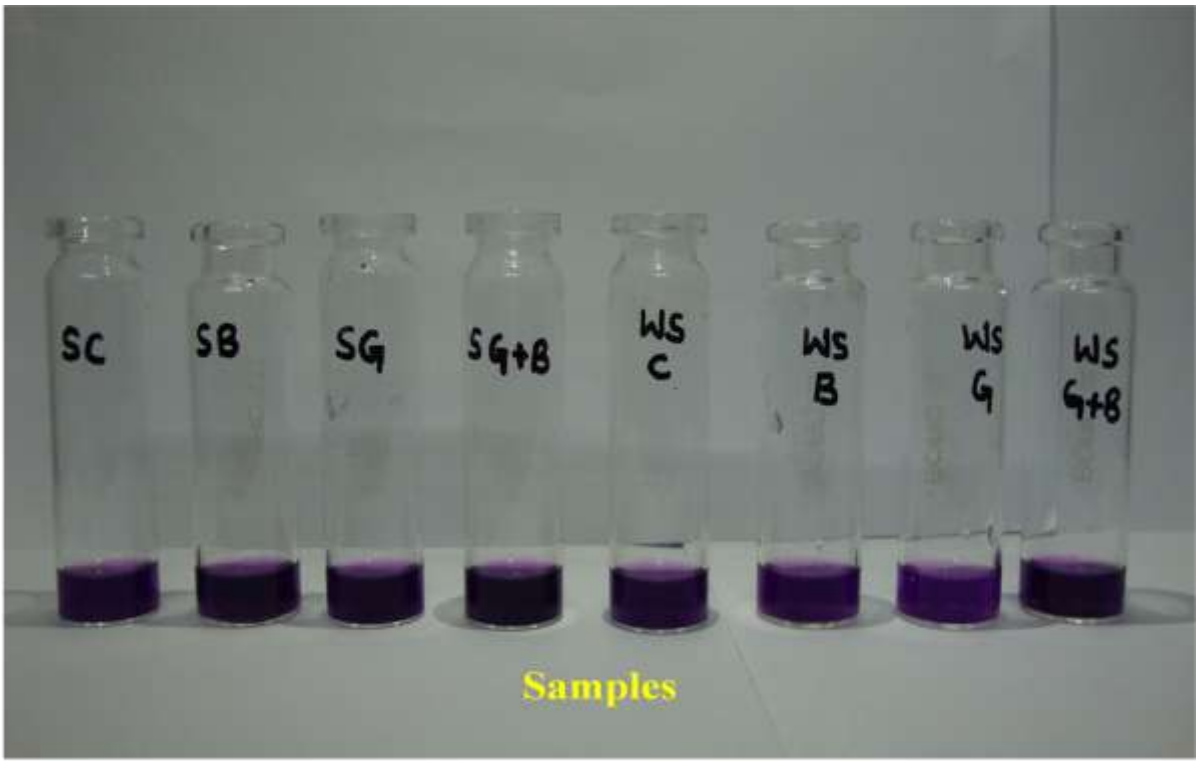


Plate.4.12: Determination of soil carbohydrate content

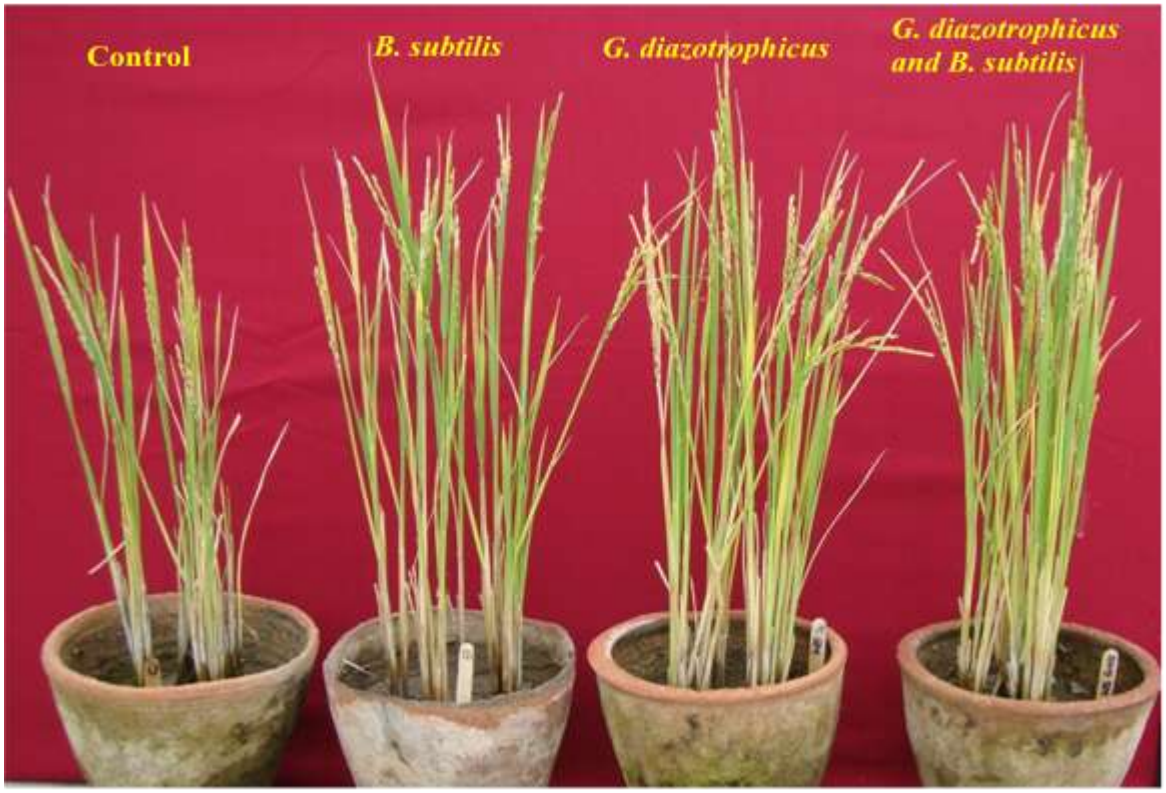


Plate.4.13: Treatments grown under non stressed condition

Plate.4.14: Recovery of rice plants after imposing stress.

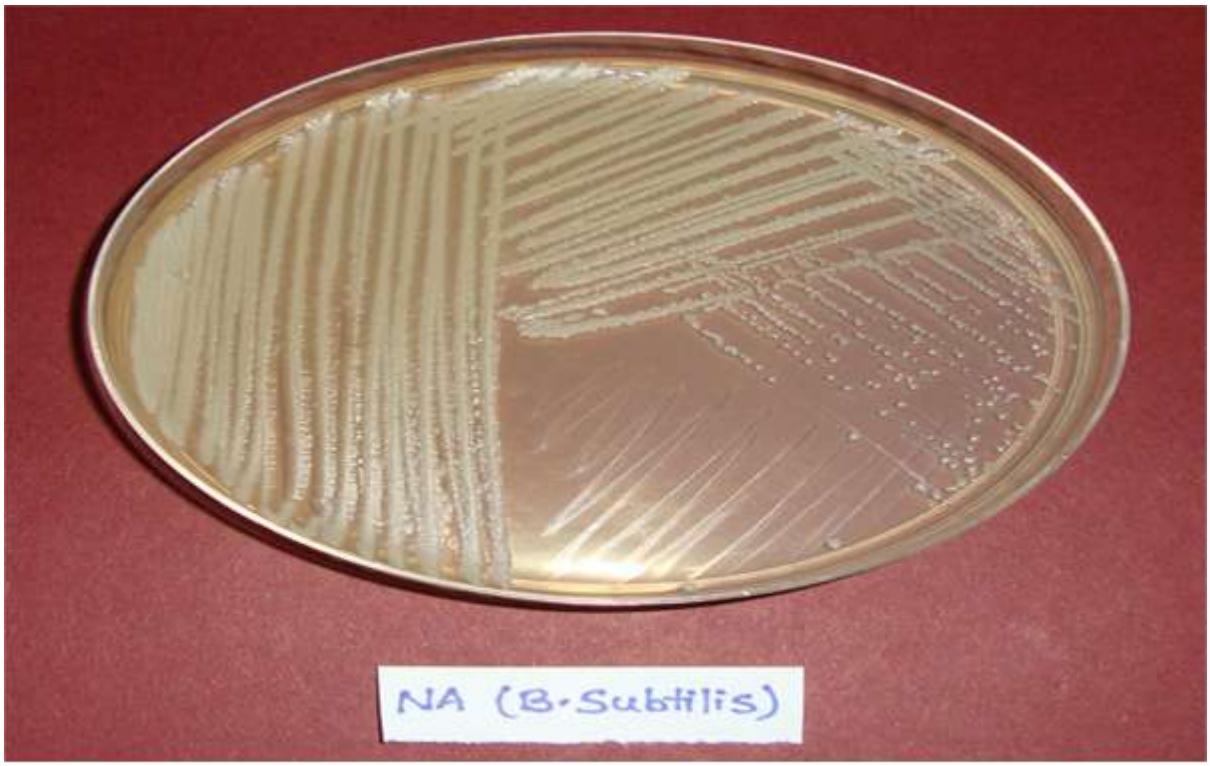


Plate.4.15: Growth of *B.subtilis* in nutrient agar medium

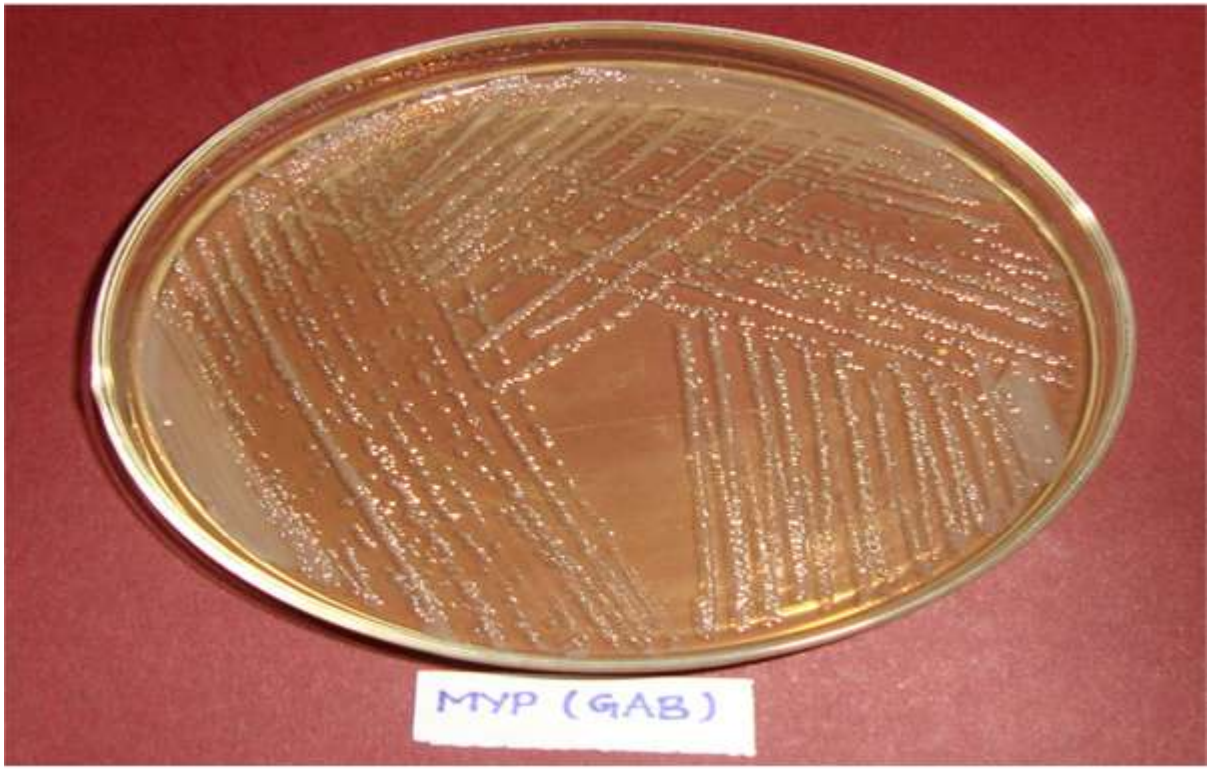


Plate.4.16: Growth of *G. diazotrophicus* in MYP medium

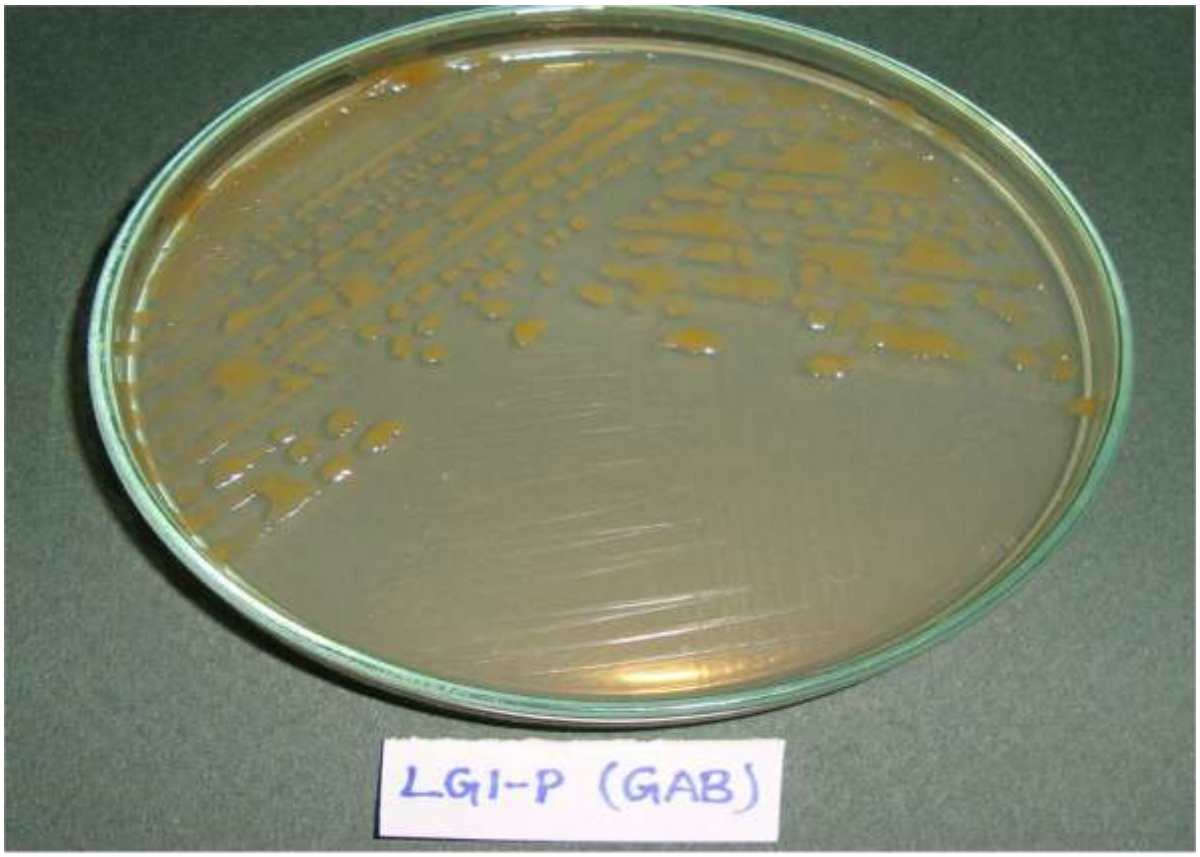


Plate.4.17: Growth of *G.diazotrophicus* in LGI medium

CHAPTER- V

SUMMARY AND CONCLUSION

The present investigation entitled **"Evaluation of *Gluconacetobacter diazotrophicus* and *Bacillus subtilis* for enhancing water deficit stress tolerance in rice (*Oryza sativa* L)"** was conducted in the Department of Soil Science Microbiology, Indian Institute of Rice Research, Rajendranagar, Hyderabad, during the year 2016-2017 comprising 8 treatments and 3 replications laid out in Completely Randomized Block Design with the objective to find out the Swarna the most popular Indian rice variety of high productivity and grown on large acreage in rainfed environments due to its high yield potential and preferred grain quality. However, the variety is susceptible to drought and imparting drought tolerant trait to this variety will be highly useful to the farming community, it is a microbial based approach to mitigate drought stress can serve as a novel solution for improving rice plant tolerance to restricted water availability. Although the roles of PGPR in plant growth promotion, nutrient management, and disease control are well known, their roles in the management of abiotic stress such as drought has only recently gained importance.

Pure cultures of *Gluconacetobacter diazotrophicus* Pal 5 (Type strain) and *Bacillus subtilis* (GenBank accession number- MF171124) maintained in the Microbiology lab, Soil Science Section, IIRR were used for in vitro and pot culture experiments.

The results have been furnished and discussed in the preceding chapter. The salient findings of the investigation have been summarized in this chapter in following points:

1. Swarna seeds were overnight inoculated with *(B.subtilis, G.diazotrophicus* and combination of *B.subtilis + G.diazotrophicus*) cultures and were treated with different concentrations of PEG (0% (control or no PEG), 15% PEG, 20% PEG). Under 0% PEG concentration the highest shoot length was observed in T_2 (5.55cm) followed by T_4 (5.42), T_3 (5.15).In 15% PEG and

20% PEG concentration highest shoot length was recorded in T_4 (5.95%), (5.82%) followed by T_2 (5.74%), (5.65%), T_3 (5.50%), (5.76%).

2. Similarly under 0% PEG the highest root length was recorded in T_2 (8.59%) and the lowest value of root length, was recorded for Control 0% PEG T_1 (8.03%). Under 15% PEG concentration highest root length was recorded in treatment T_4 (9.04%) and the lowest value of root length was observed in T_1 (7.35%). Under 20% PEG concentration highest root length was recorded in treatment T_3 (8.64%) and the lowest value of root length was observed in T_1 (6.42%).Inoculation of plant growth promoting bacteria significantly influenced the seedling vigor index. Seed inoculated with *B.subtilis* (T_2) showed higher vigor index in all the three concentrations of PEG (0%, 15%, 20%)

3. Both the selected cultures (*G. diazotrophicus, B. subtilis)* were IAA producers. There was a significant increase in both the cultures after adding L-Tryptophan. The highest IAA production was observed in *G. diazotrophicus* (748.04µg / ml) followed by *B.subtilis* (529.33 µg / ml) with tryptophan. The highest value of deamination was observed in *G.diazotrophicus* (4.10 µg α-ketobutrate /µg cell protein) followed by *B.subtilis* (1.67 µg α-ketobutrate /µg cell protein). Highest exopolysaccharides production was observed in *G.diazotrophicus* (2.4g/L)

4. Plant biochemical parameters include antioxidant activity and total soluble sugar. The total soluble sugar with highest value under stress was obtained in T_4 (3.82 mg/g FW) and T_4 (4.63 mg/g FW) under without stress condition. The total antioxidant values of the leaf extract under stress are generally higher in T_4 (194.46 mg/g extract) and T_4 (255.33 mg/g extract) under without stress condition.

5. Plant physiological parameters include chlorophyll content, relative water content and electrolyte leakage. Treatment T_3 (S) showed highest Chlorophyll a, Chlorophyll b, Carotenoid content and Total chlorophyll content under stress condition. The highest relative water content was recorded under stress and without stress condition is T_2 (S). The highest

electrolyte leakage under stress was recorded in T_1 (32.19%) and under without stress condition highest electrolyte leakage was recorded in T_4 (20.21%)

6. Plant morphological parameters include plant height, shoot biomass, root biomass, number of tillers per plant, number of panicles per plant and grain yield per plant. The results shows that, the inoculation had a positive impact on plant height. *B.subtilis* T_2 (S) 55.66 cm at water stress condition and T_2 (WS) 57 cm at without stress condition produced highest plant height. The maximum number of tillers $hill^{-1}$ was recorded in *B.subtilis* T_2 (WS) 3.77 $hill^{-1}$. Maximum number of panicles was recorded in *B.subtilis* T_2 (WS) 3.66 $hill^{-1}$. The highest shoot biomass was recorded in T_3 (WS) 17.93gm. The highest root biomass was recorded in T_2 (WS) 14.97gm. The total number of grains $hill^{-1}$ was recorded significantly higher under T_4 treatment both under water stress and without water stress condition.

7. Soil analysis parameters include rhizospheric soil moisture content and soil carbohydrate content. The highest soil carbohydrate content was recorded in T_4 (S) 7.14% under stress condition followed by T_3(S) 6.65%, T_2 (S) 5.02% and lowest carbohydrate content was recorded in T_1 (S) 4.29%. The highest soil carbohydrate content was recorded in T_4 (S) 7.75% under non stress condition followed by T_3(S) 7.28%, T_2 (S) 5.45% and lowest carbohydrate content was recorded in T_1 (S) 4.4567%.

Conclusion

The present finding clearly indicated that application of *B.subtilis and Gluconacetobacter* significantly improves plant growth parameters in both water stress and without stress condition. However the performance was found to be better stressed condition. Inoculated plants showed better results as compared to control. Both strains *B.subtilis* and *G. diazotrophicus* produce high amount of Indole acetic acid, ACC deaminase and Exopolysaccharide. EPS provides a micro environment that holds water and dries up more slowly than the surrounding environment thus protecting the bacteria and plant roots against desiccation. IAA stimulates cell elongation by modifying certain conditions like, increase in osmotic

contents of the cell, increase in permeability of water into cell, decrease in wall pressure, an increase in cell wall synthesis and inducing specific RXA and protein synthesis. It promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting. Bacteria containing ACC deaminase attach to plant cells, act as a sink for plant ACC to uptake and cleave the ACC secreted by plant cells and thus reduce plant ACC concentration, ethylene evolution and the extent of ethylene inhibition of plant growth, particularly, under a variety of abiotic and biotic stresses.

Suggestions for future research work

1. The present experimental observation should be validated at field condition to confirm the results.
2. Molecular work should be carried on for gene expression in relation to the colonization of rice roots.

REFERENCES

Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D., et al. 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. Front. Plant Sci. 6:868. doi: 10.3389/fpls.2015.00868.

Alami, Y., Achouak, W., Marol, C., Heulin, T. 2000. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide producing *Rhizobium sp*. strain isolated from sunflower roots. Appl. Environ. Microb. 66, 3393–3398.

Alquéres, S., Meneses, C., Rouws, L., Rothballer, M., Baldani, I., Schmid, M. and Hartmann, A. 2013. The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* PAL5. Mol Plant-Microbe Interact 26:937- 945.

Anonymous, 2004, Soil Survey Laboratory Method Manual

Apel, K., Hirt, H. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol; 55: 373-99.

Arabzadeh, N. 2012. The effect of drought stress on soluble carbohydrates (Sugars) in two species of *Haloxylonpersicum*and *Haloxylonaphyllum*. Asian J. Plant Sci. 11(1):44-51.

Aron, D. 1949. Copper enzymes isolated chloroplasts, polyphenoloxidase in Beta vulgaris. Plant Physiology. 24: 1-15.

Arshad, M., Frankenberger, W.T. 1992. Microbial production of plant growth regulators. In: Metting FB Jr(eds). Soil Microbial Ecol, Marcel Dekker Inc., New York. pp: 307-347.

Ashraf, M. 1994. Breeding for salinity tolerance in plants. Crit. Rev. Plant Sci. 13, 17–42.

Ashraf, M., and O'Leary, J.W. 1996. "Effect of Drought Stress on Growth, Water 1996 Relations and Gas Exchange of Two Lines of Sunflower Differing in Degree of Salt Tolerance," International Journal of Plant Sciences, Vol. 157, No. 6, pp. 729-732. doi:10.1086/297395

Ashwell, G. 1957. Colorimetric analysis of sugars. In: Colowick, S.J., Kaplan, N. O., (eds) Methods in Enzymology, vol.3, Academic Press, New York.

Austin, R.B., 1989. Prospect for improving crop production in stress full environments. In: Hamyln, G.J., Flowers, T.J., Jones, M.B. (Eds.), Plants Under Stress. Biochemistry, Physiology and Ecology and Their Application to Plant Improvement. Cambridge University Press, Cambridge, pp. 235–248.

Aza, M., Abdel-Fattah, Amira, M., Gamal-Eldeen, Wafaa, A., Helmy, Mona, A., Esawy. 2012. Antitumor and antioxidant activities of levan and its derivative from the isolate *Bacillus subtilis* NRC1aza, Carbohydrate Polymers, 89: 314-322.

Bacon, C.W. and Hinton, D.M. 2006 Bacterial endophytes: The endophytic niche, its occupants, and its utility. In: Gnanamanickam SS (Ed) Plant-Associated Bacteria. Springer, Netherlands, pp 155-194.

Bal, H. B., Subhasis, Das., Tushar, K., Dangar and Tapan, K., Adhya. 2013. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. J. Basic Microbiol. 53, 972–984.

Barazani, O. and Friedman, J. 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? J. Chem. Ecol. 25:2406.

Barber, S.A. 1995. Soil nutrient bio availability a mechanistic approach. 2nd edn.,New York: John Wiley and Sons, Inc.

Barnawal, D., Deepamala, Maji., Nidhi Bharti, Chandan Singh, Chanotiya and Alok Kalra. 2013. ACC Deaminase-Containing *Bacillus subtilis* Reduces Stress Ethylene-Induced Damage and Improves *Mycorrhizal* Colonization

and *Rhizobial* Nodulation in *Trigonella foenum-graecum* Under Drought Stress. Journal of Plant Growth Regulation, 32: 809–822

Barrs, H. D. and Weatherley, P.E. 1962. "A re-examination of the relative turgidity technique for estimating water deficits in leaves," Australian Journal of Biological Science 15: 413– 428.

Beauregard, P.B., Chai, Y., Vlamakis, H., Losick, R. and Kolter, R. 2013 *Bacillus subtilis* biofilm induction by plant polysaccharides. Proc Natl Acad Sci USA 110:E1621-E1630.

Bensalim, S., Nowak, J., Asiedu, S.K., 1998. A plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato. Am. J. Potato Res. 75, 145–152.

Berjak, P. 2006. The challenge of recalcitrant germplasm cryopreservation. J Hortic Sci Biotech;81:781-2.

Bian, S. and Jiang, Y. 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns and recovery. Sci. Hortic. 120, 264–270. doi: 10.1016/j.chemosphere.2016.02.072.

Bianco, C. and Defez, R. 2009, *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *J. Exp. Bot*., 60 (11): pp 3097-3107.

Birthal, P.S., Negi, D.S., Md. Tajuddin Khan and Agarwal, S. 2015. Is Indian agriculture becoming resilient to droughts? Evidence from rice production systems. Food Policy 56: 1–12

Bottner, P., Couteaux, M.M., Vallejo, V.R., 1995. Soil organic matter in Mediterranean-type ecosystems and global climatic changes: a case study-the soils of the Mediterranean basin. In: Jose, M., Oechel, W.C. (Eds.), Global Change and Mediterranean-type Ecosystems. Ecological studies, 117.Springer-Verlag, New York, pp. 306–325.

Cavalcante, V.A. and Dobereiner, J. 1988. A new acid tolerant nitrogen-fixing bacterium associated with sugarcane. Plant and Soil, Vol. 108, no. 1, pp. 23-31.

Chezen, O., Hartwig, W. and Newman, P.N. 1995. "The Different Effects of PEG-6000 and NaCl on Leaf Development Are Associated with Differential Inhibition of Root Water Transport," Plant Cell, Vol. 18, No. 7, pp. 727-735. doi:10.1111/j.1365-3040.1995.tb00575.x

Compant, S., Clément, C. and Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42:669-678.

Costacurta, A. and Vanderleyden, J. 1995. Synthesis of phytohormones by plant associated bacteria. Crit. Rev. Microbiol. 21:1–18.
Costacurta, A. and Vanderleyden, J. 1995. Synthesis of phytohormones by plant associated bacteria. Crit. Rev. Microbiol. 21:1–18.
Couée, I., Sulmon, C., Gouesbet, G. & El Amrani, A. 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. Journal of Experimental Botany 57, 449–459.

Crane, T. A., Roncoli, C. and Hoogenboom, G. 2011. Adaptation to climate change and climate variability: the importance of understanding agriculture as performance. NJAS–Wag. J. Life Sci. 57,179–185.doi: 10.1016/j.njas.2010. 11.002

Danhorn, T. and Fuqua, C. 2007. Biofilm formation by plant associated bacteria. Annu Rev Microbiol 61:401-422.

Datta, C. and Basu, P. 2000. lndole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub *Cajanus cojan*. Microbiol. Res. 155, 123 – 127.

Dimkpa, C., Weinand, T., Asch, F. 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ. 32, 1682–1694.

Domínguez-Porras, J.R., Ángela Ávila-Fernández, Afonso Miranda-Molina, María Elena, Rodríguez-Alegría, and Agustín López Munguía. 2015. *Bacillus subtilis* 168 levansucrase (SacB) activity affects average levan molecular weight. Carbohydrate Polymers 132 :338–344.

Dong, Z., Zelmer, C.D., Canny, M.J. et al. 2002. "Evidence for protection of Nitrogenase from O_2 by colony structure in the aerobic diazotroph *Gluconacetobacter diazotrophicus*," Microbiology, vol. 148, no. 8, pp. 2293–2298.

Dworkin, M. and J. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol., 75: 592-601.

Eskin, N., Vessey, K. and Tian, L. 2014. Research Progress and Perspectives of Nitrogen Fixing Bacterium, *Gluconacetobacter diazotrophicus*, in Monocot Plants. International Journal of Agronomy, http://dx.doi.org/10.1155/2014/208383

Gagné-Bourque, F., Mayer, B.F., Charron, J.B., Vali, H., Bertrand, A., Jabaji, S. 2015. Accelerated Growth Rate and Increased Drought Stress Resilience of the Model Grass *Brachypodium distachyon* Colonized by *Bacillus subtilis* B26. PLoS ONE 10(6): e0130456. doi:10.1371/journal.pone.0130456.

Gamalero, E. and Glick, B.R. 2010. Bacterial ACC deaminase and IAA: interactions and consequences for plant growth in polluted environments, in Handbook of Phytoremediation, IA. Golubev, (ed), Nova Science, New York, NY, USA. pp. 763–774.

Gayen, D., Ali, N., Ganguly, M., Paul, S., Datta, K. and Datta, S.K. 2014. RNAi mediated silencing of lipoxygenase gene to maintain rice grain quality and viability during storage. Plant cell, Tissue and Organ Culture (PCTOC), 118 (2), 229-243.

Gillis, M., Kersters, K., Hoste, B., et al., 1989 "*Acetobacter daizotrophicus* sp.nov, a nitrogen-fixing acetic acid bacterium associated with sugarcane",

International Journal of Systematic Bacteriology, Vol.39, no.3, pp. 361-364.

Glick, B. 2012. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica 2012:1-15.

Glick, B.R. 2004. Bacterial ACC deaminase and the alleviation of plant stress. Adv Appl Microbiol 56:291–312.

Glick, B.R. 2005. Modulation of plant ethylene levels by the enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7.

Glick, B.R., Cheng, Z., Czarny, J., Duan, J. 2007 a. Promotion of plant growth by ACC deaminase-containing soil bacteria. Eur J Plant Pathol 119:329–339.

Glick, B.R., Penrose, D.M. and Li, J. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theor Biol 190: 63–68.

Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J. and McConkey, B. 2007. Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26, 227–242.

Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J., McConkey, B. 2007 b. Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242.

Gordon, A.S. and Weber, R.P. 1951. Colorimetric estimation of indole acetic acid. Plant physio.26:192-195.

Gordon, R. E. 1981. One hundred and seven years of the genus *Bacillus*. Berkeley, R.C. and Goodfellow, M. (ed.) The aerobic endospore forming bacteria. Academic Press. London.

Gray, E.J. and Smith, D.L. 2005. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant bacterium signaling processes. Soil Biol Biochem 37:395- 412.

Grover, M., Ali, S.K.Z., Sandhya, V., Rasul, A. and Venkateswarlu, B. 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231-1240.

Gusain, Y.S, Singh, U.S. and Sharma, A.K. 2015. Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.). African Journal of Biotechnology 14 (9), 764-773.

Hendry, G.A. 2005. Oxygen free radical process and seed longevity. Seed Sci. J. 3,141–147.

Hepper, C.M. 1975. Extracellular polysaccharides of soil bacteria. In: Walker, N. (Ed.), Soil Microbiology, A Critical Review. Wiley, New York, pp. 93–111.

Hernandez, L., Arrieta, J., Menendez, C., et al., 1995. "Isolation and enzymic properties of levansucrase secreted by *Acetobacter diazotrophicus* SRT4, a bacterium associated with sugar cane," Biochemical Journal, vol. 309, no. 1, pp. 113–118.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., *et al.* 2015. Priming and memory of stress responses in organisms lacking a nervous system. Biol. Rev. 91, 1118–1133. doi: 10.1111/brv.12215

Honma, M. and Shimomura, T. (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. Agric Biol Chem 42, 1825–1831.

Hontzeas, N., Saleh, S.S. and Glick, B.R. 2004. Changes in gene expression in canola roots induced by ACC deaminase containing plant growth-promoting bacteria. Mol Plant–Microbe Interact 17, 865–871.

Idogawa, N., Amamoto, R., Murata, K. & Kawai, S. 2014. Phosphate enhances levan production in the endophytic bacterium *Gluconacetobacter diazotrophicus* Pal5. Bioengineered, 5(3), 173–179. http://doi.org/ 10.4161/bioe.28792

Jacobson, C.B., Pasternak, J.J. and Glick, B.R. 1994. Partial purification and characterization of 1-aminocyclopropane- 1-carboxylate deaminase from

the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Can J Microbiol 40, 1019–1025.

James, E. K., Olivares, F. L., Oliveira, A. L. M., Reis, F. B., Jr., Silva, L. G. and Reis, V. M. 2001. Further observations on the interaction between sugar cane and *Gluconacetobacter diazotrophicus* under laboratory and greenhouse conditions. J. Exp. Bot. 52:747-760.

James, E. K., Reis, V. M., Olivares, F. L., Baldani, J. I. and Döbereiner, J. 1994. Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. J. Exp. Bot. 45:757-766.

Jha, Y., Subramanian, R. B., Patel S. 2011. Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiologiae Plantarum, 33: 797–802

Jiang, Q.Y., Zhuo, F., Long, S.H., Zhao, H.D., Yang, D.J., Ye, Z.H., et al. 2016. Can *arbuscular mycorrhizal* fungi reduce Cd uptake and alleviate Cd toxicity of *Lonicera japonica* grown in Cd-added soils? Sci. Rep. 6:21805. doi: 10.1038/srep21805

Jiang, Y., Macdonald, S.E. and Zwiazak, J.J. 1995. "Effects of Cold Storage and Water Stress on Water Relations and Gas Exchange of White Spruce (*Picea glauca*) Seed-lings," Tree Physiology, Vol. 15, No. 4, pp. 267- 273.

Kakar, K. U., Ren, X.l., Nawaz, Z. CuiZ, Q., Li, B., Xie, G.L., Hassan, M. A., Ali E. and Sun, G.C. 2016. A consortium of rhizobacterial strains and biochemical growth elicitors improve cold and drought stress tolerance in rice (*Oryza sativa* L.). Plant Biology, 18: 471–483

Kasim, W.A., Osman, M.E., Omar, M.N., Abd El-Daim, I.A., Bejai, S., Meijer, J., 2013. Control of drought stress in wheat using plant growth promoting bacteria. J. Plant Growth Regul. 32, 122–130

Kersters, K., Lisdiyanti, P., Komagata, K. and Swings, J. 2006, "The family acetobacteraceae: the genera Acetobacter, Acidomonas, Asaia, *Gluconacetobacter*, *Gluconobacter*, and *Kozakia*", in The prokaryotes,

Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H. and Stackebrandt, E., Eds., Springer, New York, NY, USA, pp. 163-200.

Khalid, A., Arshad, M. and Zahir, Z.A. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol., 96: pp 473-480.

Khan, M.H. and Panda, S.K. 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl- Salinity stress. Acta Physiol Plant; 30: 81-9.

Kim, Y.C., Glick, B., Bashan, Y., Ryu, C.M. 2013. Enhancement of plant drought tolerance by microbes. In: Aroca, R. (Ed.), Plant Responses to Drought Stress. Springer Verlag, Berlin.

Koji, Y., Shiro, M., Michio, K., Mitsutaka, T. and Hiroshi, M. 2009. Antioxidant capacity and damages caused by salinity stress in apical and basal regions of rice leaf. Plant Prod Sci., 12: 319–326.

Lee, S., Flores-Encarnación, M., Contreras-Zentella, M., Garcia-Flores, L., Escamilla, J.E., Kennedy, C. 2004. Indole-3-Acetic Acid Biosynthesis Is Deficient in *Gluconacetobacter diazotrophicus* Strains with Mutations in Cytochrome c Biogenesis Genes. Journal of Bacteriology;186 (16):5384-5391.

Lichtenthaler, H. K. 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. Methods In Enzymology, 148: 350-382.

Lipiec, J., Doussan, C., Nosalewicz, A. and Kondracka, K. 2013. Effect of drought and heat stresses on plant growth and yield: A review. Int. Agrophys. 27:463-477.

Lisar, S. Y. S., Motafakkerazad, R., Hossain, M. M. & Rahman, I. M. M. 2012. Water stress in plants: Causes, effects and responses. Water stress, Prof. Ismail Md. Mofizur Rahman, (Ed.), ISBN: 978-953-307-963-9, InTech,

Available from: http://www.intechopen.com/books/water-stress/water-stress-inplants-causes-effects-and-responses.

Lucy, M., Reed, E., Glick, B.R. 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86:1–25.

Luna, M.F., Aprea, J., Crespo, J.M. and Boiardi, J. L. 2012. Colonization and yield promotion of tomato by *Gluconacetobacter diazotrophicus.* Applied Soil Ecology, 61: 225–229.

Lynch, J.M. 1985. Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In: Vaughan, D., Malcom, R. E. (Eds). Soil Organic Matter and Biological Activity. Martinus Nijhoff /Dr. W. Junk Publishers. Dordrecht, Boston, Lancaster. pp. 151-174.

Lynch, J.M. 1990. The rhizosphere. Wiley-Interscience, Chichester, 458 p.

Madhaiyan, M., Saravanan, V.S., Jovi, D.B.S.S., Lee, H., Thenmozhi, R., Hari, K. 2004. Occurrence of *Gluconacetobacter diazotrophicus* in tropical and subtropical plants of Western Ghats, India Microbiological research 159 (3), 233-243.

Martínez-Fleites, C., Ortíz-Lombardía, M., Pons, T., et al., 2005. "Crystal structure of levansucrase from the Gram-negative bacterium *Gluconacetobacter diazotrophicus*," Biochemical Journal, vol. 390, no. 1, pp. 19–27.

Mattsson, A. 1996. Predicting field performance using seedling quality assessment. New Forests. 13: 223-248.

McCord, J. M. 2000. The evolution of free radicals and oxidative stress. Am. J. Med., 108: 652–659.

McNabb, K., Takahashi, E. 2000. Freeze damage to loblolly pine seedlings as indicated by conductivity measurements and out planting survival. Auburn University Southern Forest Nursery Management Cooperative. Research Report 00-4.

Meneses, C., Gonçalves, T., Alquéres, S., Rouws, L., Serrato, R., Vidal, M. and Baldani, J.I. 2017. *Gluconacetobacter diazotrophicus* exopolysaccharide protects bacterial cells against oxidative stress in vitro and during rice plant

colonization. Plant Soil DOI 10.1007/s11104-017-3201-5

Meneses, C.H., Rouws, L.F., Simões-Araújo, J.L., Vidal, M.S. and Baldani, J.I. 2011. Exopolysaccharide production is required for biofilm formation and plant colonization by the nitrogenfixing endophyte *Gluconacetobacter diazotrophicus*. Mol Plant-Microbe Interact 24:1448-1458.

Miller, K.J., Wood, J.M., 1996. Osmo adoptation by rhizosphere bacteria. Annu. Rev.Microbiol. 50, 101–136.

Morsy, M.R., Jouve, L., Hausman, J.F., Hoffmann, L. & Stewart, J.M. 2007 Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. Journal of Plant Physiology 164, 157–167.

Naghavi, M. R., Aboughadareh, A. P. and Khalili, M. 2013. Evaluation of drought tolerance indices for screening some of corn (*Zea mays* L.) cultivars under environmental conditions. NotulaeScientiaBiologicae 5(3): 388-393.

Ngumbi, E. and Kloepper, J. 2016. Bacterial-mediated drought tolerance: Current and future prospects. Applied Soil Ecology, 105:109–125.

Nye, P.H., Tinker, P.B. 1977. Solute movement in the soil-root system. Oxford: Blackwell Scientific Publications.

Onofre-Lemus, J., Hernández-Lucas, I., Girard, L., Caballero-Mellado, J. 2009. ACC (1 aminocyclopropane- 1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth-promoting effect on tomato plants. Appl Environ Microbiol; 75:6581–90. http://dx.doi.org/ 10.1128 /AEM.01240-09.

Osakabe, Y., Osakabe, K., Shinozaki, K. and Tran, L.S. P. 2014. Response of plants to water stress. Front. Plant Sci. 5:86. doi: 10.3389/fpls.2014. 00086.

Pandey, M. P., Verulkar, S. B. and. Sarawgi, A. K. 2009. Status Paper on Rice for Chhattisgarh .Rice Knowledge Management Portal (RKMP), ICAR-IIRR

Pandey, V. and Shukla, A. 2015. Acclimation and tolerance strategies of rice under drought stress. Rice Science, 22(4): 147-161.

Pandey, V., Ansari, M., Tula, S., Yadav, S., Sahoo, R.K., Shukla, N., Bains, G., Badal, S., Chandra, S., Gaur, A. K., Kumar, A., Shukla, A., Kumar, J. and Tuteja, N. 2016. Dose-dependent response of *Trichoderma harzianum* in improving drought tolerance in rice genotypes. Planta, 243 (5): 1251-1264.

Patten, C. L., and B. R. Glick. 1996. Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42:207–220.

Patten, C.L., Glick, B.R. 2002. The role of bacterial indoleacetic acid in the development of the host plant root system. Appl. Environ. Microbiol. 68: 3795-3801.

Pattern, C.L., Glick, B.R. 2002. Role of *Pseudomanas putida* indo lactic acid in development of the host plant root system. App. Eniron. Microbeal. 68, 3795-3801.

Pereira, J.S., Chaves, M.M., 1993. Plant water deficits in Mediterranean ecosystems. In: Smith, J.A., Griffiths, H. (Eds.), Plant Response to Water Deficits-from Cell to Community. BIOS Sci. Ltd., Oxford, pp. 237–251.

Pereira, J.S., Chaves, M.M., 1995. Plant responses to drought under climate change in Mediterranean-type ecosystems. In: Jose, M., Oechel, W.C. (Eds.), Global Change and Mediterranean-type Ecosystems. Ecological studies, 117.Springer-Verlag, New York, pp. 140–160.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E, Analytical Biochemistry, 269 : 337-341.

Ranjbarfordoei , R ., Samson , P. , Damne , V. and Lemeur , R . 2000. “Effects of Drought Stress Induced by Polyethylene Glycol on Pigment Content and Photosynthetic Gas Exchange of *Pistacia khinjuk* and *P.mutica*,” Photosynthetic,Vol.38,No.3, pp. 443-447. doi:10.1023/A:1010946209484.

Redman, R.S., Kim, Y.O., Woodward, C.J.D.A., Greer, C., Espino, L., et al., 2011. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6, e14823.

Reetha, S., Bhuvaneswari, G., Thamizhiniyan, P. and Ravi, T., Mycin. 2014. Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allim cepa*.L) Int.J.Curr.Microbiol.App.Sci 3(2): 568-574

Reis, V. M., Olivares, F. L., de Oliveira, A. L. M., Reis, F. B., Baldani, J. I., and Döbereiner, J. 1999. Technical approaches to inoculate micro propagated sugar cane plants with *Acetobacter diazotrophicus*. Plant Soil 206:205-211.

Reis, V.M., Lee, S., Kennedy, C. 2007. Biological nitrogen fixation in sugarcane. In: Elmeerich C, Newton WE (eds) Associative and endophytic nitrogen-fixing bacteria and *cynobacterial* associations. Springer, Dordrecht, The Nrtherlands, pp 213-232.

Roberson, E.B., Firestone, M.K., 1992. Relationship between desiccation and exopolysaccharide production in soil *Pseudomonas sp.* Appl. Environ.Microbiol. 58, 1284–1291.

Rouws, L.F.M., Meneses, C.H.S.G., Guedes, H.V., Vidal, M.S., Baldani, J.I. and Schwab, S. 2010. Monitoring the colonization of sugarcane and rice plants by the endophytic diazotrophic bacterium *Gluconacetobacter diazotrophicus* marked with gfp and gusA reporter genes. Letters in Applied Microbiology 51: 325–330

Rubinstein, B., Turner, N.C. 1982. Regulation of H+ excreation. Effects of osmotic shock. Plant Physiol. 99, 355–360.

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J. and Dowling, D.N. 2008. Bacterial endophytes: recent developments and applications. *FEMS. Microbiol. Lett*, 278: pp 1-9.

Saleem, M., Arshad, M., Hussain, S. and Bhatti, A.S. 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635-648.

Sandhya, V., Ali, S.K.Z., Grover, M., Reddy, G., Venkateswarlu, B. 2009. Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biol. Fertil. Soils 46, 17–26.

Selvakumar, G., Panneerselvam, P., Ganeshamurthy, A.N., 2012. Bacterial mediated alleviation of abiotic stress in crops. In: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Stress Management. Springer-Verlag, Berlin Heidelberg, pp.205–224.

Serraj, R., McNally, K.L., Slamet-Loedin, I., Kohli, A., Haefele, S.M., Atlin, G. and Kumar, A. 2011. Drought resistance improvement in rice: An integrated genetic and resource management strategy. Plant Prod Sci, 14(1): 1–14.

Sevilla, M., Gunapala, N., Burris, R.H. and Kennedy, C. 2001. Comparison of benefit to sugarcane plant growth and 15N2 incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild-type and Nif_ mutant strains. Mol. Plant-Microbe Interact. 14:358–366.

Sgherri, C.L.M., Maffei, M., Navari-Izzo, F., 2000. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. J. Plant Physiol. 157,273–279.

Shaharoona. B., Arshad, M. and Zahir, Z.A. 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Lett Appl Microbiol 42:155-159.

Shende, S.T., Apte, R.G. and Singh, T. 1977. Influence of *Azotobacter* on germination of rice and cotton seeds. Current Science. 46(19):675-676.

Shih-Yung, H. 2010. IAA production by *Streptomyces scabies* and its role in plant microbe interaction. Msc thesis, Cornell University.

Singh, A., Gupta, R., Pandey, R. 2016. Rice Seed Priming with *Picomolar Rutin* Enhances Rhizospheric *Bacillus subtilis* CIM Colonization and Plant Growth. PLoS ONE 11(1): e0146013. doi:10.1371/journal.pone.0146013

Singh, R., et al., 2015. From QTL to variety-harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multiinstitutionalnetwork,PlantSci,http://dx.doi.org/10.1016/j.plantsci.2015.08.008.

Smirnoff, N. 1993. The role of Reactive Oxygen in the response of plants to water deficit and desiccation. J. New Phytol. 125, 27–30.

Staub, A.M. 1965. Removal of protein – Sevag method. Methods Carbohydr. Chem. 5, 56.

Stephan, M.P., Oliveria, M., Teixeira, K.R.S., Martinez-DretsM, G., Doberriner, J. and 1991. Physiology and nitrogen fixation of *Acetobacter diazotrophicus.* FEMS Microbial Lett 77:67-72

Tarkow, H., Feist, W.C. and Southerland, C.F. 1996. Interaction of wood and polymeric materials. Penetration versus molecular size. Forest Prod. J. 16, 61–65.

Thimmaiah, S. R. 2006. Standard methods of biochemical analysis, Kalyani Publishers, New Delhi, p.54-55.

Timmusk, S., Islam, A., Abd El, D., Lucian, C., Tanilas, T., Ka nnaste, A., et al., 2014. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9, 1–13.

Timmusk, S., Nevo, E. 2011. Plant root associated biofilms. In: Maheshwari, D.K.(Ed.), Bacteria in Agrobiology. Plant Nutrient Management, 3. Springer Verlag, Berlin, pp. 285–300.

Timmusk, S., Timmusk, K. and Behers, L. 2013. Rhizobacterial Plant Drought Stress Tolerance enhancement: Towards Sustainable Water Resource Management and Food Security. Journal of Food Security, 1(1): 6-9.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water stable aggregates in soils.J. Soil Sci. 33, 141–163.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A. and Dufresne, A. 2015. The importance of the microbiome of the plant holobiont. New Phytologist : doi: 10.1111/nph.13312

Vargas, L., Santa Brı´gida, A.B., Mota Filho, J.P., de Carvalho, T.G., Rojas, C.A., et al., 2014. Drought Tolerance Conferred to Sugarcane by Association with *Gluconacetobacter diazotrophicus*:A Transcriptomic View of HormonePathways.PLoSONE9(12):e114744.doi:10.1371/journal.pone.0114744.

Velázquez-Hernández, M.L., Baizabal-Aguirre, V.M., Cruz-Vázquez, F., *et al.*, 2011. "*Gluconacetobacter diazotrophicus* levansucrase is involved in tolerance to NaCl, sucrose and desiccation, and in biofilm formation," Archives of Microbiology, vol. 193, no. 2, p.p. 137–149.

Viikari, L., Gisler, R. 1986. By–Products in the fermentation of sucrose by different Zymomonas strains. Appl Microbiol Biotechnol 23:240-244.

Vinocur, B., Altman, A. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr. Opin. Biotechnol. 16,123–132.

Xonostle-Cazares, B., Ramirez-Ortega, F. A., Flores-Elenes, L. & Ruiz-Medrano, R. 2011. Drought tolerance in crop plants. Am. J. Plant Physiol. 1-16.

Xu, Z., Jiang, Y., and Zhou, G. 2015. Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO2 with environmental stress in plants. Front. Plant Sci. 6:701. doi: 10.3389/fpls.2015.00701.

Xu, Z., Jiang, Y., Jia, B. and Zhou, G. 2016. Elevated-CO2 response of stomata and its dependence on environmental factors. Front. Plant Sci. 7:657. doi: 10. 3389/fpls.2016.00657.

Yamada, Y., Hoshino, K.I. and Ishikawa, T. 1997. "The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA; the elevation of the subgenus *Gluconacetobacter* to the generic level",

Bioscience, Biotechnology and Biochemistry, Vol.61, no.8, pp. 1244-1251.

Yang, J., Kloepper, J.W. and Ryu, C.M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14, 1–4.

Yuwono, T., Handayani, D. and Soedarsono J. 2005. The role of osmotolerant rhizobacteria in rice growth under different drought conditions. Australian Journal of Agricultural Research 56, 715–721.

Zhao, Y. 2010. Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol. 61, 49-64.

APPENDIX - I

Chemical composition of media

1. Yoshida media: g/L

NH_4NO_3	91.4
K_2SO_4	71.4
$NaH_2\ PO_4.2H_2O$	23.1
$CaCl_2.6H_2O$	175
$MgSO_4.7H_2O$	324
Minor nutrients	
$MnCl_2.4H_2O$	1.5
$(NH_4)_6.Mo_7O_{24}.4H_2O$	0.074
H_3BO_3	0.93
$ZnSO_4.7H_2O$	0.035
$CuSO_4.5H_2O$	0.03
FeNa EDTA	10.5
$FeSO_4$ (made fresh)	2.5

1.25 ml of each of the stock was added per litre of the medium. The pH of the solution was adjusted to 4.5 before the addition of minor nutrients and iron solution

2. Nutrient agar Media: g/L

Peptone	5
Beef Extract	3
NaCl	5
Agar agar	15
Distilled water	1000 ml
pH maintained at	7.4±0.2 at 25°C

3. Manitol Yeast extract Peptone agar Media: g/L

Yeast Extract	5
Peptone	3
Mannitol	25
Agar agar	15
Distilled water	1000 ml

4. LGI media: g/L

Sucrose	5
K_2HPO_4	0.2
KH_2PO_4	0.6
$MgSO_4 .7H_2O$	0.2
$CaCl_2.2H_2O$	0.02
$Na_2MoO_4.2H_2O$	0.002
Bromthymol blue solution (0.5% in 0.2 N KOH)	5 ml
$FeCl_2$	0.01
Agar	15
Distilled water	1000 ml
pH maintained at	6.0

5. Dworkin and Foster (DF) salt medium:

$(NH_4)_2S0_4$	2g
KH_2P0_4	4g
Na_2HP0_4	6g
$MgS0_4.7H_2O$	02g
$FeS0_4.7H_2O$	1 mg
H_3B0_3	10 µg
$MnS0_4$	10 µg
$ZnS0_4$	70 µg
$CuSO_4$	50 µg
MoO_3	10 µg
Distilled water	1000 ml

VITA

Name	:	**Mahapatra Smruthi Sagarika**
Date of birth	:	15-08-1994
Present Address	:	Saraswati Girls Hostel College of Agriculture, Raipur (C.G.) Krishak Nagar Zora Pin no – 492012
E-mail	:	sagarika.s22@gmail.com
Peramanent Address	:	D/O Shri Mahapatra Rabindranath, Jemadaipur, Nayagarh, Bhubaneswar, Odisha

Academic Qualification :

Degree	Year	University/Institute
1. Higher Secondary	2011	A.P. Board, Hyderabad
2. B.Sc. (Ag.)	2015	I.G.K.V., Raipur (C.G.)
3. M.Sc. (Ag.)	Course work completed, Thesis being submitted for the partial fulfillment of the degree	I.G.K.V., Raipur (C.G.)

Professional Experience	:	Rural Agriculture Work Experience (RAWE)
Membership of Professional Societies	:	No
Awards/Recognitions	:	No

Signature