

**“CHARACTERIZATION OF FLUORESCENT
PSEUDOMONAS FROM RED LATERITIC SOIL
FOR PGPA AND INCREASED RESILIENCE OF
WATER STRESS IN RICE AND WHEAT”**

M.Sc. (Ag) Thesis

by

Rugma S B

**DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
FACULTY OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
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Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

Rugma S B

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF**

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In

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(Plant Pathology)

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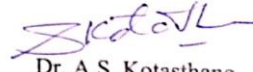
July, 2019

CERTIFICATE – I

This is to certify that the thesis entitled “**Characterization of fluorescent *Pseudomonas* from red lateritic soil for PGPA and increased resilience of water stress in rice and wheat**” submitted in partial fulfilment of the requirements for the degree of **Master of science in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bona fide research work carried out by **Rugma S B** 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 or certificate course. All the assistance and help received during the course of the investigations have been duly acknowledged.

Date: 30/07/19

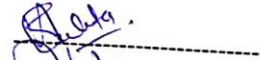

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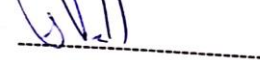
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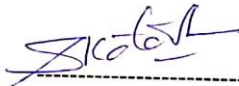
CERTIFICATE – II

This is to certify that the thesis entitled “**Characterization of fluorescent *Pseudomonas* from red lateritic soil for PGPA and increased resilience of water stress in rice and wheat**” submitted by **Rugma S B** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfilment of the requirements for the degree of **Master of science in Agriculture** in the Department of Plant Pathology has been approved by the external examiner and Student’s Advisory Committee after oral examination.

Date: 4/8/19


Signature External Examiner
(Name JAGDISH KUMAR)

Major Advisor



Head of the Department



Faculty Dean

Approved/Not approved

Director of Instructions

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Acknowledgement for a few might be just a trifle think written on a piece of paper, but in its true essence it give us an opportunity to remember and express our feeling for those whom we love and revere.

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Department of Plant Pathology
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Rugma S B

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LIST OF NOTATIONS AND ABBREVIATIONS

%	Per cent
@	At the rate of
/	Per
°C	Degree Celsius
µg	Microgram
±	Plus minus
µl	Microlitre
ACCD	1-amino cyclo propane-1 carboxylic acid deaminase
ADW	Autoclaved distilled water
BCP	Bromo cresol purple
CAS	Chrome azurol sulphonate assay
C.G.	Chhattisgarh
CD	Critical difference
cm	Centimeter
CMA	Chloromolybdic acid
CSA	Chlorostannous acid
CV	Coefficient of variance
DAS	Day after sowing
et al.	And Co-Worker/And Other
Fig.	Figures
IAA	Indole-3 acetic acid
i.e.	That is
M	Molar
mM	Millimolar
ml	Millilitre
mg	Milligram
OD	Optical density
PDA	Potato Dextrose Agar
rpm	Revolution per minute
PGPR	Plant growth promoting rhizobacteria
PGPA	Plant growth promoting activity.
KMB	King's medium B base

THESIS ABSTRACT

Title of the Thesis : "Characterization of fluorescent *Pseudomonas* from red lateritic soil for PGPA and increased resilience of water stress in rice and wheat"

Name of the Student : Rugma S B

Major subject : Plant pathology

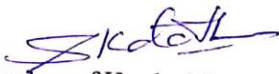
Name and Address of the Major Advisor : A.S. Kotasthane, Professor and Head,
Department of Plant pathology, CoA, IGKV,
Raipur.

Degree to be awarded : M.Sc. Plant Pathology


Signature of the Student


Signature of the Major Advisor

Date: 30/07/19.....


Signature of Head of the Department

ABSTRACT

The plant health is adversely affected by biotic and abiotic stresses. Among the abiotic stresses, drought is one of the major problems that threaten the world food security. By 2050, drought may cause serious problems for growth of the plants on more than 50% of the cultivable areas of earth. Drought can drastically decrease the productivity of the crops and also lead to land degradation. Drought also restructures the bacterial diversity in the soil. Will the fluorescent *pseudomonas*

derived from rhizosphere of the plants naturally growing in lateritic soil with harsh water stress condition can be the potential isolates contributing water stress tolerance in plants? Are the fluorescent *pseudomonas* derived from rhizosphere of the plants naturally growing in lateritic soil strong phosphate solubilizes and siderophore producers? Will the isolation and the identification of the rhizospheric microorganisms survive under stressed environment may lead to the identification of potential microbes with beneficial properties? Nevertheless, these issues prompted us to isolate phosphate-solubilizing plant-associated fluorescent *Pseudomonas* from lateritic soil rich with iron content and to assess their PGPR activity.

Seventeen isolates of fluorescent *pseudomonas* were derived from the rhizosphere of the plant surviving in water stress condition. Potential isolate of fluorescent *Pseudomonas* imparting water stress tolerance in Rice and Wheat were identified. Three varieties of rice were used IR64 (drought susceptible), B15 and RRF75 (drought tolerant). It was observed that IR64 a drought susceptible variety IR-64 showed greater responses to the water stress although it has no genes to overcome water stress.

Recurrent capability to solubilize fixed forms of phosphates by the release of organic acids is another characteristic feature of fluorescent *Pseudomonas* and it helps to increase the available form of phosphorous as nutrition under different fertilization

conditions. In addition, as an indirect plant growth promoting trait fluorescent *Pseudomonas* suppresses plant diseases by their antagonistic activity towards phytopathogens. Biochemical screens helped us to identify potential ACC deaminase, Indole-3-acetic acid producers and phosphate solubilizes. Potential siderophore producing iron chelators were also identified all the fluorescent *Pseudomonas* isolates were screened for the plant growth promoting activity following seed bio-priming of Rice, Wheat Tomato and Brinjal and observed positive correlation with ACCd, IAA and phosphate solubilizing ability. Confrontation assays (*in-vitro*) against *S. rolfii* and *R. solani* identified potential isolates with mycoparasitic abilities.

शोध सारांश

शोध का शीर्षक	:	लाल लाटरैट मृदा से एकत्र किये गये प्रतिदीप्त स्यूडोमोनास को इसकी पादप वर्धन गतिविधि एवं धान और गेहूं में जल तनाव के लिये प्रतिरोध के लिये अभिलक्षित करना
छात्रा का पूरा नाम	:	रुग्मा एस बि
मुख्य विषय	:	पादप रोगविज्ञान
मुख्य सलाहकार का नाम और पता	:	डॉ. अनिल एस. कोटस्थाने, प्राध्यापक एवं विभागाध्यक्ष, (पादप रोगविज्ञान), कृषि महाविद्यालय, इंदिरा गांधी कृषि विश्वविद्यालय, रायपुर (छ.ग.)
उपाधि का नाम	:	एम. एस. सी. (कृषि)


छात्रा का हस्ताक्षर

मुख्य सलाहकार के हस्ताक्षर

दिनांक:- 30/07/19


विभागाध्यक्ष के हस्ताक्षर

सारंश

वनस्पति स्वास्थ्य बायोटेक और अजैविक तनाव से प्रतिकूल रूप से प्रभावित होता है अजैविक तनावों के बीच, सूखा एक बड़ी समस्या है जो विश्व खाद्य सुरक्षा के लिए खतरा है। 2050 तक, सूखे के कारण धरती की पचास प्रतिशत से अधिक कृषि योग्य भूमि पर फसलों के लिए बहुत गंभीर विकास समस्याएं पैदा हो सकती हैं। सूखे से फसलों की उत्पादकता में भारी कमी आ सकती है और भूमि क्षरण भी हो सकता है। सूखे की स्थिति में मिट्टी में बैक्टीरिया की विविधता का पुनर्गठन होता है। क्या पौधों के राइजोस्फीयर से निकले हुए फ्लोरोसेंट स्यूडोमोनास प्राकृतिक रूप से कठोर जल तनाव की स्थिति के साथ बाद की मिट्टी में बढ़ रहे हैं, जो पौधों में पानी के तनाव सहिष्णुता में योगदान देने वाले संभावित अलगाव हो सकते हैं?

अलगाव और राइजोस्फीयर सूक्ष्मजीवों की पहचान तनाव वाले वातावरण में जीवित रहने से लाभकारी गुणों के साथ संभावित रोगाणुओं की पहचान हो सकती है? फिर भी, इन मुद्दों ने हमें फॉस्फेट सोलुबिलाइजिंग संयंत्र से जुड़े फ्लोरोसेंट सोलुबिलाइजिंग संयंत्र से जुड़े फ्लोरोसेंट स्ट्रुडोमोनास को लौह सामग्री से समृद्ध लेटरिटिक मिट्टी से अलग करने और उनकी पीजीपीआर गतिविधि का आकलन करने के लिए प्रेरित किया।

फ्लोरोसेंट स्ट्रुडोमोनास के सत्रह आइसोलेट्स पानी के तनाव की स्थिति में जीवित रहने वाले पौधे के प्रकंद से प्राप्त किए गए थे। चावल और गेहूं में पानी के तनाव सहिष्णुता प्रदान करने वाले फ्लोरोसेंट स्ट्रुडोमोनास के संभावित अलगाव की पहचान की गई थी। चावल की तीन किस्मों का उपयोग ऐआर-64 (सूखा अतिसंवेदनशील), बी-15 और आरआरएफ-75 (सूखा सहिष्णु), किया गया। यह देख गया कि ऐआर-64 ने पानी के तनाव के प्रति अधिक प्रतिक्रिया दिखाई है, हालांकि इसमें पानी के तनाव को दूर करने के लिए कोई जीन नहीं है।

कार्बनिक अम्लों की रिहाई के द्वारा फॉस्फेट के निश्चित रूपों को घोलने की आवर्तक क्षमता फ्लोरोसेंट स्ट्रुडोमोनास की एक और विशेषता है और यह विभिन्न विषेचन परिस्थितियों में पोषण के रूप में फॉस्फोरस के उपलब्ध रूप को बढ़ाने में मदद करता है। इसके अलावा, एक अप्रत्यक्ष पौधे के विकास के रूप में, पारगमन फ्लोरोसेंट स्ट्रुडोमोनास को बढ़ावा देने से पादप रोगों को फाइटोपैथोगन्स के प्रति उनकी विरोधी गतिविधि द्वारा दबा दिया जाता है। बायोकेमिकल स्क्रीन ने हमें संभावित एसीएसी डेमिनमिनस, इंडोल-3 एसिटिक एसिड उत्पादकों और फॉस्फेट सॉल्यूबल की पहचान करने में मदद की। लोहे के शीशियों का निर्माण करने वाले संभावित सिडरोफोर की पहचान करने में मदद की। लोहे के शीशियों का निर्माण करने वाले संभावित सिडरोफोर की पहचान सभी फ्लोरोसेंट स्ट्रुडोमोनास आइसोलेट्स से की गई, जो कि राइस, व्हील टोमेटो और बैंगन की सीड बायो-प्राइमिंग की गतिविधि को बढ़ावा देने वाले प्लांट ग्रोथ के लिए जांचा गया था। और इसमें एसीसीडी, आईएए और फॉस्फेट सॉल्यूबिलिंग क्षमता के साथ पॉजिटिव कॉर्डिनेशन देखा गया। एस रॉल्फसी और आर सलानी के खिलाफ टकराव परख (इन-विट्रो) ने मायकोपरसिटिक क्षमताओं के साथ संभावित आइसोलेट्स की पहचान की।

CHAPTER – I

INTRODUCTION

In India Agriculture is the back bone of the economy. Majority of the people or a bulk of the working population in the India is depending on agriculture for their survival. There is a saying that “if you are eating, you are involved in agriculture”. So directly or indirectly we all are involved in agriculture. Agriculture plays a pivotal role in the growth of the country and it is the primary sector which contributes a significant amount to the Gross Domestic Product (GDP). As the population of the country increases the production of the agricultural produce should be increased. Modern agriculture practices are trying to increase the production in more efficient and eco- friendly manner. There are many efficient approaches like plant breeding, tissue culture, molecular breeding and genetic engineering etc. for getting more output from agriculture.

India ranks second in the production of cereals in the world, especially rice and wheat contributes major part of the production. They are rich in carbohydrates, vitamins, minerals, protein, fat, oil and fiber. In cereals rice and wheat having the highest potential to serve entire world and having the brand of staple food (FAO). India is the leading country in the export of the cereals and it contributes a very large income to the Indian economy. Dominant crop of our country is rice. The production of rice in India recorded in 2017 was 110.15 million tonnes by Department Of Agriculture, Cooperation & Farmers welfare in their annual report. Heavy annual rainfall is needed for the rice production and that is the reason why it is basically a kharif crop in India. Rice requires above 100cm rainfall and temperature more than 25°C. In the area which receives less rainfall rice is grown under irrigation. Low land or wet rice is grown in well watered low land areas and upland rice is grown in the hilly areas. Per hectare basis the yield of the low land rice is more than the upland rice. For producing per kg of grain rice utilizes about 4,000 – 5,000 liters of water.

About one third of the fresh water of our country is utilized for rice cultivation. Rice production faces many constraints like abiotic and biotic stresses. Wheat is a worldwide staple food. In world it is the second most cereal produced after maize. Wheat production in the world recorded in 2016 was 749 million tonnes. Gluten protein in the wheat facilitates the production of processed food because of the adhesive and viscoelastic properties. So the global demand of wheat is increasing. In India it is grown on 13% of the cropped area. Total production of wheat recorded in the country in 2017 was 98.38 million tonnes under 305.97 lakh hectare (annual report of Department Of Agriculture, Cooperation & Farmers welfare).

Growth of these cereals and their yield is majorly affected by constraints like biotic and abiotic stresses. These stresses prevent the plants from attaining their maximum genetic potential. We know that when plants can't express full of their genetic potential then they are not healthy. So mitigation of these stresses is important for getting the maximum productivity from the crops. Among abiotic stresses drought stress is one of the most serious problems for the plants and it deleteriously affects the food security of the world. Agricultural drought is defined as a condition when soil moisture availability to plants reduced to a level which can affect the normal crop physiology and yield which eventually leads to crop failure. The drought in plants may results in the reduction in the proliferation of root, growth of the shoot and size of the leaves which ultimately leads to cause disturbance in plant-water relation, reduction in water use efficiency, nutrient intake of the plants, photosynthesis, respiration and leads to the reduction in growth and productivity. A primary constraint that affects the productivity and growth of rice growing under rainfed condition is drought stress due to the low rainfall distribution and in irrigated area due to lack of proper delivery of water. Among the different growth stages the drought stress decreased the agronomic traits differently in rice and wheat and yield of wheat and rice also decreased by 27.5 and 25.4 respectively (Zhang et al., 2018).

For mitigating this drought stress there is methods like developing tolerant varieties through breeding and genetic engineering. But these are time consuming and tedious processes. Recently plant associated microbes are gaining global attention in the management of abiotic stress like drought stress. Mainly the plant growth promoting rhizobacteria (PGPR) playing an important role in plant growth promotion as well as in the management of drought stress. These plant associated microbes improves the soil health and promote the plant growth by enhancing phosphate solubilisation ability, production of different plant hormones, produce an arsenal of antimicrobials HCN, pyoluteorin, phenazines, pyrrolnitrin, siderophores ,cyclic lipopeptides and 2,4-diacetylphloroglucinol (DAPG) and by inducing systemic resistance (ISR) (Glick, 2014; Raaijmakers et al., Paulitz, 2009).

The PGPR like fluorescent *Pseudomonas* having a mutualistic association with the plant roots through several mechanisms promote the plant growth and also helps to manage the abiotic stresses like drought. These bacteria improve several physiological as well as morphological traits of the plants and ultimately improve the plant-water relationship, water use efficiency and nutrient uptake by plants. There are several mechanisms by which the microbes can impart the drought tolerance to the plant. Root colonizing bacteria like fluorescent *Pseudomonas* decreases the level of the ethylene which is a stress hormone in the plants. The bacteria produces ACC (1-aminocyclopropane-1-carboxylate) deaminase enzyme which cleaves ACC (precursor of ethylene) into ammonia and α -ketobutyrate. Also these microbes can increase the proline levels in the plants, increases the tolerance of drought stress.

Plant growth promoting bacteria also play very important role in bio-control activity by suppressing the phytopathogens. Use of these bio-control agents will be a favorable approach for sustainable agriculture. Certain members of fluorescent *Pseudomonads* show biological control activity against plant pathogens. By protecting the seeds and the roots from the fungal infection it suppresses the plant diseases. There are many mechanism by which these bacteria can act as bio-control

agents. In *Pseudomonas* fluorescence the anti-fungal metabolites like 2, 4-diacetyl phloroglucinol play an important role in the capability for bio-control activity (Delany et al., 2000). Also these bacteria were able to produce some antibiotic compounds which help to suppress many of the plant diseases. Some isolates have high potential to produce strong siderophores which act as strong chelating agents of iron. These potential strains compete with the pathogen for iron uptake and make them less available to these pathogens and suppress their growth. So these microbes are having multi roles in sustainable agriculture by promoting the growth, managing biotic and abiotic stress. So in an attempt to identify the potential strains which have these abilities some fluorescent pseudomonads were collected from the red lateritic of water stressed areas of Raipur and characterized and maintained in the department of plant pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur. The overall aim of the present investigation will therefore be undertaken with following major objectives:-

1. Collection and isolation of fluorescent *Pseudomonads* from plant rhizosphere surviving in water stressed lateritic soils.
2. Characterization and Identification of potential strains of fluorescent *Pseudomonas* sp. imparting water stress tolerance in rice and wheat and with PGPA
3. DNA fingerprinting using gene specific markers.
4. Evaluation of bio-control activity *in-vitro*.

CHAPTER II

REVIEW OF LITERATURE

Among abiotic stress, drought is one of the most destructive one which increases in intensity year after year. In many parts of the world drought is a major obstacle for good crop growth and productivity (Naveed et al., 2014, Vinocur, 2005) . By 2050 almost 50% of the arable land may affected by drought stress by causing serious plant growth problems (Ashraf, Sciences, & 1994, n.d.; W. A. Kasim et al., 2013; Vinocur et al.). Drought affect the plant-water potential and turgor as a result interfere with the normal functions by changing physiological and morphological traits of the plant (Rahdari, and, & 2012, n.d.). Several studies have been conducted on growth reduction as a result of drought in different crops like barley (Samarah, 2005), maize (Kamara et al.,2003) , rice (Lafitte,2006) and wheat (Rampino,2006). Drought is having a negative effect on the growth parameters like fresh weight and water content (Jaleel, 2009). Nutrients are transported to plant roots with the help of available water in the soil. So scarcity of the water leads to the unavailability of soil nutrients such as nitrate, sulfate, Ca , mg and Si (Barber, 1995; Selvakumar, Panneerselvam, & Ganeshamurthy, 2012) . Drought also causes oxidative stress by inducing the free radicals which affect the antioxidant defenses and reactive oxygen species (super oxide, hydrogen peroxide and hydroxyl radicals). There is a decrease in the chlorophyll content in the plants under drought stress due to the photo-oxidation (Anjum et al. 2011, n.d.; Rahdari et al., 2012) . Biochemical activities like nitrate reduction ,due to lower uptake of nitrate from the soil are also affected by drought stress (Ali, Sandhya, & Venkateswar Rao, 2014) . It is a multidimensional stress which affects whole plant and mainly on different sub cellular compartment, cell organs (Choluj et al.,2004, nd. Rahdari et al.). Ultimately the quantity and quality of the plant growth is negatively affected by drought stress. So drought stress is a major problem which threatens the food security of the world. As the population increases

the demand for food increases, so the mitigation of the drought stress is getting more importance.

2.1 The effect of plant associated microbes on drought stress and plant growth promoting activity.

So many researches have been carried out worldwide to develop strategies which can cope with the drought stress. Development of drought tolerant varieties, shifting of the crop calendars, resource management practices etc. done by the researchers but they are highly cost-intensive (Venkateswarlu & Shanker, 2012). Mainly the development of high yielding drought tolerant crop varieties through conventional plant breeding method is time consuming, labor intensive may also lead to the loss of the other desirable traits from the host's gene pool (M. Ashraf, 2010; Eisenstein, 2013; Philippot, Raaijmakers, 2013). Theoretically the genetic engineering is also faster but it is also having the disadvantages or challenges like time and labor (M. Ashraf, 2010; Eisenstein, 2013).

Recent studies have been reported that plant-associated microbes can influence the responses of the plant to the environmental conditions including drought stress (Cooper et al., 2014). So the microbial based approach for mitigating the drought stress is needed. By the plant interactions and metabolic activities the millions of microbes that inhabit the plant root system which forms a complex ecological community influences the plant growth and productivity (Berg, 2009; Lugtenberg & Kamilova, 2009; Schmidt et al., 2014).

The plant associated microbes are getting more attention for providing good crop growth as well as stress resistance (B. Glick et al. 2007, n.d.; Marulanda et al. 2009; Mayak et al. 2004, Yang et al. 2009). These plant associated microbial communities include mycorrhizal fungi (Azcón, 2013), nitrogen fixing bacteria (Lugtenberg & Kamilova, 2009) and plant growth promoting rhizobacteria

(B. Glick, Cheng, et al., n.d.; Kloepper, 2004) . Plant growth promoting rhizobacteria are wide range of root colonizing bacteria and they have received the global attention because of their root colonizing ability and the ability to produce wide range of enzymes, providing biotic and abiotic stress resistance (Chauhan, Bagyaraj, 2015, n.d.; B. Glick, Cheng, et al., n.d.; Kim, Glick, Bashan, stress, & 2012, n.d.; Mayak, Tirosh, Biochemistry, & 2004, n.d.; Pineda, Dicke, Pieterse, & Pozo, 2013) . PGPR are having the well-known roles like plant growth promotion , nutrient management and disease control , and their role in management of abiotic stress is also gaining attention recently (Dimkpa, Weinand, & Asch, 2009; Grover, Ali, Sandhya, Rasul, & Venkateswarlu, 2011; Yang et al., n.d.). Advantage of using PGPR is that they have the ability to confer the drought tolerance to their host such as monocotyledonous ,dicotyledonous and vegetable crop species (W. A. Kasim et al., 2013; Mayak, Tirosh, Science, et al., n.d.; Sandhya, SK. Z., Grover, Reddy, & Venkateswarlu, 2009; Timmusk & Wagner, 1999) .

PGPR improve physiological processes which are closely associated with drought resistance. Among those physiological traits root system architecture is one of most important (Bacon, 2009; Huang, DaCosta, & Jiang, 2014; Yu, Zhuang, Nakayama, & Jin, 2007). Root system architecture integrates topology of the root system , primary and lateral root's spatial distribution , and also the length and number of various diameter of the roots (Dorlodot, Forster, Pagès, 2007, n.d.; Vacheron et al., 2013). In response to soil physical condition roots exhibit morphological plasticity (Bengough et al., 2005, n.d.; Tuberosa, 2012) . Particularly under drought conditions such features allows the plant to adapt better to soil properties like chemical and physical properties (Yu et al., 2009) . Increase in the number of roots with smaller diameters and a deeper root system are the specific root traits associated with maintaining plant productivity under drought conditions (Comas et al., 2013). These traits by increasing the surface area in contact with soil water as well as by increasing the volume of soil that can be explored for water, enable the

plants undergoing drought to increase hydraulic conductance (Comas, Becker, Cruz, Byrne, & Dierig, 2013). It has been reported that the treatment of the plants with PGPR promotes the root growth and alter the root architecture (S. Zang et al., 2004). There will be improved water and nutrient uptake ,with positive effect on whole plant growth as the consequence of bacterial induced alterations in root architecture leading to an increase in total root surface area (Somers, Vanderleyden, & Srinivasan, 2004; Timmusk et al., 2014) . Naseem and Bano (2014) conducted an experiment on maize by applying the strain *Alcaligenes faecalis* to the seeds in growth chamber tests. There was 10% increase in the root length of the drought stressed PGPR treated plants as compared to the drought stressed control plants after three weeks of planting. They argued that the development in the root system of the treated plant increased the water uptake that allowed them to tolerate the drought stress.

For measuring the plant water status one of the best criteria is relative water content (RWC) in plant leaves which is involved in the metabolic activity in tissues. The decrease in the RWC results in loss of turgor that leads to limited cell expansion and consequently leads to reduced plant growth (advances & 2010, n.d.; Castillo et al., 2013, n.d.; Lu et al., n.d.). So as an important drought tolerance enhancement strategy the increase in the RWC can be considered. The studies have shown that as compared to non- treated plants, the PGPR treated plants showed higher RWC. This leads to the conclusion that the PGPR strains increase the RWC in plant as result it improves the survival of the plant under drought stress.

Studies showed that the plant treated with PGPR has been shown an increased level in the proline content. And increased levels of proline would be better able to tolerate drought stress. It has demonstrated in maize (Naseem & Bano, 2014; Sandhya et al., 2010, n.d.; Vardharajula et al., 2011) , sorghum (Grover et al., 2014) , potato plants (Gururani et al., 2013),, mung bean (Sarma, soil, & 2014.) .

Drought stress causes the increased production of reactive oxygen species such as hydrogen peroxide , singlet oxygen ,superoxide radical and the

hydroxyl radical (Cruz de Carvalho, 2008) . The oxidative damages to lipids, proteins and other macromolecules by the ROS ultimately cause cell death (Farooq, 2009; Hasanuzzaman, 2013; science & 2002). The PGPR treated plants led to an increase in the antioxidant enzyme levels. Enzymes such as CAT, POX serves to minimize oxidative injury and contributes to drought tolerance. The gram plants treated with *Pseudomonas fluorescens* pf1 and *Bacillus subtilis* EPB showed increase in the activity of CAT. The increase in the enzyme was correlated with the observed drought tolerance. An association of the drought tolerance and CAT production has also been observed in cucumber plants (Wang et al., 2012), wheat (W. Kasim et al.2013) . Over production of the ROS scavenging enzymes by the selected PGPR treated plants results in reduction of the over – produced ROS, thereby conferring the drought tolerance.

Plant growth regulators and several phytohormones, including auxin, gibberellins, cytokinins, ethylene and abscisic acid control the plant growth and development including shoot growth. Plant growth is promoted by gibberellins and cytokinins while it is inhibited by ethylene and abscisic acid. The increase in the concentration of the substances that inhibit growth as a result of the drought stress allows the plants to regulate their water budget (Farooq et al., 2009). By manipulating and modifying the phytohormones content, PGPR treatment promote the plant growth in the presence of the drought stress (Bresson et al., 2013; Dodd et al., 2010). Decreasing ET production (Belimov et al., 2009; B. Glick et al. 1998) and changing the balance of CKs and ABA (Cohen et al., 2009; Figueiredo, 2008) or IAA signaling are the main modification by the PGPR. All these are associated with bacterial-mediated drought tolerance contributed by the modifications associated with drought stress tolerance when PGPR are applied. Auxin which is also referred as indole-3-acetic acid (IAA) , regulates the development and growth of the plant , also influences diverse cellular functions like vascular tissue differentiation , lateral and adventitious root initiation , and root and shoot growth orientation in response to light and gravity (B. R. Glick, 1995) . There is an increased root and shoot biomass and water content

under drought stress in the clover (*Trifolium repens*) plants treated with PGPR (*P. putida* and *B. megaterium*) and these increases were correlated with PGPR elicited IAA production (A Marulanda et al., 2009). IAA concentration in rhizobacteria treated plants was correlated with increase in root length and modifications of root architecture. These results show that the bacterial – mediated drought tolerance may partly be mediated by IAA (Contesto et al., 2010).

As a result of several stress signals like mechanical wounding, chemicals and metals, flooding, extreme temperature, pathogen infection and drought ethylene (ET) is synthesized in higher rates (Johnson & Ecker, 1998). The immediate precursor of ET in higher plants is 1- Aminocyclopropane-1-carboxylate (ACC). Bacteria exert beneficial effects on plants under abiotic stress including drought stress by its regulation (Saleem et al., 2007). Instead of converting into ethylene ACC is hydrolyzed into ammonia and alpha-ketobutyrate by ACC deaminase that is contained in some PGPR. The endogenous ET levels are reduced as a result of reduction in the ACC level which eliminates the inhibitory effect of higher ET concentration (B. Glick, 2006). As a consequence the normal growth of the plants is maintained (Siddique et al., 2011). The treatment of the pea plants with ACC deaminase containing *Pseudomonas* spp. partially eliminated the effect of the drought stress (Arshad et al., 2008). *Achromobacter piechaudii* ARV8 treated tomato and pepper seedlings showed reduced production of ET, that may have contributed to the observed drought tolerance (Mayak, Tirosh, Science, 2004).

In many physiological processes in plants abscisic acid (ABA) plays an important role and it is very crucial for the response to environmental stress such as drought (Cohen et al., 2015; Porcel et al., 2014). Under drought stress there is an elevation in the ABA contents in plant organs results in physiological changes that modulate plant growth (Farooq et al., 2009). ABA regulates the leaf transpiration and root hydraulic conductivity and enhances the drought tolerance (Aroca et al., 2006). One more explanation is like ABA up regulate the aquaporins and enhances the drought tolerance (Zhou et al., 2012). Cytokinins also have positive effect on plant

growth under drought stress conditions (Arkhipova et al., 2007, n.d.; Liu et al., n.d.; Timmusk & Wagner, 1999) .

Bacterial communities that are associated in the root zone changes its structure towards the selection of assemblages which have more potential to get adapt with the abiotic stress, increase the resistance against stressors to promote health and water stress tolerance in plants (Cherif et al., 2015; Schmidt et al., 2014). The drought-sensitive pepper plants cultivated in desert showed more variation in the distribution of bacteria at endosphere, rhizosphere and the soil just around the root as compared to uncultivated soil that indicates a selective pressure on the structure of micro biome by the activity of the plant (Marasco et al., 2012). The bacterial isolates from deserts inoculated on the pepper plants and the plants showed high tolerance to water shortage as compared with the control (Marasco et al., 2013). In high saline conditions of Tunisia the salicornia plant associated microbes showed a wide set of abiotic stress resistance. They performed well in different plant growth promoting activities and their dense root colonization indicated that the salt tolerant bacteria of arid and salty ecosystems are having high potential in promoting the growth of plant under saline and drought condition (Mapelli et al., 2013).

2.2 Management of drought stress and plant growth promoting activity by *Fluorescent Pseudomonads*

Pseudomonas fluorescence biotype G (ACC 5) was identified to produce ACC deaminase enzyme and were inoculated to *Pisum sativum*. These treated ones were induced to produce longer roots led to increased uptake of water under drought stress (Zahir et al., 2008). *Cicer arietinum* varieties (Kabuli L-552 and Desi GPF-2) were co-inoculated with Bacillus isolate 23-B and Pseudomonas 6-P that produce ACC deaminase with *Mesorhizobium ciceris*. It was observed that under axenic condition on these varieties it helped in mitigation of the drought stress and also with the plant growth promotion by improving germination, root shoot length and fresh weight. Under water stress the PGPR treated varieties showed increased amount

of proline (P. Sharma et al., 2013). In maize plants treated with *Pseudomonas putida* GAP-P45 showed increase in the proline content due to which there was an improved relative water content, plant biomass and leaf water potential under drought stress (Ansary et al., 2012). *P. fluorescens* treated maize plants showed increased quantity of proline under drought stress (Sandhya et al., 2010). The varieties of rice IR-64 (drought sensitive) and Sahabhagi when treated with the consortium of *Pseudomonas jessenii* R62, *Pseudomonas syxantha* R-81 and *Arthrobacter nitroguajacolicus* strain YB3, strain YB5 promoted the plant growth and accumulated high quantity of proline indicated the drought tolerance (Gusain et al., 2015). The treatment with *P. fluorescens* YX2 enhanced the biosynthesis and accumulation of choline in maize plants. More amount of choline content in maize plants increased the relative water content and under drought stress as a food additive it supplied more nutrition. (Zeisel, 2006; Zhang et al., 2010). Five plant growth promoting drought tolerant strains of *Pseudomonas* spp. namely *P. entomophila*, *P. stutzeri*, *P. putida*, *P. syringae* and *P. montelli* were used to treat the maize plants. Under the drought stress as compared to control plants there was decrease in the activity of antioxidant enzymes (Sandhya et al., 2010). Wheat plants showed increase in the thickness of epidermis, ground, phloem and mesophyll tissues, dimensions of vascular bundles of root system when treated with *Azotobacter chroococcum* and *Pseudomonas* sp. resulted in the alleviation of drought stress (El-Afry et al., 2012). As compared to the single PGPR strains the consortia of bacterial strains *P. penneri*, *P. aeruginosa* and *A. faecalis* showed more potential to drought tolerance when inoculated to maize plants (Naseem & Bano, 2014). In rice plants the treatment with PGPR consortia reduced oxidative damage and also increased the proline level which helped in promoting the plant growth under drought stress (Gusain et al. 2015). The drought tolerant strain *Pseudomonas fluorescens* DR7 associated with foxtail showed high potential in production of ACC deaminase and EPS. DR7 efficiently colonized the soil that adhered to the root surface, improved the level of soil moisture and root adhering soil-root tissue ratio and effective as bio-inoculants for using in arid regions (Niu et al., 2018).

Fluorescent *pseudomonas* isolates were collected from the rhizospheric and bulk soil and analyzed for plant beneficial properties. Among 698 isolates 12 plant beneficial properties were identified by molecular and biochemical methods. But in an experiment with maize it preferentially selected the *Pseudomonas* isolate with low plant beneficial properties (up to 5) (Vacheron et al., 2016). Rice plants treated with *P.aeruginosa* PW-99, *P.putida* PW-2, *P.cepacia* PW-18 and *P.fluorescence* PW-5. Each seed coated with 18 cfu of the *Pseudomonas* culture. All *Pseudomonas* showed increase in the plant growth and *P.fluorescence* PW5 was identified as more potential in producing maximum shoot, root and dry weight. These isolates were able to produce high amount of IAA, HCN, siderophores, and also showed activity like phosphorus solubilisation (Kumar Deshwal et al., 2013). From the wheat rhizosphere 3 Fluorescent pseudomonads were isolated and were identified as *P.aeruginosa* RB5, *P.putida* AF2, *P.aeruginosa* RB5, *P.aeruginosa* EH4, and *P.fluorescens* CHA0 by physiological, biochemical and 16S rRNA sequencing. They were screened for their PGPR activities. All the strains were potential in solubilizing the phosphates and the maximum was 187.9µg/ml. Maximum amount of IAA was reported in *P.fluorescens* CHA0 (88.37µg/ml). percentage siderophore production among these strains varied from 38 to 46 (Fouzia Silini Allaoua Cherif-Silini Hafsa Ghoul Mostefa, 2015). Among the 28 isolate *Pseudomonas fluorescens* SS5 isolated from the rhizosphere of tomato plants showed plant growth promoting activity and enhanced productivity in tomato plants. It produced increased amount of IAA, siderophores and HCN and also increased root length, shoot length and yield (Fouzia Silini Allaoua Cherif-Silini Hafsa Ghoul Mostefa, 2015).

2.3 Bio-control activity of Fluorescent *pseudomonads*

The pf1 strain of *Pseudomonas fluorescens* showed maximum inhibition of mycelial growth of *Macrophomina phaseolina*, causal organism of coleus root rot. The efficacy was tested under greenhouse condition and the strain pf1 was applied as talc based formulation. Test for siderophore, antibiotics and HCN was done and it was observed that this isolate was able to produce good quantity of siderophores

(R Ramjegathesh, 2014). From the healthy plants of tomato the different strains of *Fluorescent pseudomonas sp.* were isolated and screened for their efficacy in bio control activity against *Fusarium solani*, *Alternaria alternate*, *Sclerotium rolfsii*, phytopathogens of tomato and pepper. The strain PC12 was identified as the potential antagonist for the *Sclerotium rolfsii* which causing damping off in tomato plants (N.A. Pastor et al., 2010). From the wheat rhizosphere *Ralstonia solanacearum*, *Fusarium oxysporum*, *Rhizoctonia solani* were isolated. It was observed that the *Pseudomonas flourescens* 2P24 inhibiting these phytopathogens. Under greenhouse condition the biological control activity of *Pseudomonas fluorescens* Bak150 was screened against *Phytophthora infestance* in-vitro. It inhibited the radial growth of the pathogen by 88%. Foliar spray is also sprayed under greenhouse condition on healthy potato plants as suspension and after three days the plants were inoculated with pathogen. This application was significantly reduced the disease as compared to control (Zegeye et al., 2011).

2.4 Biochemical and molecular characterization of *Fluorescent pseudomonas* isolates.

The identification of the new plant growth promoting strain increases the use of microbial technologies in agriculture (Uzair et al., 2018). For the characteriasation of fluorescent *Pseudomonas* different biochemical tests are carried out including IAA production, Gelatin liquefaction, nitrate reduction, HCN production, TSI glucose, Oxidase test, casein hydrolysis, carbohydrate utilization test (Goudar, Jones Nirmal Nath, & Patil, 2017). IAA production by the *Pseudomonas* isolates from green gram analyzed in LB medium broth supplemented with tryptophan. The treatment with the identified potential isolates showed increase in the nodule umber and biomass (Malik & Sindhu, 2011). From the rhizosphere of apple tree 15 phosphate solubilizing *pseudomonas* isolates were collected and characterized biochemically. Isolates were maintained in King's B medium. The phosphate

solubility was tested on pikovskya's agar medium which contains 0.5% tri-calcium phosphate. In plate assay the strain An-15-Mg(46mm) and in liquid assay the same strain showed maximum value (76µg/ml) (R. Sharma, Pal, & Kaur, n.d.,2017).

The strains of Fluorescent *Pseudomonas* have been delineated as *Pseudomonas fluorescens* lineage which includes *Pseudomonas fluorescens* together with *P.tolaasi*, *P.chlororaphis*, *P.marginalis*, *P.viridizflava* on 16s RNA basis (Moore et al., 1996). The strains of *Pseudomonas* isolated from root nodules of different plants. 8 bacterial isolates were screened for growth promoting activity and the data obtained from 16S rDNA sequencing data showed that those 8 bacterial isolates belonged to *Pseudomonas* genus (Choudhary, Kumar Gautam, & Gaur, 2012) . Fluorescent *pseudomonas* associated with rice was analyzed for plant growth promoting activity and were differentiated based on 16S rDNA sequence analysis. Based on the similarity in sequence the isolates were designated as *Pseudomonas aeruginosa*, *P. monteilii*, *P.plecoglossicida*, *P.mosselii*, *P.libaniensis*, *P.aeruginosa*. Through the different genomic DNA fingerprinting techniques the isolates belonging to same group were differentiated. These techniques includes repetitive extra genic palindrome (REP), randomly amplified polymorphic DNA (RAPD), enterobacterial repetitive intergenic consensus (ERIC), amplified ribosomal DNA restriction analysis (ARDRA), bacterial repetitive BOX elements (BOX). The rep- PCR generated fingerprinting pattern and the genetic diversity observed among the isolates revealed that the fluorescent pseudomonads associated with the rhizosphere of rice was *P.plecoglossicida* (Rameshkumar et al., 2014).

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled “**Characterization of fluorescent *Pseudomonas* from red lateritic soil for PGPA and increased resilience of water stress in rice and wheat**” was carried out in Molecular Plant Pathology Laboratory at the Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur (Chhattisgarh). At the time of course study, King’s B medium was used for maintaining the collected isolates of fluorescent *pseudomonads*. The media were sterilized by autoclaving at 121°C at 15 psi for 20 minutes. Before using the glass wares were sterilized in hot air oven at 180°C for two hours. Metallic instrument like forceps, inoculation needles etc. were sterilized by dipping them in 70% ethanol and heating over the flame during isolation, multiplication and other studies.

Material

Fluorescent *pseudomonas* isolates.

The material under investigation consisted of seventeen purified isolates of fluorescent *pseudomonas* species which were collected from the rhizosphere of the weed hosts in red lateritic soil surviving under highly water stressed conditions and from the different locations of Raipur. These isolates were used for the biochemical and molecular characterization, screening the increased resilience of water stress in Rice and wheat, Plant growth promoting activity in Rice, Wheat, Tomato and Brinjal and for the evaluation efficacy in bio – control activity *in-vitro*.

Plant materials

Three varieties of rice (IR-64, B-15, and RRF-75) and one variety of wheat (RATAN) were used for the screening of drought tolerance and PGP activity in pot as well as field experiments. Tomato and brinjal were used to evaluate the PGPA in the pot trial. The materials were kindly provided by Dr. A. S. Kotasthane, Professor, Department of Plant Pathology, College of Agriculture, IGKV, Raipur.

3.1 Collection and isolation of the fluorescent *pseudomonads*.

The fluorescent *pseudomonas* isolates were collected in the 2018 summer season from the rhizosphere of the plants which were still green and good in growth under such a harsh water stress condition. These cultures are isolated from the red lateritic soils and from the non-cultivable areas. The hard surface of the soil was loosened by the pickaxe and the plant is gently uprooted with the rhizospheric soil. The plants or these weed hosts were collected in different envelopes and the isolates were numbered according to the order of collection. Isolation is done by scraping the soils from the roots of the collected plant and spreading it in to the plates of King's B media. The plates were incubated at 28° C for 24-48 hours. Fluorescent *pseudomonas* isolates were identified under the UV light (366nm) and sub cultured it by streaking on to the KMB plates. Pure cultures of these isolates were made by picking the single colony from the sub culture and maintained it in the slants of King's B media at culture collections of the Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India.

3.2 Biochemical characterization of *Pseudomonas* isolates

All the 17 isolates of *Pseudomonas* are biochemically characterized by various biochemical tests as detailed below. The composition of different media used for the tests is mentioned in the method itself or else summarized in Table 3.4

3.2.1 Gelatin liquefaction test

Gelatin is a protein which dissolves in warm water and exists as a liquid above 25° C and solidifies (gel) when cooled below 25°C. Gelatin hydrolysis was tested by growing *Pseudomonas* isolates in nutrient gelatin medium. The gelatin hydrolysis was detected by observing liquefaction at low temperature (4° C) after incubation of the pseudomonas isolates in it at 28±2° C for 48 hrs. The tubes that are gelatin inoculated and remained liquefied produced gelatinase and showed positive test for gelatin hydrolysis and tubes that remained solid were negative.

3.2.2 Nitrate reduction test

On the basis of the ability to reduce nitrate to nitrite or nitrogenous gases bacterial species may be differentiated. This test was performed using the nitrate broth which is composed of peptone 5g/l, beef extract 3g/l, potassium nitrate 1g/l and prior to autoclaving pH was adjusted to 7. The broth is prepared and in large test tubes 20 ml is distributed. Sterilized by autoclaving at 15 lbs pressure and 121°C for 30 minutes. The test tubes were inoculated with fresh cultures of the selected isolates. Then incubated at 28±2°C for 48 hrs. The culture containing test tubes are mixed with 5 drops of sulfanilic acid and N, N-dimethyl 1-1 naphthylamine and it is shaken very well. In a few minutes there should be red or pink coloration, indicated nitrate reduction. If the inoculated broth turned to pink-red in color before adding Zn powder then the reaction is positive and the test finished. Then Zn powder was added to the test tubes which remained colorless and shaken vigorously and kept at room temperature for 15 minutes. After adding the Zn powder if the medium remains colorless then the test result is positive and if it changes to pink then the result is negative.

3.2.3 Carbohydrate utilization test

For testing the carbohydrate utilization profiles Hicarbohydrate™ kit (Himedia Laboratories, Mumbai, India.) was used. King's B broth is prepared and the bacterial cells were grown in that broth to reach the density of 0.5 O.D. at 600 nm. 50 µl of aliquot of the cell suspension was inoculated to each well in the kit. The kit was incubated at 28°C for 24-48 hrs. The results were recorded by going through the instructions given by the manufacturer.

3.2.4 Antibiotic sensitivity test

For distinguishing the species of fluorescent *Pseudomonas*, this rapid antibiotic test was performed. Kanamycin and carbenicillin sensitivity was tested by mixing the KMB media with 0.1 mg/ml of kanamycin and 0.1 mg/ml of carbenicillin respectively. Antibiotic sensitivity studies were performed by streak plate method Bauer et al. (1996). If the *Pseudomonas* spp. showed positive growth on either of the medium supplemented with antibiotic was recorded as resistant.

3.2.5 Egg yolk test

75 grams of the egg yolk medium is suspended 900 ml distilled water and heat to boil for dissolving thoroughly. From the dissolved media 90ml is poured in to different flask and autoclaved at 15 lbs pressure. The sterilized media is cooled to 50-55°C and 10 ml of sterile egg yolk emulsion/90 ml of medium is added. Poured this medium into the petri plates. The plates were then spot inoculated with the selected *pseudomonas* cultures and incubated at 28±2°C for 48 hrs. In the surface of the colonies “oil and water” is seen because of the breakdown of free fats by lipase. It is suggested to incubate 7 more days before concluding negative result for lipase production. The formation of the clear zone around the growth in the medium indicated proteolysis.

3.2.6 Phenylalanine test

Added few drops of 10% aq. ferric chloride to the cultures grown medium amended with phenylalanine. Appearance of green color indicated the positive reaction and no change in the color indicated the negative reaction. Organisms deaminate phenylalanine into oxidative form pyruvic acid. The green color fades quickly, so the result should be interpreted within 5 minutes after the addition of ferric chloride.

3.2.7 Oxidase test

Pseudomonas isolates are grown in Kings B broth. 0.2 ml of 1% α -naphthol in 95% ethanol and 0.3 ml of 1% aq. solution of Wurster's reagent was added to 1 ml of the culture. And then shaken vigorously. The reagent gets oxidized into coloured compound indophenol blue. So the development of blue colour indicated positive result for the oxidase test.

3.2.8 Triple sugar iron agar medium

Triple sugar agar iron media is used to distinguish the microbes by determining their ability for carbohydrate fermentation and hydrogen sulfide production. Triple sugar iron agar medium was prepared by taking 16.13 g of TSI medium and dissolved in 1000ml of distilled water. Medium in the test tubes were Sterilized by autoclaving. Prepared the slants by put it in sloped form with a butt about 1 inch long. Then those slants were streaked with the selected cultures and incubated at $28 \pm 2^\circ \text{C}$ for 48 hrs. Then results were noted.

The reaction can be summarized as follows:-

S. No.	Reaction	color	Description
1.	Acid butt Alkaline slant	Yellow Red	Only Dextrose fermented
2.	Acid butt Acid slant	Yellow yellow	Glucose and lactose are fermented or all the three sugar glucose, sucrose, and lactose fermented
3.	Bubbles or Cracks		Gas production
4.	Black Precipitate		H ₂ S gas production

3.2.9 Screening and characterization of Pseudomonas isolates for Siderophore production.

All the collected fluorescent pseudomonas isolates were screened for their potential to produce siderophores.

Qualitative estimation

3.2.9.1 Hydroxyquinoline mediated siderophore test

Among the selected Pseudomonas isolates the isolates which have high ability to produce siderophores were identified by inoculating the isolates on King's B medium supplemented with 8-Hydroxyquinoline (50mg/L) which is a strong chelater. After supplementation with this strong chelater it reduces the availability of iron in the medium. So only the isolates which have high ability to produce siderophores can grow on this medium. Inoculated isolates were then incubated Inoculated at 28±2°C for 48-72hrs.

3.2.9.2 Arnow's assay

This assay was used to quantify the catechol type of siderophore production. Arnow's assay was used for quantification of catechol type of siderophore production. For qualitative estimation of siderophore quantification, actively growing selected *Pseudomonas* cultures was inoculated to 20 ml King's B medium in 50 ml tarson tubes. The inoculated tubes were incubated for 72hrs at $28\pm 2^{\circ}\text{C}$. Centrifuged at 3000 rpm for 5 minutes to remove the bacterial cell. 3 ml of supernatant of the culture was mixed with 0.3 ml 5N HCl solution, 1.5ml Arnow's reagent (10g NaNO_2 , 10g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ dissolved in 50 ml distilled water) and 0.3 ml of 10 N NaOH . The presence or absence of pink colour was observed and noted after 5-10 minutes.

3.2.9.3Tetrazolium test

The tetrazolium test is based on the ability of the hydroxamic acid to reduce the tetrazolium salt by hydrolyzing the hydroximate groups using strong alkali. To a pinch of tetrazolium salt when 1-2 drops of 2N NaOH and 0.1 ml of test sample is added. The red colour showed the reduction and release of alkali and the instant appearance of this deep red indicated the presence of hydroximate siderophore.

3.2.9.4 Quantitative assay for siderophore production (liquid assay)

For the quantification of siderophore *Pseudomonas* cultures was inoculated to 20 ml of King's B broth (Hi Media) in flask of 100ml capacity and incubated for 72 hrs at $28\pm 2^{\circ}\text{C}$. Centrifugation was carried out at 3000rpm for 5 minutes to remove the bacterial cells. Took 0.5 ml of the supernatant of the culture and then mixed with 0.5 ml of CAS solution and 10 μl of shuttling solution (sulfosalicylic acid). After the incubation for 20 minutes, the colour formed was determined by using the spectrophotometer at 630 nm. King's B broth was used as blank and reference solution was made by adding CAS dye and shuttling solution to the King's B broth.

Then absorbance was recorded. Values of siderophore released in King's B was expressed in percent siderophore units and calculated using the formula.

$$\frac{(Ar-As) \times 100}{Ar}$$

Where,

Ar = OD of reference solution,

As = OD of samples

3.2.10 Determination of Indole Acetic acid production

An active culture of *Pseudomonas* spp. was inoculated to 20 ml DF salt minimal media (Dworkin and Foster, 1958) in 100 ml conical flasks for the production of indolic compound. For the production of indolic compounds. Kept for incubation up to 3 days at 28±2°C. The DF salt minimal media was supplemented with L-Tryptophan in a concentration of 1.02g/L from a 5Mm filter sterilized stock prepared in warm distilled water. After 3 days of incubation centrifuged it at 5,000 rpm for 5 minutes and the bacterial cells were removed from the medium. pH of the medium of all isolates was recorded. 4 ml of Salkowski's reagent was added to 1 ml of aliquot from the supernatant. Uninoculated DF salts minimal media is used as blank. Before taking the absorbance at 535 nm in calorimeter it was allowed to stand at RT for 20 minutes. The standard curve was prepared by adding 1M IAA in the increasing concentration: 0.0µl, 5µl, 10µl, 20µl, 30µl, 40µl, 50µl, 60µl, 70µl, 80µl, 90µl, 100µl in DF salt minimal media with decreasing concentration: 1 ml, 995µl, 990µl, 980µl, 970µl, 960µl, 950µl, 940µl, 930µl, 920µl, 910µl, 900µl respectively. In each tube 4

ml of Salkowski's reagent is added and mixed vigorously. Incubated at RT for 20 minutes after which the absorbance at 535 nm was measured in calorimeter. The concentration of IAA in each culture was determined by comparison with standard curve.

3.2.11 Screening isolates of *Pseudomonas* spp. for Phosphate solubilisation

3.2.11.1 Qualitative screening

The qualitative screening of phosphate solubilizing fluorescent *Pseudomonas* was done in pikovskya's agar medium. The medium contains tri-calcium phosphate as a phosphate source and Bromo Cresol (0.1 g/l) as a pH indicator for acidification. The plates containing the medium spot inoculated with the fresh cultures of the fluorescent *Pseudomonas* isolates. After incubation of the inoculated plates at $28 \pm 2^\circ\text{C}$ for 2 days the phosphate solubilizing isolates turned the media color from purple to yellow in the zone of acidification.

3.2.11.2 Quantitative screening

Quantitative screening was done in three different medium.(liquid assay)

1. Pikovskya's medium
2. NBRIP (National Botanical Research Institute's phosphate growth) medium.
3. NBRIY (National Botanical Research Institute's phosphate growth medium devoid of yeast extract)

3.2.11.2.1 Quantitative screening in pikovskya's medium

To evaluate the ability of the *Pseudomonas* cultures to solubilize the supplemented phosphate in-vitro the isolates were inoculated into Pikovskya's broth.

In 100 ml of the conical flask 50 ml of the broth was poured and sterilized. After sterilizing the media it was cooled and inoculated with different *Pseudomonas* isolates and incubated at $28 \pm 2^\circ\text{C}$ and 100rpm. After 7 days of incubation the amount of inorganic phosphate (Pi) released in the broth was estimated in comparison with uninoculated control. Then broth culture in the flask was transferred to 50 ml Tarson tubes and centrifuged at 10,000 rpm for 10 minutes. It separated the supernatant from the bacterial growth and insoluble phosphates. 5ml of chloromolybdic acid (CMA) was added to the 0.5 ml of the culture supernatant and mixed thoroughly. Distilled water is added and the volume was made up to 10 ml and 125 μl of chlorostannous acid (CSA) was added to it. With distilled water immediately the final volume was made up to 25 ml and mixed thoroughly. The blue color developed after 15 minutes was read in a colorimeter at 610 nm. The DW was used instead of sample supernatant to prepare the blank and the reference or control was prepared with uninoculated broth supernatant. The standard solution was prepared with KH_2PO_4 (10mg/ml) in distilled water with increasing concentration of stock 0.01 μl , 1 μl , 2.5 μl , 5 μl , 10 μl , 20 μl , 30 μl , 40 μl , 50 μl , 60 μl , 70 μl , 80 μl , 90 μl and the concentration of KH_2PO_4 was 0.0 μg , 10 μg , 25 μg , 5 μg , 100 μg , 200 μg , 300 μg , 400 μg , 500 μg , 600 μg , 7000 μg , 800 μg , 900 μg . Measurement of optical density (OD) was taken at 610 nm.

3.2.11.2.2 Quantitative screening in NBRIP and NBRIY medium

Quantitative estimation of phosphate solubility of different collected *pseudomonas* spp. were done in the National Botanical Research Institute's phosphate growth medium (NBRIP) as well as National Botanical Research Institute's phosphate growth medium without yeast (NBRIY). National Botanical Research Institute's phosphate growth medium devoid of yeast extract (NBRIY) medium contained l^{-1} : glucose, 10 g; $\text{Ca}_3(\text{PO}_4)_2$, 5 g; $(\text{NH}_4)_2\text{SO}_4$, 0.5 g; NaCl, 0.2 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g; KCl, 0.2 g; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.002 g and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g. National Botanical Research Institute's phosphate growth medium (NBRIP) contained l^{-1} : glucose, 10 g; $\text{Ca}_3(\text{PO}_4)_2$, 5 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 g; KCl, 0.2 g and $(\text{NH}_4)_2\text{SO}_4$, 0.1 g. The pH of the medium was adjusted to 7 before autoclaving. Procedure for testing the

phosphate solubility in these two media is same as that of PVK medium test for phosphate solubility.

3.2.12 Screening for 1- Aminocyclopropane-1-carboxylic acid deaminase (ACC Deaminase) containing Fluorescent *Pseudomonas* isolates.

Because of the enzymatic activity of ACC deaminase the ACC becomes available as a nitrogen source. The materials required and the protocol for utilization of ACC is as follows:

Materials required

1. Trypticase soya broth
2. DF salt Minimal Media
3. 0.5M solution of ACC
4. 0.1M Tris HCl (pH 7.5)
5. (NH₄)₂SO₄ (0.2% w/v)
6. 5% toluene
7. DNF solution
8. 0.56 N HCl
9. 2N NaOH
10. 0.1 N NaOH
12. α -keto butyrate

Preparation of ACC:

A 0.5 M solution of ACC (Sigma –Aldrich Co., Mumbai, India), which is labile in solution, was filter-sterilized through a 0.2- μ m membrane and the filtrate collected, aliquoted and frozen. 0.5M solution of ACC (Sigma-Aldrich Co., Mumbai, India), which is labile in solution, was filter-sterilized through a 0.2- μ m membrane and the filtrate collected, aliquoted and frozen at -20°C . Just prior to inoculation, the ACC solution was thawed.

3.2.12.1 Qualitative estimation of ACC Deaminase activity

1. *Pseudomonas* isolates were grown in 5 ml of TSB medium and incubated at 28°C at 120 rpm for 24 h.
2. The cells were harvested by centrifugation at 3000 g for 5 min and washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and resuspended in 1 ml of 0.1 M Tris-HCl (pH 7.5) and spot inoculated on petri plates containing modified DF (Dworkin and Foster) salts minimal medium (Dworkin and Foster, 1958) supplemented with 2 mM ACC as sole nitrogen source.
3. Plates containing only DF salts minimal medium without ACC as negative control and with $(\text{NH}_4)_2\text{SO}_4$ (0.2% w/v) as positive control.
4. The plates were incubated at 28°C for 72 h.
5. Growth of isolates on ACC supplemented plates was compared to negative and positive controls and was selected based on growth by utilizing ACC as nitrogen source.

3.2.12.2 Quantitative estimation

- In 5ml of the TSB medium the selected *Pseudomonas* isolates were grown and incubated at 28°C until they attained stationary phase.

- Centrifugation was carried for the cultures and the cells were collected and washed twice with 0.1M Tris-HCl(pH 7.5). Then it was suspended 2ml of modified DF salt minimal medium supplemented with 2mM final concentration of ACC.
- Kept for incubation at 28°C and placed in a shaker for 36-72hrs.
- Again did centrifugation at 3000 rpm for the incubated bacterial cells for 5 minutes. Using 0.1M Tris- HCl (pH 7.5) it was washed twice and resuspended in 200µl of 0.1M Tris- HCl (pH 8.5)
- With 5% Toluene (v/v) the cells were labilized and the vortex it for 30 sec.
- Took 50µl from the labilized cell suspension and was incubated in eppendorf tube with 5µl of 0.3M ACC at 28°C for 30 minutes.
- For this assay the negative control included 50µl of labilised suspension without ACC. Blank for this assay was prepared by 50µl of 0.1M Tris- HCl (pH 8.5) with 5µl of 0.3 M ACC.
- Then to the samples 500µl of 0.56N HCl is added and mixed well by vortexing and centrifugation was done at 12,000g for 5 minutes and the cells were removed.
- From the supernatant 500µl of aliquot was transferred to a glass test tubes and mixed with 400µl of 0.56 N HCl and 150µl of DNF solution and the obtained mixture was incubated at 28°C for 30 minutes.
- Then before the absorbance measurement at 540nm, 1 ml of 2N NaOH was added to the sample.

Standard preparation: - In each sample the alpha ketobutyrate concentration was determined by comparing with a standard curve which generated as follows.

- 100mmol/l alpha ketobutyrate stock solution was made in 0.1 mol/l Tris- HCl (pH 8.5) and it was stored at 4°C. The stock solution was diluted just prior to use with the above mentioned buffer and made a 10m mol⁻¹ solution and from that a standard curve was generated.

- Then different concentrations of the α ketobutyrate was made like 1,5,10,15,20,25,30,35,40,45 and 50 μ M. From each this 500 μ l was taken in different tubes and mixed with 400 μ l of 0.56 N HCl and 150 μ l of DNF solution.
- Then before the absorbance measurement at 540nm , 1 ml of 2N NaOH was added to the sample
- Constructed a standard curve by using the values of absorbance and concentration of α -ketobutyrate.

3.3 Identification of potential strains of fluorescent *pseudomonas* sp. imparting water stress tolerance in rice and wheat.

Three rice varieties namely IR-64, B-15 and RRF-75 grown in pots during kharif season. Wheat variety RATAN is used in the rabi season.

3.3.1 Seed treatment

Equal amount of seeds are weighed and filled in different 18 glass bottles which are numbered serially. Add same quantity of sterilized talc powder and the fresh culture of the collected isolates in the bottles. The different culture is added in the different bottles according to the number. Mix it thoroughly so that it forms slurry. Among the 18 bottles one kept for control. In control instead of the culture distilled water is added. Then kept the treated seeds for 4-5 hours.

3.3.2 Pot experiment

Same quantity of soil will be filled in equally sized pots. Equal quantity of DAP (5g) and FYM (10g) is mixed with the soil in the pots. Pots are numbered serially including the variety's name. One pot is kept for the control in each variety. This experiment conducted in three replications. The treated seeds are then sown by direct sowing method. The pots are kept under the rainout shelter for screening the drought tolerance. Equal amount of water (450ml) made available to each pot. Same procedure is followed for wheat during the Rabi season. After 15-20 days started giving water stress to the plants in the pots. From next day onwards started taking observation on

rolling of the leaves. Drought tolerance of rice is evaluated in the field condition also. The observation is taken at 12 pm for the rolling of the leaves for the treated as well as control plants.

3.4 Assessment of plant growth promoting activity in Rice, Wheat, Brinjal and tomato

Plant growth promoting activity by each isolate is assessed in field condition for rice and wheat. Pot trial is carried out for the evaluation of PGPA in tomato and brinjal. Seed treatment is done with the different 17 isolates and sowing is done in the field under rain fed condition for rice and wheat. Pot experiment is conducted in three replication for tomato and brinjal. The plant growth promoting activity is assessed by taking the observation on plant height, number of tillers, number of panicles emerged, length of the roots, yield in case of rice and wheat and in tomato and brinjal root length, shoots length and root volume is measured. These observations were compared with the control plants.

3.5 Evaluation of bio-control activity *In-vitro*

The bio-control efficacy of the selected 17 isolates was tested against the phytopathogens *Sclerotium rolfsii* and *Rhizoctonia solani*. The equal volume of the sterilized potato dextrose agar (PDA) and King's B medium was mixed and poured to the sterilized petri plate under laminar air flow cabinet. After the solidification of the media the mycelia disc is placed in the center of the plates. With the help of the sterilized glass funnel the actively growing fluorescent *pseudomonas* cultures are inoculated around the mycelia disc. The disc should be at the center of the funnel or the ring made by it. Kept control of these pathogens without inoculating with the *Pseudomonas* spp. After the incubation the percent inhibition of the pathogen by *Pseudomonas* was calculated by the formula of Vincent (1947):

$$\frac{[(\text{Growth of pathogen in control} - \text{Growth of pathogen with Pseudomonas isolates}) / \text{Growth of pathogen in control}] \times 100}{}$$

3.6 Molecular Characterization of Fluorescent *Pseudomonas* isolates

3.6.1 Procedure for genomic DNA isolation of Fluorescent *Pseudomonas*

DNA extraction of fluorescent pseudomonas is done using the microbial DNA isolation kit. The user protocol for bacterial DNA extraction is as following:-

1. Added 2 ml of microbial culture to a 2 ml tube , centrifuged at 13000 rpm for 3 minutes, decanted the supernatant(add more culture if pellet is small) and repeated the process.
2. Resuspended the pellet in 300 µl of micro bead solution, gently vortexed to mix.
3. Transferred homogenized mixture to micro bead Tube.
4. Added 50 µl of solution MD1 to micro bead tube.
5. Homogenized the solution in micro bead tubes for 5-7 minutes in tissue lyser.
6. Centrifuged at 13,000 rpm for 1 minute.
7. Transferred the supernatant to clean 2 ml tube (provided)
8. Added 100µl solution MD 2 to the supernatant and vortexed for 5 seconds.
9. Incubated at 4° C for 5 minutes.
10. Centrifuged at 13000rpm for 1 minute.
11. Transferred the entire supernatant to a clean 2 ml tube (provided).
12. Added 900µl of solution MD3 (shaked before use) to the supernatant and vortexed for 5 seconds.
13. Loaded about 700µl into spin filter and centrifuged at 13,000 rpm for 1 minute.
14. Discarded the flow through.
15. Added remaining solution (700µl at a time) from step 11 again in the same spin filter and centrifuged at 13,000rpm for 1 minute. Discarded all the flow through.
16. Repeated till all the solutions of the step 11 is spin filtered.
17. Added 300µl solution MD4 and centrifuged at 13000rpm for 1 minute.

18. Discarded the flow through.
19. Again centrifuged at 13,000 rpm for 1 minute.
20. Placed the spin filter in new 2 ml tube (provided)
21. Added 50µl of solution MD5 to the center of the filter membrane inside spin filter
22. Centrifuged at 13,000 rpm for 1 minute.
23. Discarded the spin filter
24. Stored the DNA in 2 ml tube at -20 °C

3.6.2 Quantification of genomic DNA

The samples of DNA were quantified using Nano drop spectrophotometer (ND 1000). Over the lower pedestal of Nano drop one microliter of isolated DNA was placed to record absorbance at 260nm with TE as blank. To estimate the purity of DNA the absorbance ratio (A260/A280) was recorded for each sample of DNA. For pure DNA the acceptable absorbance ratio (A260/A280) is 1.8. After quantification: the DNA was diluted with TE buffer such that final concentration of DNA was approximately 40ng/µl for PCR analysis.

3.6.3 Procedure for making Whole cell template (WCT) for PCR amplification for antibiotic-related genes

- Young viable cultures of *Pseudomonas* was inoculated into 4 ml of King's B broth and incubated for 48hrs at 27°C.
- Then took 5µl of bacterial colonies from the incubated cultures and transferred into 100µl of lysis solution (50 Mm KCl, 10Mm Tris HCl (pH 8.3), 0.1 %(v/v) Tween 20).
- Resulting suspension was centrifuged @ 1200 rpm for 1 minute. Incubated for 10 minutes at 99°C in thermal cycles.
- Heat lysed bacterial suspension was frozen at -20°C for 30 minutes. After thawing 4µl volume of supernatant carefully taken for PCR.

3.6.4 Characterization of fluorescent *Pseudomonas* using genus and species specific loci

To confirm the strains as fluorescent *Pseudomonas*, 16S-23S rRNA intervening sequence specific primers 27 F and 1492 R were used to get an amplicon size of 1500 bp.

ERIC-PCR- based genotypic analysis-

ERIC primers were used to detect difference in the number of repetitive sequence in the bacterial genome. This is a PCR based marker and was carried out using primers ERIC –F and ERIC-R as described by Brujin in 1992.

REP and BOX PCR analysis-

In REP PCR two families of repetitive sequence were used including BOX element and repetitive extragenic palindromic (REP) sequences. To differentiate closely related microbial strain, to deduce phylogenetic relationships and to study their diversity this REP-PCR is a simple method. It is highly reproducible also.

Table: 3.1 Sequence of the primers needed for characterization of fluorescent *Pseudomonas* spp. using genus, species specific and antibiotic genes.

Primer	Sequence	Tm(°C)
27 F	5'-AGAGTTTGATCMTGGCTCAG-3'	56
1492 R	5'-CTACGRRACCTTGTTACGAC-3'	56
ITS 2 R	5'-AAGGAGGTGATCCAGCCGCA-3'	53.2
ITS 1 F	5'-AGAGTTTGATCCTGGCTCAG-3'	46.4
REP R	5'-TTTTCGTCGTCATCTGGC-3'	32.2
REP F	5'-TCGTCTTATCTGGCCTAC-3'	41.9
BOXAIR	5'-CTACGGCAAGGCGACGCTGACG-3'	76.5
Primer	Sequence	Tm(°C)
PCA2a	5-TTGCCAAGCCTCGCTGCCCCAAC-3	62
PHZ 1	5-GGCGACATGGTCAACGG-3	62
PrnA R	5-TGCCGGTTCGCGAGCCAGA-5	62
PrnA F	5-GTGTTCTTCGACCTTCCTCGG-3	62
PrnC R	5-GAGAAGAGCGGGTCGATGAAGCC-3	62
PrnC F	5-CCACAAGCCCGGCCAGGAGC-3	62

Table: 3.2 **Reaction mixture needed for performing polymerase chain reaction using different primers**

Reaction mixture needed for performing PCR REP,BOX, ITS, ERIC, primers(DNA)		
components	stock concentration	volume per reaction(10µl)
ADW	-	4.5µl
DNA	40ng/µl	2µl
dNTP	1mM	1µl
PCR buffer containing 15 Mm MgCl ₂	10X	1µl
reverse primer	10µM	0.5µl
forward primer	10µM	0.5µl
Taq polymerase	1U/µl	0.5µl
Reaction mixture needed for performing PCR PrnC, PrnA, Plt 1+2,PltB primers(WCT)		
components	stock concentration	volume per reaction(10µl)
ADW	-	3.5µl
DNA	40ng/µl	2µl
dNTP	1mM	1.5µl
PCR buffer containing 15 Mm MgCl ₂	10X	1µl
Reverse primer	10µM	0.75µl
Forward primer	10µM	0.75µl
Taq polymerase	1U/µl	0.5µl
Reaction mixture needed for performing PCR hcn AC,hcn PM2&hcn PM7-26R primers(WCT)		
components	stock concentration	volume per reaction(10µl)
ADW	-	4.75µl
DNA	40ng/µl	2µl
dNTP	1mM	1µl
PCR buffer containing 15 Mm MgCl ₂	10X	1µl
reverse primer	10µM	0.5µl
forward primer	10µM	0.5µl
taq polymerase	1U/µl	0.25µl

Reaction mixture needed for performing PCR P-GS primer (DNA)		
components	stock concentration	volume per reaction(10µl)
ADW	-	9.5µl
DNA	40ng/µl	4µl
dNTP	1mM	2µl
PCR buffer containing 15 Mm MgCl ₂	10X	2µl
reverse primer	10µM	1µl
forward primer	10µM	1µl
taq polymerase	1U/µl	0.5µl

Table 3.3 **Amplification thermal profile for primers.**

16S-23S rRNA intervening sequence specific internal transcribed(ITS)				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	94	5	1
2	Final denaturation	92	4	
3	Annealing	40	1	1
4	Extension	7	2	
5	Final extension	72	10	1
6	storage	4		
Repetitive extragenic palindromic sequence (REP sequence)				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	94	3	1
2	Final denaturation	94	45s	
3	Annealing	38	1	40
4	Extension	72	2	
5	Final extension	72	8	1
6	storage	4		
BOX element				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	94	3	1
2	Final denaturation	94	45s	
3	Annealing	53	1	40
4	Extension	72	2	
5	Final extension	72	8	1
6	storage	4		

PG-S				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	95	5	1
2	Final denaturation	95	1	
3	Annealing	60	1	35
4	Extension	72	1	
5	Final extension	72	7	1
6	storage	4		
Plt 1-Plt 2				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	95	3	1
2	Final denaturation	95	1	
3	Annealing	52	1	35
4	Extension	72	1	
5	Final extension	72	5	1
6	storage	4		
PHz, PltB, Prnc, PrnA				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	95	3	1
2	Final denaturation	95	1	
3	Annealing	62	1	35
4	Extension	72	1	
5	Final extension	72	5	1
6	storage	4		
ERIC				
Steps	Activity	Temperature(°C)	Time(min.)	Repeats
1	Initial denaturation	94	3	1
2	Final denaturation	94	45s	
3	Annealing	53	1	45
4	Extension	72	1	
5	Final extension	72	8	
6	storage	4		

3.6.5 Polyacrylamide gel electrophoresis (PAGE) for visualizing PCR products.

- For preparation of PAGE CBS scientific dual vertical unit is used.
- With a detergent clean the glass plates thoroughly and also wipe it with alcohol.
Apply gasket around the sides of the dried glass plate.

- Gasketed plate was placed on a flat surface with tubing side up and places the spacers on two sides of the plates.
- Notched plate was placed on top off the bottom assembly and on bottom of the plate assembly two clamps were placed.
- Made the assembly stand on the base of the clamps.
- Add PAGE solution (65-70 ml polyacrylamide gel solution, 80 μ l TEMED and 800 μ l solution of freshly prepared 10% ammonium persulphate) to the gel plate and insert the comb of required size and allow the gel to solidify.
- Disassemble the clamps and gasket after 20 to 30 minutes.
- Placed the plates in electrophoresis unit with notched plate facing the upper buffer reservoir and clamp on sides.
- In upper and lower chambers poured freshly prepared buffer and after removing the comb load the gel samples mixed with dye.
- Connected with power supply and run at 150 to 200 volts for 20-30 minutes.
- After completing the run power supply is switched off and glass plates were disassembled.
- After removing the side clamps pulled the notched plate outside and took out the gel very gently.
- Kept the gel for staining in 1X TBE buffer with 4 μ l/100ml ethidium bromide (10mg/ml stock). For 5-10 minutes staining was done. Gel was rinsed with distilled water. The gels were scanned on gel documentation system.

DATA ANALYSIS

All the experiment conducted in laboratory and pots under the rainout shelter were arranged in completely randomized block design. The pot trial carried in three replications and lab experiment in two replications. The statistical analysis of the data was done using WASP (Web Agri Stat Package) software (<http://icargoa.res.in./wasp/index.php>). Critical difference at 0.01 and 0.05% level of

significance was calculated for the data collected along with average and standard deviation.

Table: 3.4 **Composition of different media used in the present investigation**

King's B Base medium	
Dipotassium hydrogen orthophosphate	1.5g
Agar	20g
Magnesium sulfate	1.5g
Protease peptone	20g
Glass distilled water	1000ml
Glycerol	15 ml
Gelatin broth (pH 6.8)	
Peptone	5g
yeast extract	3g
Glass distilled water	1000ml
Gelatin	120g
Phenylalanine agar medium	
NaCl	5g
yeast extract	3g
Na ₂ HPO ₄	1g
DL-Phenylalanine	2g
Glass distilled water	1000ml
Agar	15g
Potato Dextrose agar medium	
Potato	250g
Dextrose	20g
Agar	20g
Glass distilled water	1000ml
Nitrate Broth(HIMEDIA)	
Beef extract	3g/l
Peptone	5g/l
Potassium nitrate	1g/l
Sulphanilic acid(HIMEDIA)	
Zinc (HIMEDIA)	1g
Final pH at 37°C	7.0±0.2

Pikovskya's agar medium	
Na ₂ HPO ₄	3g/l
KH ₂ PO ₄	5g/l
Gluconic acid	2.0g
Glucose	1g/l
MgSO ₄ .7H ₂ O	0.2g
Citric acid	2g
FeSO ₄ .7H ₂ O	7.0±0.2
(NH ₄) ₂ PO ₄	0.001g
MnSO ₄ .H ₂ O	11.19
H ₃ BO ₃	1mg
CuSO ₄ .5H ₂ O	78.22
ZnSO ₄	124.6
Agar	15g
MoO ₃	10µg
Glass distilled water	1000ml
NBRIY MEDIUM	
Glucose	10g
calcium phosphate	5g
ammonium sulfate	0.5g
sodium chloride	0.2g
magnesium sulfate	0.1g
potassium chloride	0.2g
manganese sulfate	0..002g
ferrous sulfate	0.002g
NBRIP MEDIUM	
Glucose	10g
Calcium phosphate	5g
Ammonium sulfate	0.1g
Magnesium sulfate	0.25g
Potassium chloride	0.2g
Magnesium chloride	5g

3.6.7 Solutions used for different test

Chrome azurol sulfonate(CAS) solution

1. **1M HCl (stock):-** 0.36ml of conc.HCl diluted in 10ml distilled water.
2. **1M FeCl₃.6H₂O (stock) :-** 2.703g FeCl₃.6H₂O
3. **Iron (Fe) (III) solution**

Chemical	Stock solution	working concentration	volume required per 1000ml
FeCl ₃ .6H ₂ O	1M	1Mm	10µl
HCl	1M	1mM	100µl

Both components are mixed and made up the volume to 10 ml with autoclaved distilled water.

Chrome azurol sulphonate solution

50 ml of autoclave distilled water poured in a beaker. To the beaker 60.5mg of CAS and 10ml of iron (III) solution is added and mixed well by stirring. HDTMA (in 40ml autoclaved water 72.9 mg was dissolved.) Solution was added to it slowly. There was a formation of dark blue color.

Arnow's reagent

Sr.No.	Composition	Amounts(g/l)
1	sodium molybdate	10g
2	distilled water	50ml
3	sodium nitrate	10g

Salkowski's reagent

Sr.No.	Composition	Amounts(g/l)
1	FeCl ₃ .6H ₂ O	7.5 ml of 0.5M
2	Conc.H ₂ SO ₄	150ml
3	Autoclaved distilled water	250ml

3.6.8 Reagents required for inorganic phosphate quantitative estimation

1) Chloromolybdic acid:- In 150ml autoclaved distilled water 7.5g of ammonium molybdate was dissolved. Then it was mixed into 162 ml conc.HCl. final volume was adjusted to 1000ml with autoclaved distilled water and it was stored in amber colored bottle at 4°C.

2) Chlorostannous acid:- In 100ml of concentrated HCl 25g of stannous chloride was dissolved and adjusted final volume to 1000 ml by adding autoclaved distilled water. And stored at 4°C in an amber colored bottle.

3.6.9 Solution and stocks needed for PAGE:-

10X TBE:-

Sr.No.	Composition	Conc.required	Quantity
1	Boric acid	89mM	5.5g
2	Tris-base	89mM	10.8g
3	0.5M EDTA(pH 8.0)	2mM	4ml

These components are mixed and the volume adjusted to 100m by adding distilled water.

1)1X TBE: - 10ml of 10X TBE was diluted with 100ml of distilled water.

2) Polyacrylamide stock solution (40%):- 2g of Bis-acrylamide and 38 g of Acrylamide was dissolved in100 ml of autoclaved distilled water then it was filtered through Watman filter paper no.1. It was stored in brown bottle in the refrigerator.

3) 5% PAGE preparation:-

Sr.No.	Composition	Final volume
1	10% APS	700μl
2	40% Polyacrylamide solution	12.5
3	TEMED	70μl

4) By mixing the following components the staining solution was prepared

Sr.no.	Stock solution	Final conce.
1	I X Buffer	100ml
2	Ethidium bromide	4μl

5) 10% APS

Sr.no.	Composition	Final conce.
1	Distilled water	10ml
2	Ammonium persuphate	1g

6) Formaldehyde dye

Sr.No.	Stock	Final Concentration	For 10ml
1	0.5M EDTA	10mM	200 μ l
2	Formamide	98%	9.8ml
3	Bromo phenol blue	0.10%	4ml
4	Xylene cynol	0.10%	0.01g

CHAPTER –IV

RESULTS AND DISCUSSION

Agriculture is the major sector on which the whole world is depended for the survival. The growing human population and the climate changes are the major challenges faced by this sector and these are the two major risks to the global food security. Major constraints like biotic and abiotic stresses decrease the food production worldwide. In order to solve these problem in agriculture sector there should be sustainable solutions. So for raising the agricultural production one of the best way is to exploit the beneficial potential of the root micro biome(Philippot, Raaijmakers, 2013, n.d.).The beneficial microbes associated with the root rhizosphere increase the soil productivity majorly by preserving the soil structure, recycling the nutrients, controlling the diseases and by degrading the pollutants. These microbes can also act synergistically to impact plant health and development. Drought is one of the major constraints that decrease the food production. Majorly the reduction in the cereal production by drought affects the country like India where the staple foods are rice and wheat. The plant associated microbes especially the plant growth promoting bacteria are well known for their nutrient management and antagonistic activity. But recently it is also gaining attention because of their ability to manage the abiotic stress like drought. So the use of these beneficial microbes will be more eco- friendly method for the mitigation of drought as well as the promotion of the plant growth. These plant associated microbes produce various enzymes and other metabolites which make them able to cope up with the biotic as well as abiotic stresses. Among the PGPR Fluorescent pseudomonads are getting attention for the plant growth promoting activity and management of the abiotic stresses. The application of these beneficial microbes is very useful in the case of plants like rice which is highly susceptible to water deficit.

4.1 Collection and isolation of fluorescent *Pseudomonads* from plant rhizosphere surviving in water stressed red lateritic soils.

Our earlier experience (Kotasthane personal communication) with the collected fluorescent *Pseudomonas* spp. from the root rhizosphere indicated that the isolates were able impart water stress tolerance in rice and wheat. During our survey for collection of soils from root rhizosphere, a unique feature was observed along the road side regarding weed plants naturally survived which in very harsh water stress conditions. Weeds hosts are commonly seen growing under such conditions along the road side or in fellow uncultivable land. This formed the basis of our present investigation. We raised the issues like 1) Are the fluorescent *pseudomonas* derived from rhizosphere of the plants naturally growing in lateritic soil strong phosphate solubilizes and siderophore producers? 2) Will the isolation and the identification of the rhizospheric microorganisms which survive under stressed environment may lead to the identification of potential microbes with beneficial properties? Nevertheless the issue prompted us to isolate plant-associated phosphate-solubilizing *Pseudomonas* from red lateritic soil which is rich in iron and to evaluate their potential as PGPR. The plants were collected with rhizospheric soil adhered to the root surface. Because of the unavailability of water the soil surface was so hard and for loosening the soil pick-axe was used. Sixty five weed hosts were collected in brown paper envelopes with the rhizospheric soil. Rhizosphere and rhizoplane soils inoculated on KMB medium expressed the bacterial colonies with strong fluorescence when visualized against UV illumination. Seventeen plant samples expressed association of fluorescent *pseudomonas*. Fluorescing colonies were observed and were picked using a sterilized needle and streaked on fresh KMB containing petriplate. Isolates were further purified following streak plate method. Single colonies were isolated and transferred to slants containing KMB medium for future use. The isolates were designated as (P1to P17) (Table 4.1).

Many researchers reviewed research on exploring the potential of such PGPR (Bhattacharyya and Jha 2012; Gray and Smith 2005; (Bhattacharyya and Jha

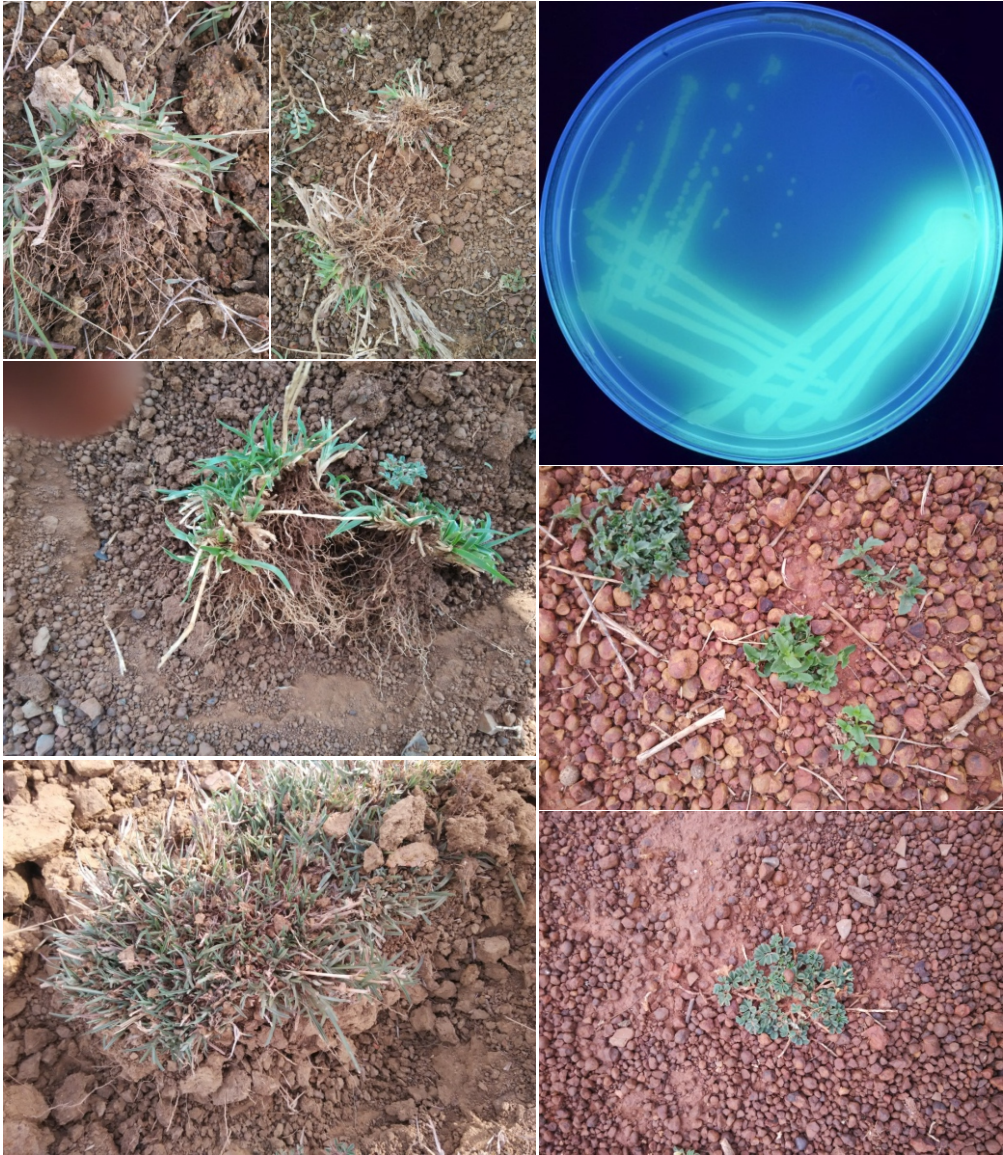


Fig 4.1 Collection and isolation of fluorescent *Pseudomonads* from plant rhizosphere surviving in water stressed redlateritic soils

2012; Gray and Smith 2005; Johri et al. 2003; Lugtenberg and Kamilova 2009) Maroniche et al., reported plant associated fluorescent *Pseudomonas* derived from the red lateritic soil belonged to *P.fluorescent* and *P.putida* phylogenic group. The isolates derived were strong phosphate solubilizers and displayed a rich array of plant growth promotion traits. 75 strains of fluorescent pseudomonas spp. were isolated from different ecosystems of India and tested for various PGPR traits (Kumar, 2015).

4.2 Biochemical characterization fluorescent pseudomonas isolates

4.2.1 The collected 17 fluorescent pseudomonads were screened for different Biochemical tests.

- The isolated bacteria were tested for nitrate reduction in the nitrate broth. The inoculated bacteria reduced the nitrate into nitrite by the production of the nitrate reductase by the bacteria. Before addition of the zinc powder nitrate reduction was showed by 70.5% of the total isolates. Before addition of the zinc powder only 5% of the isolates turned into red color and showed the nitrate reduction. But after adding the zinc powder the colorless samples showed the nitrate reduction constitutes 64.7% of the isolates. So overall 69.7% of the tested isolates (P1, P2, P3, P6, P7, P8, P9, P11, P12, P14, P15, and P17) showed positive result for the nitrate reduction. Out of 17 isolates only P17 showed positive result before addition of the zinc powder and may be it comes under *P. aeruginosa*. After the addition of zinc 12 isolates showed positive result and can be categorized as *P. putida*. The isolates (P4, P5, P10, P13, and P16) which showed negative result for the test may be *P. fluorescens*.
- These isolates were tested for the gelatinase liquefaction. Inoculated tubes of gelatin that were remained in the liquid form under low temperature (4°C) produced gelatinase and showed positive result for the test and the solid form after the incubation showed negative result. 52% of the isolates showed positive result for the test. The isolates which showed positive results are P2,

P3, P5, P8, P10, P11, P12, P13, and P14. The collected 17 isolates were grown in the King's B medium in replicates. One set kept at 4°C and the other at 42°C. Isolates P2, P3, P4, P5, P6, P7, P8, P9, P11, P12, P14, P15, P16 were able to grow at 4°C and P2, P3, P7, P9, P15 were able to grow at 42°C. Rest of them showed negative result.

- The isolates were tested for the sensitivity against two antibiotics. 76.4% of the isolates were able to grow in the kanamycin supplemented medium and 35.2% were able to grow in carbenicillin supplemented medium. so the isolates resistant to kanamycin were P1, P2, P3, P4, P5, P6, P8, P11, P12, P13, P14, P16, P17. The isolates resistant to carbenicillin were P1, P2, P4, P11, P12, and P17. Among the 17 isolates 5 isolates (P1, P2, P11, P12, and P17) were resistant to both the antibiotic. P7, P9, P10, P15 were unable to grow in the presence of both the antibiotic. So these 4 isolates were sensitive to these antibiotics.
- All the isolates were able to grow in the fluorescein and pyocynine medium. The yellow green pigment production in the fluorescein medium was the most favorable result for the fluorescein test and blue green for the pyocynin test. Isolates P11, P12, P16, P17 showed good growth as well as high pigmentation in the fluorescein medium. The isolates P3, P10, P13, and P16 showed high amount of pigmentation in the pyocyanin medium. P16 isolate showed the best result in both of the test, so the P16 can be *P.aeruginosa* as it gave positive result in both medium.
- The oxidase test of these isolates formed blue color in the inoculated medium after the addition of the reagents showed positive result for the test. The blue color indicated the oxidation of the reagents and the formation of the indophenol. Except P2 all other isolates showed the positive result for the test.

- Except P1, P9, P10, P13 showed positive result for the cetrimide agar base.
- None of the isolates showed positive result for the Hugh-Leifson's test.
- In TSI agar test isolates P 5, P 6, P 7, P 10, and P17 produced the acid slant and acid butt and indicated that the glucose and lactose were fermented by the bacteria or all the three, glucose, sucrose, and lactose were fermented. P2, P3, P4, P8, P9, P11, P12, P13, P14, P15, P16 produced acid butt and alkaline slant indicated that only dextrose is fermented by the bacteria. P1 and P10 produced bubbles or crack in the slants and it indicated the gas production by the isolates. Isolates P2, P8, P11, and P16 produced black precipitate in the slants and indicated the production of H₂S gas production. .
- Present Biochemical screens filtered out 9 isolates (P2, P3, P5, P8, P10, P11, P12, P13, and P14) positive for gelatin liquefaction and the rest expressed negative reaction. Nine isolates were +ve isolates were therefore designated as *P. fluorescens* and the rest as *P. putida*.
- Fluorescent Pseudomonas isolates (P2, P3, P7, P9, P15) that were able to grow at 42°C qualifies for a species identity as *P. aeruginosa* whereas the following isolates # , P4, P5, P6, P8, P11, P12, P14, P16 qualify for a species identity as *P. fluorescens* because they were able to grow at 4°C. . Our present observation indicates that isolates # P2, P3, P7, P9, P15, were able to grow at 42°C and 4°C. In view of Blazevic et al. (1973) diagnostic tests *P. aeruginosa* is the only fluorescent pseudomonads that can grow at 42°C and therefore isolates P2, P3, P7, P9, P15, were included in the category of *P. aeruginosa*.
- *P. fluorescens* and *P. putida* are very resistant to carbenicillin and sensitive to kanamycine. Opposite in case of *P. aeruginosa*. Because the following isolates,

P3, P5, P6, P8, P13, P14, P16, expressed resistance to kanamycine and sensitive to carbenicillin therefore they may be designated as *P.aeruginosa*.

- Isolates P1, P2, P4, P 11, P 12 & P 17 expressed resistance to both the antibiotics and isolates P7, P9 P10 and P15 expressed sensitivity to both the antibiotics. In view of this the isolates do not qualifies to be designated based on Blazevic et al. (1973) antibiotic sensitivity test.
- Many of the strains come under Pseudomonaceae family like *P. putida* S12 shows resistance to multiple antibiotics (Kieboom and de Bont 2001). Blazevic et al. (1973) suggested that the test for sensitivity to kanamycin and resistance to carbenicillin and nitrate reduction would provide a means of identifying *P.fluorescence* and *P.putida* and separating them from *P.aeruginosa*. However, the rare nitrate negative *P.aeruginosa* and however, rare nitrate-negative *P. aeruginosa* or rare nitrate-positive *P. fluorescens* or *P. putida* should not be misidentified using both of these characteristics.

4.2.2 Hi – carbohydrate™ kit test for identifying the differential utilization of the carbon sources by fluorescent *pseudomonas* isolates.

All the 17 isolates were screened for the carbohydrate utilization. Out of these 17 isolates 16 (except P13) were able to utilize the xylose and dextrose (94.1%). Fifteen isolates were able to utilize galactose (except P10 & P13), arabinose (except P11 & P13), and mannose (except P1 & P13) that constitutes 88.2%. Fourteen isolates except P1, P10 and P13 utilized citrate (82.3%). Lactose and raffinose was used by only 5 (29.4%) isolates P6, P9, P14, P15, and P17 while the isolate P16 utilized rafinose and not Lactose. Trehalose utilization was done by 8 isolates (P6, P9, P10, P11, P14, P15, P16, P17) constitutes a total of 47%. 13 isolates (76.4%) utilized the melibiose (except P2, P10, P11, and P13), glycerol (except P5, P11, P12, P13) and D-arabinose (except P2, P4, P13, P14). Sucrose was utilized by only 6 isolates including P9, P10, P14, P15, P16, and P17 that constitutes 35.2% of total isolates. Seven isolates, P1, P3, P4,

P5, P8, P9, P15, P17 (41.17%) were identified to utilize linulin. Only 11.7% of the total isolates utilize sodium gluconate (P6 & P17), erythritol (P4 & P17), xylitol (P10 & P15), sorbose (P1 & P15). Salicilin (except P2, P3, P5, P7, P8, and P13), ONPG (except P1, P3, P4, P5, P7, P8), dulcitol(except P2, P3, P5, P7, P8, P13) and esculin hydrolysis (except P3, P4, P5, P7, P8, P12) were utilised by 11 isolates (64.7%).Sorbitol was utilised by 4 isolates (P14, P15, P16, P17) constituted 23.5%. Mannitol was utilized by 47% of the isolates (P1, P6, P9, P10, P4, P15, P16, P17). Adonitol utilizing ability was observed only in 6 isolates (35.29%), P1, P6, P7, P14, P15, and P17. Isolates P1, P14, P15, and P17 were able to utilize Arabitol accounting for the 23.5% isolate. α -methyl D-glucoside and inositol, utilizing ability was identified in isolate #P1 P14 & P17 and isolate # P14, P16 & P17 Rhamnose and cellobiose were utilized by 29.4 % (P5, P6, P14, P15, P17) and 41.17% (P5, P6, P9, P10, P14, P15, P17) respectively of the total isolates collected. None of the isolates used melizitos as a carbon source. α -methyl D-mannoside was utilized by only P17 isolate. Isolate # P10 & P15 were the only two isolates which utilized the xylitol as carbon source. Ten isolates (P2, P3, P4, P5, P6, P8, P9, P11, P15, P16) utilized by malonate utilization.

4.2.3 Numerical analysis of phenotypic characteristics revealed polymorphism among fluorescent pseudomonas.

The polymorphism among the fluorescent pseudomonas isolates are revealed by the numerical analysis of phenotypic characteristics. All the 17 pseudomonas isolates were grouped into 4 major clusters at 0.67 similarity coefficient level. Among the 17 isolates the similarity coefficient ranged from 0.57 to 0.87. Formed 3 clusters having 2, 6, and 9 isolates respectively.

Tests	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
1	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	-	+
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
3	+	-	-	-	-	+	-	-	+	-	+	-	-	+	+	+	+
4	+	-	+	+	-	+	-	-	+	+	+	+	-	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
6	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
7	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	+	+
8	+	-	-	-	-	+	-	-	+	+	+	-	-	+	+	+	+
9	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
10	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+
12	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
13	+	-	+	+	+	-	-	+	+	-	-	-	-	-	+	-	+
14	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
15	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+
16	+	-	-	+	-	+	-	-	+	+	+	+	-	+	+	+	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
18	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+
19	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
20	+	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	+
21	+	-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	+
22	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
23	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
24	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
25	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+
26	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-	+
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

[illegible]

19. Sorbitol, 20. Mannitol, 21. Adonitol, 22. Arabitol, 23. Erythritol, 24. α -methyl D-glucoside, 25. Rhamnose, 26. Cellobiose, 27. Melizitose, 28. α -methyl D-mannoside, 29. Xylitol, 30. ONPG, 31. esculin hydrolysis, 32. D-arabinose, 33. citrate utilization, 34. malonate utilization, 35. Sorbose, 36. Growth at 4°C, 37. Growth at 42°C, 38. Gelatin liquefaction test, 39. Nitrate reduction test, 40. Amow's assay, 41. Tetrazolium test, 42. Hydroxyquinoline test, 43. TSI agar (acid slant/acid butt), 44. TSI (acid butt/alkaline slant), 45. TSI (bubbles/cracks), 46. TSI (black precipitate), 47. cetrimide agar base test, 48. Oxidase test, 49. Hugh leifson's test, 50. Egg yolk test, 51. Fluorescein test, 52. . Pyocyanin test, 53. Kanamycin sensitivity test 54. Carbenicillin test

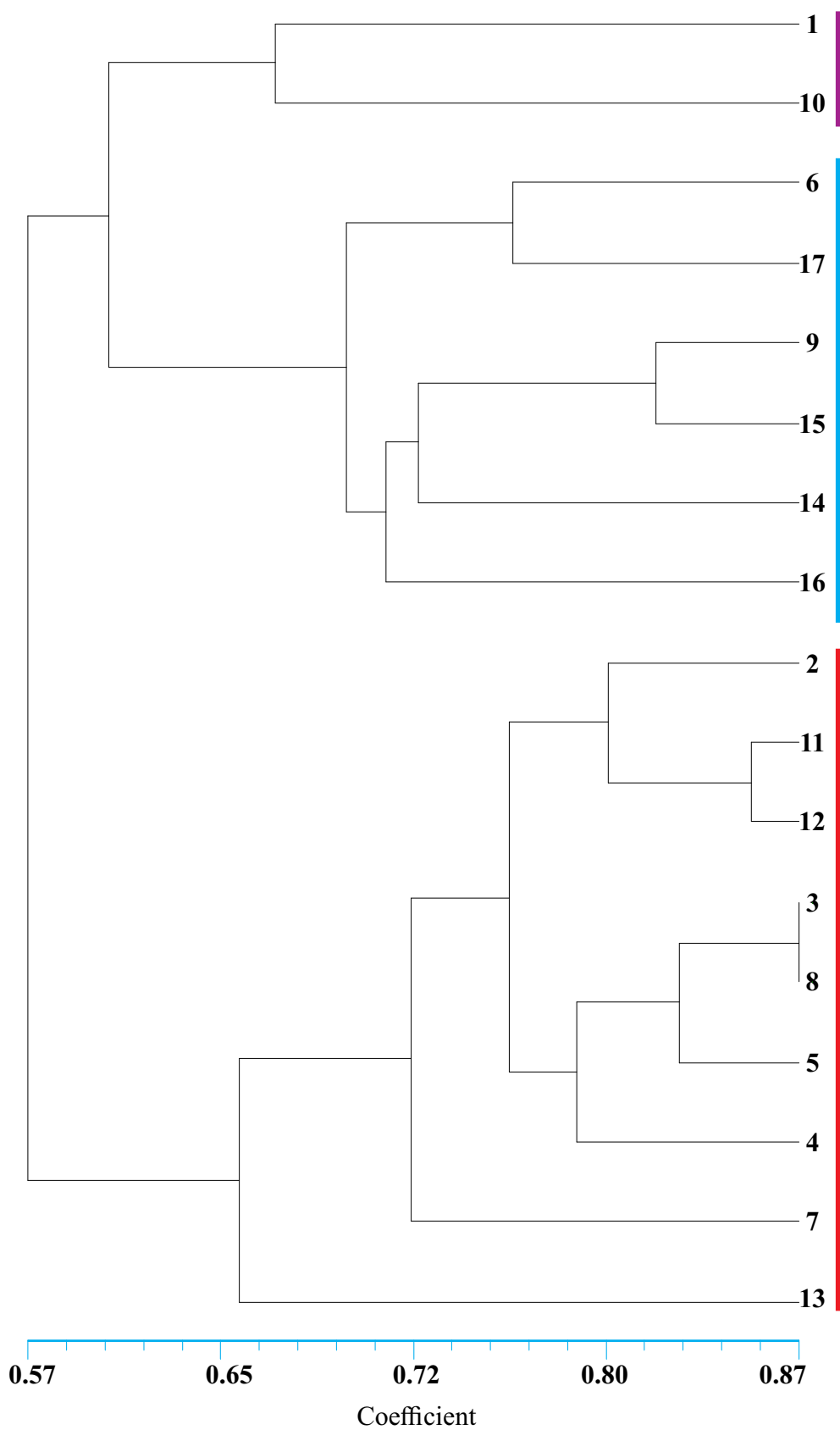


Fig 4.2: Phenogram of 17 fluorescent *Pseudomonas* isolates based on their carbon source utilization profiles and other biochemical parameters.

4.3 Screening for phosphate solubilization

Phosphorus is one of the essential macronutrient for the growth of plants. Mainly performs the functions in the structure, plant metabolism and reproduction. As compared to any other elements an unsatisfactory growth is more often due to the phosphorus shortage. Also it is a vital constituent of every cell. From the soil solution plants take phosphorus as orthophosphate ion, either H_2PO_4^- or $\text{H}_2\text{PO}_4^{2-}$. Capacity to solubilize fixed forms of phosphates through the release of organic acids is another characteristic feature of fluorescent *Pseudomonas*. All the isolates derived from red lateritic soil had the potential to differentially utilizing tri-calcium phosphates and was qualitatively estimated on PVK agar media and quantitatively estimated on NBRIP NBRIY and PVK broth medium.

4.3.1 Qualitative analysis of phosphate solubilisation

The qualitative assessment was done in the pikovskya's agar medium. This medium contains the phosphorus in the form of tri-calcium phosphates. Bromo cresol was used as the pH indicator. The fresh cultures of the isolates were spot inoculated in the plates at the center. After incubation the isolates which having potential to solubilise the phosphate produced an orange color circular zone around the inoculated area by acidification. The highly potential isolates produced large circular area in orange color around point of spot inoculation. By measuring the diameter of the circular zone in orange color, qualitatively we can find which of the isolates are good in solubilizing the phosphates. The isolates P6, P11, P13, P16, and P17 were found to make the large orange colored circular zone. So these isolates are good in phosphate solubilisation ability and the order is $\text{P11} > \text{P13} > \text{P6} > \text{P16} > \text{P17}$. The maximum diameter of the circular orange zone was 24.5 cm and the minimum was 5.75cm.

4.3.2 Quantitative analysis of the phosphate solubilisation (liquid assay)

The quantitative analysis was done in three medium.

- Pikovskya's broth
- NBRIP (National Botanical Research Institute's phosphate growth) medium.
- NBRIY (National Botanical Research Institute's phosphate growth medium devoid of yeast extract) medium.

The isolates were inoculated in these broth media and incubated for 7 days keeping on the shaker. The amount of the inorganic phosphate solubilized by the inoculated *Pseudomonas* isolates was estimated by preparing the standard curve at a wavelength of 610nm. After the screening for the phosphate solubilisation it was observed that the isolates showed variation in the ability to utilize the calcium phosphate supplemented in the medium. The soluble phosphate concentrations was expressed as µg/ml

- In pikovskya's broth medium the tri- calcium phosphate supplemented in different nutrient constituents. The phosphate solubilisation varied from 66.5 to 1328 µg/ml. The lowest was observed for P10 and highest for P11. The % increase over control of the highest valued isolate is 253%. The other isolates which shows good phosphate solubilizing ability are P13 (939.5µg/ml), P6 (979µg/ml), P16 (667.5µg/ml) and P17 (683.5 µg/ml).
- In NBRIY broth medium tri-calcium phosphate was directly supplemented and this medium is devoid of yeast extract. The yeast extract found to decrease the amount of soluble phosphate produced as a result of microbial action. The phosphate solubilisation range varied from 337.5 to 1773 µg/ml. The highest was observed for P11 and the lowest was observed for

P10, as same as obtained in the pikovskya's broth. But the percentage increase over control is too high as compared to the above method. The % increase over the control in the case of the highest phosphate solubilizing isolate was 312.3%. P13 (15845µg/ml), P16 (1215.55µg/ml), P14 (1055.55µg/ml), P3 (1296.55µg/ml), P7 (1215.55µg/ml).

- In NBRIP broth medium the tri-calcium phosphate was directly supplemented and this is a modified form of NBRIY broth medium. This medium was found to be more efficient in showing the phosphate solubilisation . The highest value shown was 1873 µg/ml (P11) and the lowest was 590.5 µg/ml (P10). The % increase over control in this case of highest valued isolate was 338.6%. Every isolates showed phosphate solubilisation ability and some of the other potential isolates were P16 (1215.5 µg/ml), P17 (1163 µg/ml), P6 (1369.5 µg/ml), P7 (1793.5 µg/ml).

P.fluorescens and *P.putida* isolated from red lateritic soil were identified as high phosphate solubilizers and they were displaying a rich array of traits which promote the plant growth (Maroniche, 2016). Three strains of fluorescent *pseudomonas* (BFPB9, FP12, and FP13) out of 80 strains showed high ability to solubilize the tri-calcium phosphate. Later these strains were identified as *P.aeruginosa*, *P.plecoglossicida* and *P.mosseli* (B.K Jha, 2009).

Table 4.2. Phosphate solubilization in three different medium.

isolates	Phosphate Solubilization InPikovskya's	Phosphate solubilization in NBRIY Broth($\mu\text{g/ml}$)	Phosphate solubilisation in NBRIP Broth ($\mu\text{g/ml}$)	Plate assay for phosphate solubilisation
P1	357.5 ^{fg} $\pm$ 1.5	995 ^{gh} $\pm$ 6.5	972 ^{efg} $\pm$ 1	17.2 ^{cd} $\pm$ 0.3
P2	825.5 ^{fg} $\pm$ 11.5	979.5 ^{gh} $\pm$ 6	937 ^{efg} $\pm$ 6	14.3 ^{fg} $\pm$ 0.3
P3	703 ^c $\pm$ 26	1296.5 ^c $\pm$ 8.5	521.5 ^{hi} $\pm$ 4	15.26 ^{ef} $\pm$ 0.26
P4	418 ^d $\pm$ 4.5	1025.5 ^{fg} $\pm$ 4.5	1145.5 ^{de} $\pm$ 4.5	16.5 ^{de} $\pm$ 0.5
P5	624.5 ^d $\pm$ 7.5	1265 ^{cd} $\pm$ 3.5	1516 ^{bc} $\pm$ 4.5	13.75 ^g $\pm$ 0.25
P6	979 ^{de} $\pm$ 114	1074 ^f $\pm$ 5	1369.5 ^{cd} $\pm$ 2	22.5 ^b $\pm$ 0.5
P7	564 ^a $\pm$ 20	1215.5 ^{de} $\pm$ 15	1793.5 ^{ab} $\pm$ 21.5	14.045 ^{fg} $\pm$ 0.515
P8	117 ^{hi} $\pm$ 7	771.5 ⁱ $\pm$ 3.5	590.5 ^{hi} $\pm$ 5.5	11.675 ^h $\pm$ 0.075
P9	569 ^e $\pm$ 1	527 ^j $\pm$ 16.5	1027 ^{ef} $\pm$ 2.5	15.25 ^{ef} $\pm$ 0.25
P10	66.5 ⁱ $\pm$ 1.5	337.5 ^k $\pm$ 6	695 ^{ghi} $\pm$ 2	5.75 ^j $\pm$ 0.75
P11	1328 ^a $\pm$ 2.0	1773.5 ^a $\pm$ 6	1873.5 ^a $\pm$ 2	24.5 ^a $\pm$ 0.5
P12	170 ^h $\pm$ 8	952.5 ^{gh} $\pm$ 2	766.5 ^{fgh} $\pm$ 3	8.815 ⁱ $\pm$ 0.385
P13	939.5 ^h $\pm$ 49.5	1584 ^b $\pm$ 47.5	1153 ^{de} $\pm$ 2.5	24.325 ^a $\pm$ 0.675
P14	569 ^e $\pm$ 15	1289 ^c $\pm$ 55	1055.5 ^{ef} $\pm$ 37	14.88 ^{fg} $\pm$ 0.13
P15	316 ^g $\pm$ 5	384 ^k $\pm$ 2.5	934 ^{efg} $\pm$ 1.5	6.035 ^j $\pm$ 0.285
P16	667.5 ^g $\pm$ 6.5	1215.5 ^{de} $\pm$ 4.5	1641 ^{abc} $\pm$ 3	18.23 ^c $\pm$ 0.73
P17	683.5 ^d $\pm$ 6	1163 ^e $\pm$ 4.5	1734 ^{ab} $\pm$ 4.5	17.5 ^{cd} $\pm$ 0.5
Max.	1328 ^a $\pm$ 2.0	1773.5 ^a $\pm$ 6	1873.5 ^a $\pm$ 2	24.325 ^a $\pm$ 0.675
Min.	66.5 ⁱ $\pm$ 1.5	337.5 ^k $\pm$ 6	590.5 ^{hi} $\pm$ 5.5	5.75 ^j $\pm$ 0.75
CD(0.01%)	127.212	75.113	414.271	1.844
CD(0.05%)	92.867	54.834	302.426	1.342
CV	7.746	2.613	12.857	4.152

Treatments found Significant at 1% and 5% level of significance

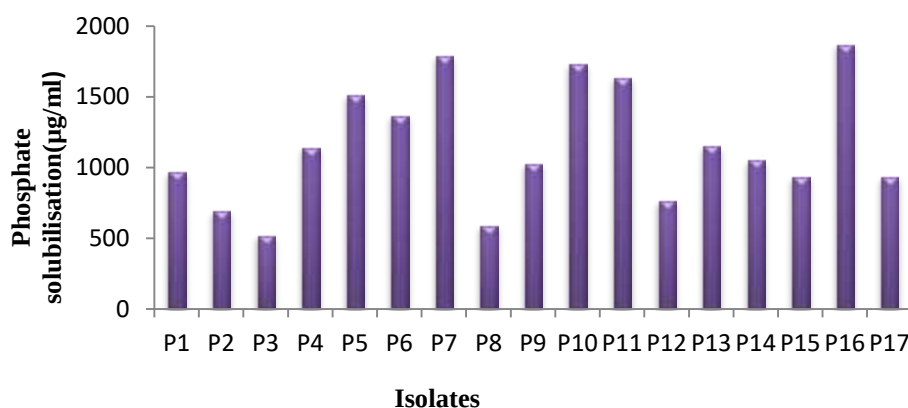


Fig 4.3. Phosphate solubilisation in NBRIP mediumn

4.4 Screening for Indole acetic acid production

Indole acetic acid is one of the most important plant growth promoting hormones. The precursor of IAA is tryptophan. The screening of the 17 isolates for the production of IAA was done in the DF salt minimal media which was supplemented by tryptophan (1.02g/L from 5mM stock solution.). This medium was inoculates with the isolates and one kept as control which contain the same or other precursor molecule. The absorbance was measured at 535nm. The concentration of the IAA produced by the 17 isolates were evaluated by comparing it with a standard curve that was prepared by serial dilution (0-100 µg/ml) using the commercially available IAA. For the quantification of the amount of the IAA produced by different isolates of the fluorescent *pseudomonas* in the medium the colorimeter readings were interpolated with the standard curve. The amount of IAA produced by the isolates ranged from 1.13 µg/ml to 72.905 µg/ml.

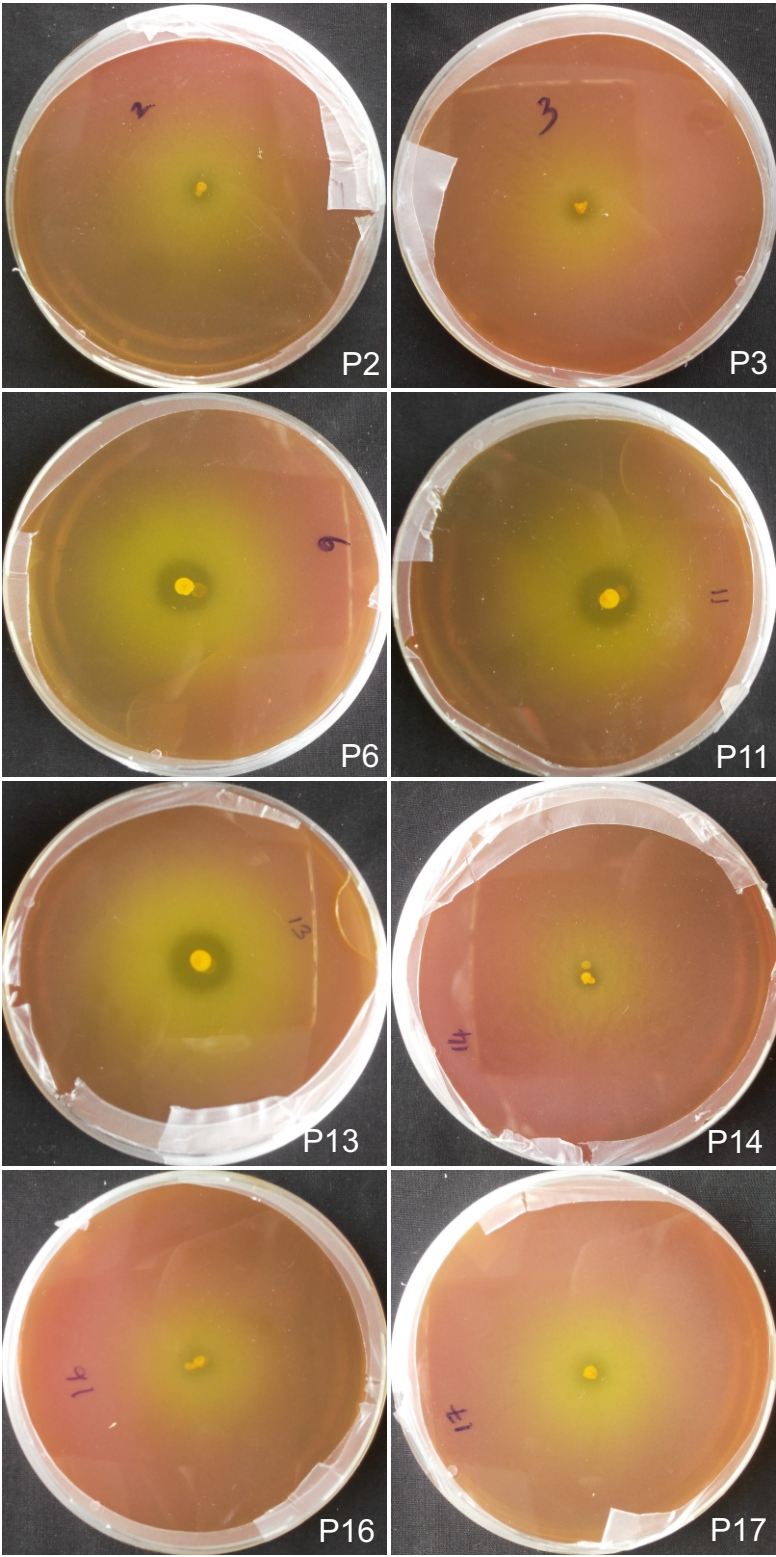


Fig 4.7 PVK agar test for phosphate solubilization

Fluorescent *pseudomonas* isolates collected were identified to increase the nutrient availability to plants and produce indole acetic acid, variety of polyamines and gibberellins (Y Bashan, agronomy, & 2010, n.d.; Namvar & Khandan, 2015b; Thuler, Floh, Handro, & Barbosa, 2003; S. Veresoglou, soil, & 2010, n.d.). 11 isolates of fluorescent *pseudomonas* sp. were isolated from rhizospheric soil and tested for the production of IAA in the tryptophan supplemented media. 5 isolates produced high amount of IAA ranging from 41.0 to 53.2 mg/ml and 6 isolates produced the IAA ranged from 23.4 to 36.2 mg/ml (F.Ahmed, 2005).

Table: 4.3 Concentration of the IAA produced by fluorescent *pseudomonas* isolates.

Sr. NO.	Isolates	IAA Production Conc. (ug/ml)	pH of medium
1	P1	6.495 ^g ±0.227	6.49
2	P2	1.225 ^h ±0.136	7.5
3	P3	0.95 ^h ±0.227	5.59
4	P4	10.956 ^e ±0.091	5.27
5	P5	13.59 ^d ±0.227	4.76
6	P6	1.13 ^h ±0.318	5.29
7	P7	5.405 ^g ±1.818	7.37
8	P8	2 ^h ±0.136	5.53
9	P9	8.765 ^f ±0.136	7.38
10	P10	1.675 ^h ±0.136	6.18
11	P11	5.135 ^g ±0.136	7.24
12	P12	1.675 ^h ±0.318	7.49
13	P13	2.04 ^h ±0.455	6.43
14	P14	11.9 ^e ±0.136	7.13
15	P15	72.905 ^a ±0.136	7.33
16	P16	22.001 ^c ±0.227	5.57
17	P17	69.705 ^b ±0.455	6.41
	Max.	72.905 ^a ±0.136	
	Min.	0.95 ^h ±0.227	
	CD(0.01%)	2.04	
	CD(0.05%)	1.486	
	CV	5.084	

Treatments found Significant at 1% and 5% level of significance

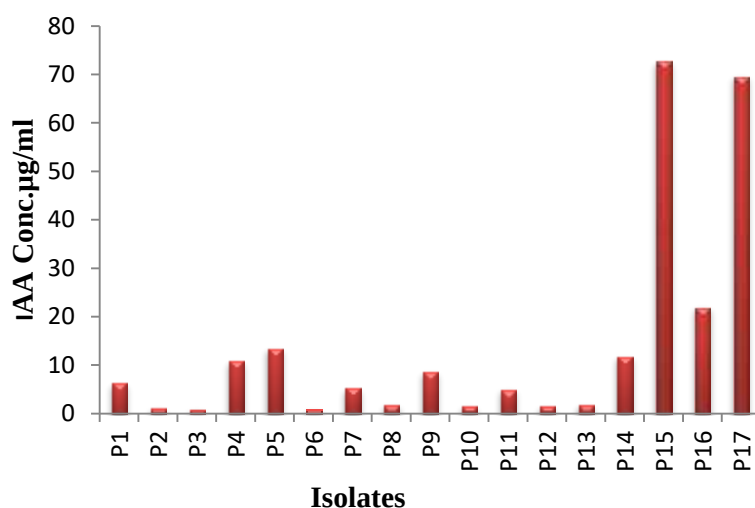


Fig 4.4 Indole acetic acid producing ability of fluorescent *pseudomonas* isolates

4.5 Screening for ACC deaminase producing isolates

4.5.1 Qualitative estimation of ACC deaminase

Ethylene acts as stress hormone in the plants. ACC (1-aminocyclopropane-1-carboxylate) acts as the precursor for the ethylene. So decrease in the amount of ACC will decrease the stress in the plants. Some of the potential plant associated microbes produce ACC deaminase which degrade this ACC. Screening of the isolates for finding the ability to utilize the ACC deaminase was done in the ACC supplemented modified DF salt minimal media. In this ACC was the sole nitrogen source. The plates containing $(\text{NH}_4)_2\text{SO}_4$ served as positive control. The plates contained only the DF salt minimal media without ACC and $(\text{NH}_4)_2\text{SO}_4$ served as the negative control. All the isolates showed growth on the positive control plates and no growth on negative plates. Out of 17 isolates 10 isolates showed growth in the plates in which ACC was supplemented indicates that only those isolates could utilize the

ACC deaminase and degrade the ACC. This activity provides nitrogen source for the growth of the isolates. The isolate which gave the positive results were P1, P2, P5, P6, P10, P11, P13, P14, P15, P17.

4.5.2 Quantitative estimation of ACC deaminase

The isolates that gave the positive result in the qualitative estimation were tested for the amount of ACC deaminase produced by them. The quantitative estimation was done in the DF salt minimal media and the concentration was estimated by comparing with the standard curve that formed by serial dilution with α -keto butyrate. The amount of ACC deaminase produced range from 0.765 $\mu\text{M/ml}$ to 82.235 $\mu\text{M/ml}$. P15 isolate produced maximum amount of ACC and the P14 produced the minimum. 32 isolates of fluorescent *pseudomonas* sp. were screened for the production of ACC deaminase in the medium where ACC was the sole nitrogen source. Only one isolate (Sorge P4) was identified to produce ACC deaminase and produced 3.71 $\mu\text{M/ml}$ of ACC (Ali, 2014). *Pseudomonas fluorescence* biotype G (ACC 5) was identified to produce ACC deaminase enzyme and were inoculated to *Pisum sativum*. These treated ones were induced to produce longer roots led to increased uptake of water under drought stress (Zahir et al., 2008).

Table: 4.4 Concentration of ACCd produced by fluorescent *pseudomonas* isolates

Sr.No.	Isolate	ACCd production
		($\mu\text{M/ml}$)
1	P1	4.02
2	P2	4.882
3	P5	16.447
4	P6	17.824
5	P10	19.822
6	P11	0.765
7	P13	19.588
8	P14	0.176
9	P15	82.235
10	P17	40.765
	Max.	82.235
	Min.	0.176

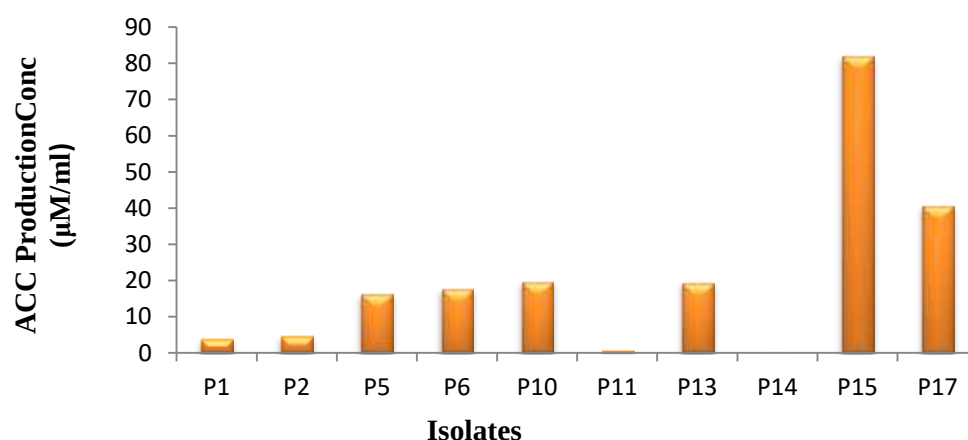


Fig 4.5 ACC deaminase producing ability of selected fluorescent *pseudomonas*

4.6 Screening for Siderophore producing fluorescent pseudomonas

4.6.1 Qualitative estimation of siderophore production

Siderophores are microbial originated iron chelating low molecular weight compounds. The PGPR associated with the plant which produces the siderophores increases the supply of Fe^{3+} to the plants and it enhances the plant growth and production. Also these strong chelates produced by some potential PGPR makes the Fe^{3+} deficient for other soil microbes and inhibit the activity of competitive microbes. The 17 isolates were screened by three qualitative assays. Hydroxylquinoline test, tetrazolium test, Arnow's assay. In hydroxyquinoline test the KMB medium was supplemented with the strong chelating agent 8-hydroxyquinoline. Then the medium was inoculated with the isolates and kept for incubation. Only those isolates will grow in that medium which produce strong iron chelating siderophores because 8-hydroxyquinolin lowers the amount of available iron. The isolates P2, P3, P4, P5, P8, P11, P12, P13, P15, P16 (58.8 %) were able to grow in this medium. All the isolate produced deep red color in tetrazolium test which indicated the presence of hydroximate type of siderophores. The deep red color was produced by the reduction

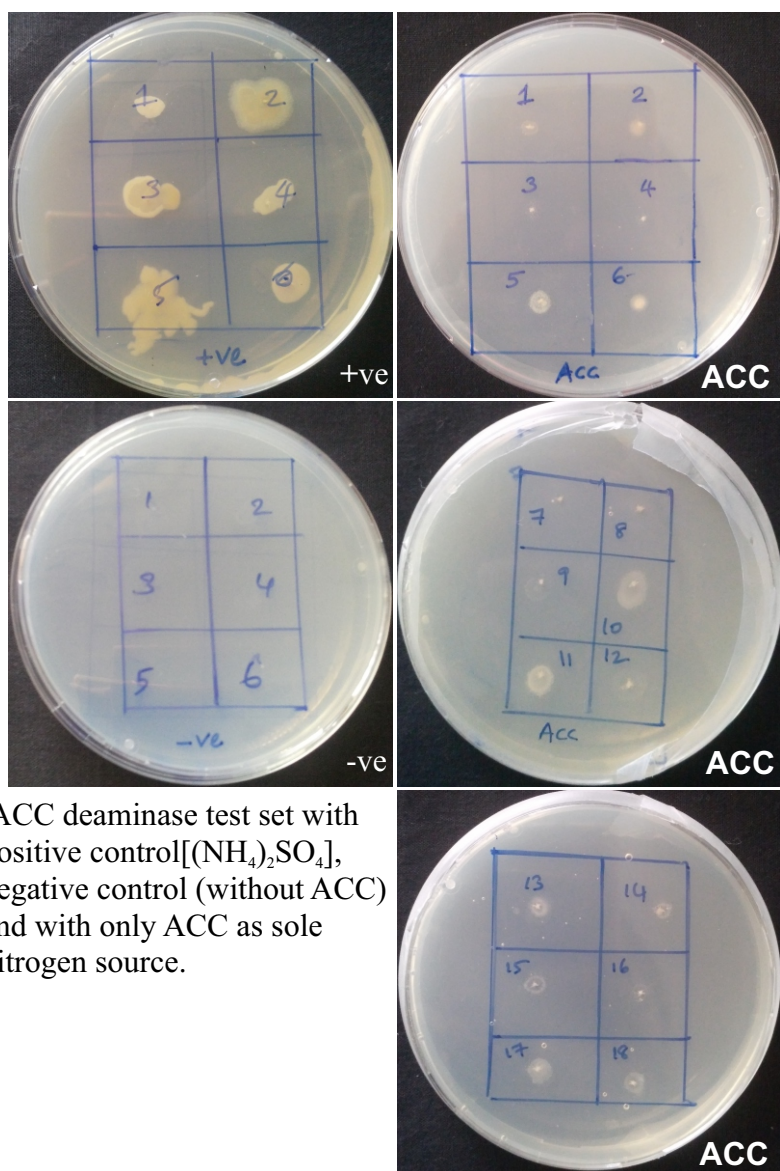


Fig 4.8: ACC deaminase test set with positive control $[(\text{NH}_4)_2\text{SO}_4]$, negative control (without ACC) and with only ACC as sole nitrogen source.

of the tetrazolium salt by hydrolysis of hydroximate group in presence of strong alkali. For detecting the catechol type of siderophore the Arnow's assay was carried out. Only P2 & P14 was showing positive result for this test.

4.6.2 Quatitative estimation of siderophore production

The quantitative estimation was done by CAS assay (Chrome Asurol assay). This is a universal assay for the detection of the siderophores. The percentage of siderophore unit produced was determined by the spectrophotometry. The siderophore units produced range from 5.68% to 67.4%. The isolate P2 produced the highest amount of siderophores and the lowest was by P7. The isolates P1, P2, P10, P12, P14, and P15 produced more than 50% of siderophore units ($P2 > P12 > P10 > P3 > P1 > P15 > P14$).

For increasing the amount of soluble iron for uptake, the microbes produce the siderophores. This increased siderophore production profits the plants and then selected these isolates for enhance the iron availability and increase the plant growth (Carvalhais et al., 2013, Hartmann et al., 2009). The rhizosphere as a plant nutrient enriched site is a highly competitive environment for microbes. Some potential microbes are capable of producing some secondary metabolites which compete with other competitors which have same niche and those metabolites includes mainly the siderophores with antibiotics and toxins (Bais et al., 2006).

Table: 4.5 Quantitative and qualitative estimation of siderophore production by fluorescent *pseudomonas*

Isolates	% siderophore units	Arnow's test	Tetrazoilum test	Hydroxyquinoline
P1	62.6 ^{bc} ±1.671	Negative	Positive	Negative
P2	67.4 ^a ±1.407	Positive	Positive	Positive
P3	65 ^{ab} ±0.483	Negative	Positive	Positive
P4	29.45 ^f ±0.778	Negative	Positive	Positive
P5	29.1 ^f ±0.112	Negative	Positive	Positive
P6	14.15 ⁱ ±0.204	Negative	Positive	Negative
P7	5.68±4.337	Negative	Positive	Negative
P8	15.6 ^{hi} ±1.004	Negative	Positive	Positive
P9	19.95 ^{gh} ±2.749	Negative	Positive	Negative
P10	65.555 ^{ab} ±0.037	Negative	Positive	Negative
P11	33.4 ^f ±1.524	Negative	Positive	Positive
P12	65.7 ^{ab} ±1.325	Negative	Positive	Positive
P13	23.25 ^g ±0.449	Negative	Positive	Positive
P14	56.4 ^{de} ±0.243	Positive	Positive	Negative
P15	58.45 ^{cd} ±0.245	Negative	Positive	Positive
P16	16.25 ^{hi} ±0.847	Negative	Positive	Positive
P17	5.68 ^j ±0.529	Negative	Positive	Negative
Max.	67.4 ^a ±1.407			
Min.	5.68 ^j ±0.529			
CD(0.01)	6.138			
CD(0.05)	4.469			

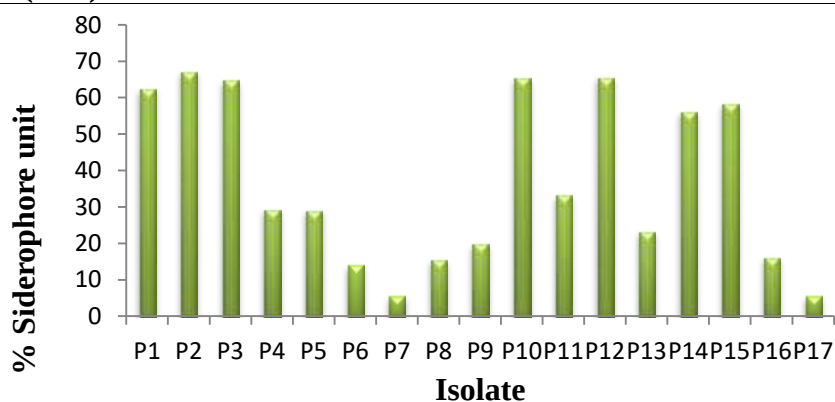


Fig 4.6 Percentage of siderophore units produced by the fluorescent *pseudomonas* isolates.

4.7 Screening of fluorescent *pseudomonas* isolates imparting water stress tolerance

Through the metabolic activities and plant interaction the microbes which inhabit the root system influences the plant growth and productivity. (Berg, 2009; Lutenberg and Kalimova 2009). The change in the structure of bacterial communities that are associated with plant roots towards the selection of assemblages that are adapted with abiotic stress, improve the resistance against those stressors to promote drought tolerance and health of the plants. (Schmidt et al., 2014; Cherif et al., 2015). At the rhizosphere, endosphere and soil around the roots observed variation in the distribution of bacteria as compared to uncultivated soil that are associated with drought – sensitive pepper plant in the desert farming condition. This indicates that there is a selection pressure which is determined by the activity of plants on the structure of microbiome (Marasco et al., 2012).

4.7.1 Identification of the potential fluorescent *pseudomonas* isolates imparting water stress tolerance in Rice

In the present investigation the isolates were collected from the water stressed red lateritic rhizospheric soils and those were screened for imparting water stress tolerance in Rice and wheat. Three cultivars of rice were used namely IR-64 (drought susceptible), B15 (drought tolerant) and RRF-75. In wheat RATAN variety was used. For rice the drought tolerance of treated plants with control was compared both in pot trial under the rainout shelter and field trial. After the seed treatment the rice was sown in the pots as well as in the field. After 15 days stopped watering to the plants in the pots. The plants were observed daily. Within two days observed that the control plants were showing the leaf rolling in three of the varieties, because it couldn't tolerate the water stress and the leaves lost their turgidity. Continued to observe the plants and in three varieties, some of the treatment showed 25 to 75% of leaf rolling within 5 days of the treatment.

The observation continued for 11 days and observed that in three of the varieties some treated plants were still green and was not showing any leaf rolling. In IR-64 variety P1, P2, P4, P5, P6, P7, P9, P10, P11, P12, P13, P15, P17 isolates were identified as potential isolates which imparts the drought tolerance. It constitutes 76.4% of the total isolates. In B-15 the potential isolates which imparted drought tolerance were P4, P5, P6, P7, and P17 (29.4%). In RRF-75 the potential isolated identified were P7, P8, P13, P14, P15, and P16 (35.3%). During the time of field trial there was a period when there was no rainfall for many days. The leaves of the control plants in the field trial were rolled by loss of turgidity. But the plants treated with P1, P2, P6, P12, P14, P15, P16, P17 of IR-64 variety were not showing any leaf rolling even in the 12 pm. In B-15 P12, P15, P16, P17 treated plants and in RRF-75 P8, P13, P14, P15 showed the similar result as in the case of IR-64. The plants that were treated with potential isolates were uprooted and the root imprint was taken in KMB medium for testing whether the isolates were colonized the roots or not. After 2 days of incubation the root imprinted plates were observed and they have shown the root colonization or recovery of those fluorescent pseudomonads isolates.

Pseudomonas fluorescence strain P2 tested for their ability to impart drought tolerance and plant growth promotion under drought stress in rice varieties IR-64 (Drought susceptible), Sahabnagi (drought tolerant). The plants were able to tolerate the drought stress and both the varieties showed higher proline content (Gusain et al, 2015). The PGPR mediated drought tolerance may be because of various mechanisms like increased levels of proline content by PGPR and the increased levels would be better able to tolerate the drought stress (Naseem et al., 2014). The ACC deaminase producing PGPR exerts beneficial effects on plants which help to tolerate the drought stress.

4.7.2 Wheat trial with fluorescent pseudomonas isolates

The wheat variety Ratan was used for the pot trial conducted for identifying the potential fluorescent *pseudomonas* isolates which impart drought tolerance in wheat. The collected 17 isolates were screened in pot trial conducted under rainout



Fig 4.9 Rice plants (derived from seed treatment with fluorescent *Pseudomonas* isolates) expressing tolerance to the water stress

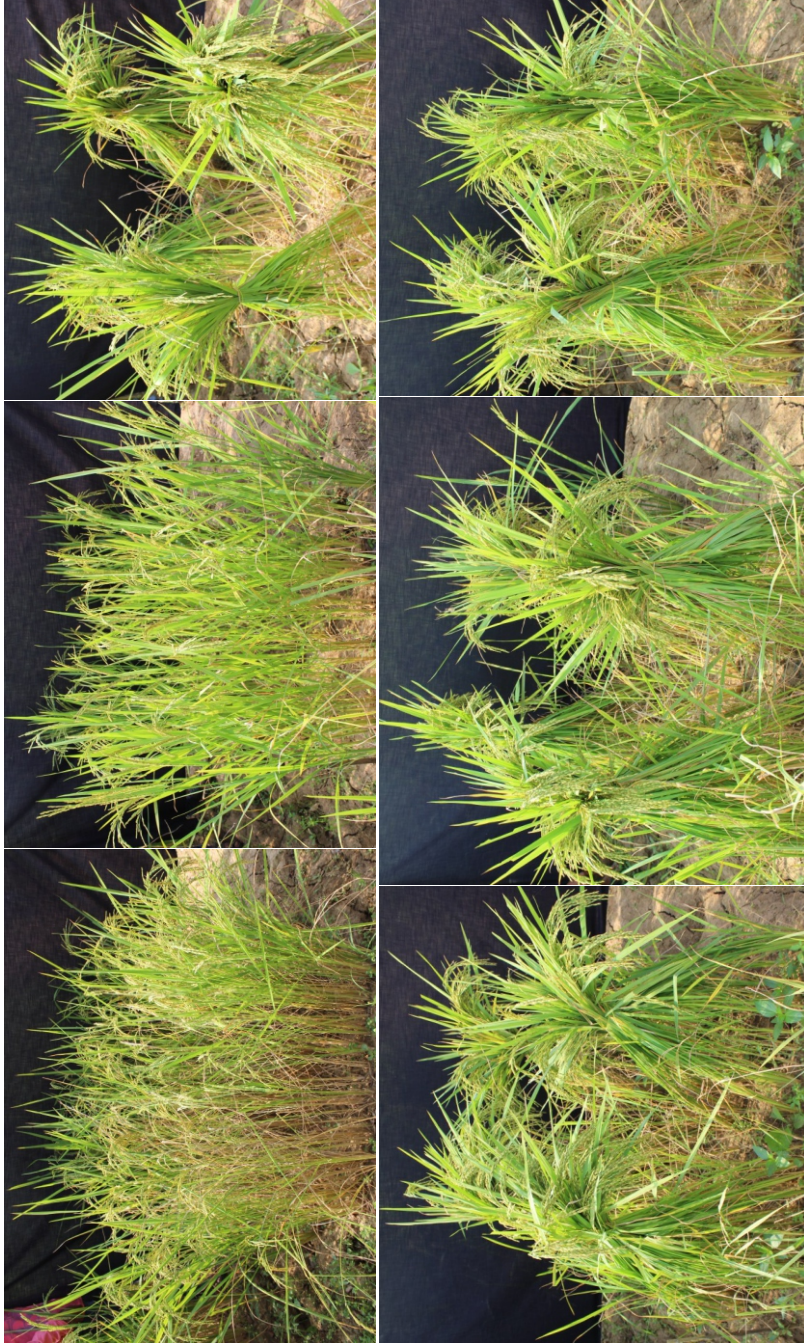


Fig 4.10: Response of the seed treated rice plants towards water stress in the field condition.

shelter. The treated seeds of wheat were sown in the pots in three replicates. The first two replicates were only treated with collected 17 isolates and in the third replicate treated the wheat with the 17 isolates along with the microbial consortia obtained from the root imprint of the rice plants that were managed to survive in water stressed condition. Then after 15 days the watering was stopped and created a water stressed condition. Observation was taken each day. In 3 to 4 days the control plants showed the leaf rolling. In 7 to 8 days some of the treatments were showing 50 to 75% leaf rolling. Observation was taken until 12th day and surprisingly when the control was dried some treated plants were still green and turgid. The treatments that were identified as potential ones were P7, P8, P13, P14, P15, P16 (35.29%). In the pots in which the microbial consortia were incorporated there was no such significant difference from the plants that were only treated with the fluorescent pseudomonas isolates. So it was assumed maybe that microbial consortia was not having any effect in imparting water stress tolerance or maybe it is not having any effect on wheat crop.

The ACC deaminase producing potential plant growth promoting rhizobacteria were identified and screened for the ability to confer the drought tolerance in wheat crop. Lab results of the gas chromatograph revealed that these rhizobacteria lowered the endogenous ethylene levels in the rhizosphere. Those potential PGPR conferred the drought tolerance to wheat plants (*Triticum aestivum* L). (Shakir et al., 2012). Wheat plants showed increase in the thickness of epidermis, ground, phloem and mesophyll tissues, dimensions of vascular bundles of root system when treated with *Azotobacter chroococcum* and *Pseudomonas* sp. resulted in the alleviation of drought stress (El-Afry et al. 2012). The wheat seeds were bio-primed with the plant growth promoting rhizobacteria and drought was induced by stopping watering for 5 to 7 days to 12 days old seedlings. This seed priming with plant growth promoting bacteria alleviated the deleterious effect of the drought stress on wheat (W.A.Kasim, 2013).

During the drought stress plant growth promoting activities of rhizobacteria was reported in maize plants (Vardharajula). Cucumber (Wang et al., 2012), Mung bean

(Sarma and Saikia, 2014), as well during salinity stress in stress in tomato (Mayak et al., 2004), Wheat (Tiwari et al., 2011) and white clover (Han et al., 2014). PGPR induces drought and salt stress tolerance in the plants through elicitation of so-called induced systemic tolerance (IST) process which involves different biochemical and physiological changes (Yang et al., 2009). Kaushal and Wani (2015) has reviewed rhizobacterial induced drought endurance and resilience (RIDER) mechanism, however it was lacking any salinity issues which is a major constraint along with the drought characteristics of drylands and recent work.

Better understanding of what makes a beneficial PGPR interaction with plant would provide an important insight to better handle microbes. Despite the recent progress and perspectives highlighted on microbial mediated drought tolerance in plants, we are still at the beginning of our understanding of PGPR mechanisms imparting drought tolerance in plants. Nevertheless, current progress in the area conveys that future research has great potential to give new insights for sustainable food production.

4.8 The Plant Growth Promoting Activity of the fluorescent *Pseudomonas* isolates on Rice, Wheat, Tomato and Brinjal.

The bacterial endophyte's plant growth promoting ability occurs as a consequence of either direct or indirect mechanisms. When a bacterium facilitates the acquisition of essential nutrients or modulates the level of hormones within the plant it promotes the plant growth directly. The 17 fluorescent *pseudomonas* isolates were screened for the plant growth promoting activity in Rice, wheat, Tomato and Brinjal. For rice and wheat large scale trials were conducted. Tomato and brinjal were grown in pots



Fig 4.11: Wheat plants (derived from seed treatment with fluorescent *Pseudomonas* isolates) expressing tolerance to water stress.

4.8.1 PGPA in Rice and wheat by fluorescent pseudomonas

In present investigation the 17 fluorescent isolates were screened for the imparting plant growth promoting activity in Rice crops. Two varieties namely IR-64 and B-15 were used in the large scale trial. In both the varieties the observation was recorded on shoot length, root length, number of tillers, bundle weight and yield weight. In all aspect the treatment dominated the control plants. In IR variety number of tillers was about double of the control plants. P15 (143.66) showed the highest tiller number. Shoot length in the treated plants ranged from 72.70 cm to 84.143 cm (P16). The length of the superior roots in the treatment showed 11 to 12 cm difference from the control plants. The treated plants also reached maturity early as compared to the treatments. After the harvest the yield was measured by recording the yield weight as well as bundle weight. The maximum bundle weight observed was 0.914kg while in control it was 0.613kg. The yield weight recorded was 0.372kg as the highest among the treatment and 0.179kg for the control plants. B15 also gave better growth as compared to the control plants. The panicle emergence was so early as compared to the control plants. Shoot length and root length of the plants ranged from 97.6 to 131.4cm and 10.3 to 24.267cm respectively. At the initial stage of panicle emergence in treated plants the highest number of panicle emerged was 66 while in control it was only 1. In control plants the tiller number was also less and the average number was 4 and in treatment it was 10. The yield weight and bundle weight ranged from 0.206 to 0.324kg and 0.644 to 0.925kg respectively.

The rice seeds coated with 10 cfu of fluorescent pseudomonas isolates were used in a pot trail to screen the plant growth promoting activity of these isolates. After 45 days the data on shoot length, shoot length and dry weight was analysed and all the strains of fluorescent pseudomonas enhanced the plant growth in the rice plants. *Pseudomonas fluorescens* PW-5 produced maximum shoot length, root length and dry weight (Dashwal et al.,2013). The PGPR *Pseudomonas fluorescens* (PGPR pf) was inoculated into the rice in the nursery and it increased the seed germination, seed vigor and most of the attributes (N.M.Elekhtyar, 2015).

Table: 4.6 Plant growth promoting response of rice (IR-64) following seed bacterization with fluorescent *Pseudomonas* isolates.

Isolate	Shoot length	Root length	Number of tillers	bundle wt.(kg)	yield wt.(kg)
P1	73.177 ^{ij} ±0.052	11.733 ^j ±0.12	102 ^e ±1.732	0.635 ^{gh} ±0.009	0.138 ^k ±0.002
P2	73.8 ^{hij} ±0.084	11.367 ^j ±0.318	95.66 ^{fg} ±1.202	0.746 ^d ±0.009	0.163 ^{ij} ±0.003
P3	75.35 ^{fgh} ±0.252	17.4 ^e ±0.265	98.333 ^{ef} ±1.202	0.648 ^g ±0.008	0.148 ^{ik} ±0.002
P4	78.76 ^d ±0.36	14.2 ^h ±0.173	124.333 ^c ±1.202	0.68 ^f ±0.007	0.174 ^{hi} ±0.003
P5	74.8 ^{fgh} ±0.0581	7.867 ^k ±0.203	91.667 ^{gh} ±3.48	0.65 ^g ±0.013	0.192 ^h ±0.001
P6	80.463 ^c ±0.532	18.933 ^d ±0.26	123.667 ^c ±1.764	0.684 ^f ±0.005	0.176 ^{hi} ±0.002
P7	73.86 ^{hij} ±0.0588	13.43 ⁱ ±0.233	116.667 ^d ±1.732	0.751 ^{cd} ±0.007	0.245 ^g ±0.011
P8	72.70 ^j ±0.726	7.233 ^k ±0.233	90 ^h ±2.404	0.807 ^b ±0.005	0.264 ^{fg} ±0.005
P9	73.8 ^{hij} ±0.239	21.6 ^b ±0.265	114 ^d ±2.646	0.813 ^b ±0.006	0.288 ^e ±0.006
P10	75.07 ^{fgh} ±0.391	20.367 ^c ±0.26	93 ^{gh} ±2.082	0.75 ^{cd} ±0.006	0.313 ^{cd} ±0.007
P11	74.457 ^{ghi} ±0.761	15.833 ^f ±0.203	118.333 ^d ±1.764	0.716 ^e ±0.004	0.333 ^{bc} ±0.005
P12	77.043 ^e ±0.229	15.833 ^f ±0.24	116.667 ^d ±1.453	0.731 ^{de} ±0.006	0.25 ^g ±0.017
P13	76.14 ^{ef} ±0.482	18.5 ^d ±0.231	117.333 ^d ±1.667	0.731 ^{de} ±0.01	0.281 ^{ef} ±0.004
P14	75.257 ^{fg} ±0.174	15.067 ^g ±0.353	113.333 ^d ±0.882	0.771 ^c ±0.011	0.315 ^{cd} ±0.005
P15	82.547 ^b ±0.439	24.133 ^a ±0.186	143.667 ^a ±2.404	0.826 ^b ±0.009	0.372 ^a ±0.014
P16	84.143 ^a ±0.544	23.867 ^a ±0.145	132.667 ^b ±2.603	0.814 ^b ±0.006	0.294 ^{de} ±0.004
P17	80.697 ^c ±0.288	20.333 ^c ±0.296	130.667 ^b ±1.202	0.914 ^a ±0.004	0.341 ^b ±0.006
Control	70.873 ^k ±0.4	11.267 ^j ±0.067	74.333 ⁱ ±1.453	0.613 ^h ±0.006	0.179 ^{hi} ±0.005
CD(0.05)	1.304	0.645	5.152	0.022	0.021
CV	1.03	2.428	2.8	1.769	5.017

Treatments found Significant 5% level of significance

Table: 4.7 Plant growth promoting response of rice (B-15) following seed bacterization with fluorescent *Pseudomonas* isolates.

Isolate	Shoot length	Root length	No. of tillers	bundle wt.	yield wt.	no.of panicle
P1	107.04±3.524	20.133±0.233	7±0.577	0.773±0.004	0.291±0.001	3.333±0.667
P2	97.66±1.202	11.833±0.348	9±1.155	0.771±0.008	0.281±0.002	2.667±0.333
P3	113.7±1.966	17.133±0.186	8±1.155	0.791±0.002	0.284±0.001	17.333±0.882
P4	102.333±2.963	15.367±0.186	8±0.577	0.709±0.002	0.206±0.003	14.667±0.882
P5	107.667±1.453	18±0.115	7.667±0.882	0.738±0.005	0.293±0.003	1.333±0.333
P6	115.66±2.202	15.9±0.208	8±1.155	0.792±0.002	0.317±0.003	31.667±0.882
P7	108.333±4.177	10.3±0.173	9±0.577	0.703±0.003	0.279±0.001	2±0.577
P8	104.667±1.764	19.6±0.321	7.333±0.667	0.778±0.006	0.321±0.001	11.333±0.882
P9	104.41±2.262	14.2±0.115	6.667±0.333	0.673±0.009	0.284±0.002	16.667±0.882
P10	117±0.577	15.4±0.265	8±0.577	0.757±0.002	0.281±0.001	26.333±1.202
P11	118±1.155	13.4±0.208	8±1.155	0.759±0.001	0.279±0.001	42±1.157
P12	119.667±0.882	17.767±0.145	6.333±0.333	0.65±0.008	0.255±0.003	33.333±1.202
P13	121.667±0.882	22.133±0.367	8.333±0.333	0.644±0.003	0.243±0.003	24.667±1.453
P14	123.667±1.202	18.867±0.203	8±1.155	0.703±0.002	0.283±0.001	56.333±1.453
P15	129±2.082	24.267±0.185	10±0.577	0.925±0.006	0.324±0.001	97±1.155
P16	131.433±1.955	22.2±0.116	9.667±0.333	0.778±0.002	0.298±0.003	66.333±0.882
P17	126.367±0.857	22.833±0.405	7±0.577	0.8±0.01	0.281±0.002	52.333±1.202
CONTROL	88.5±1.041	10.667±0.273	4±0.577	0.61±0.005	0.206±0.004	1±0.667
CD(0.05)	5.735	0.664	1.792	0.015	0.007	2.590
CV	3.05	2.323	13.887	1.222	1.496	5.616

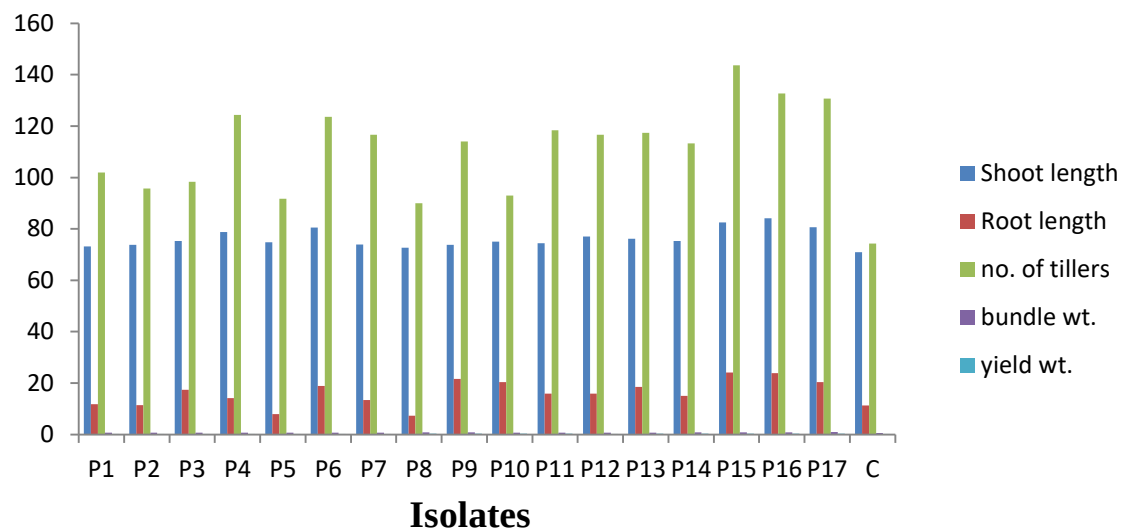


Fig 4.12 Plant growth promoting response in rice (IR-64) by fluorescent *Pseudomonas* isolates.

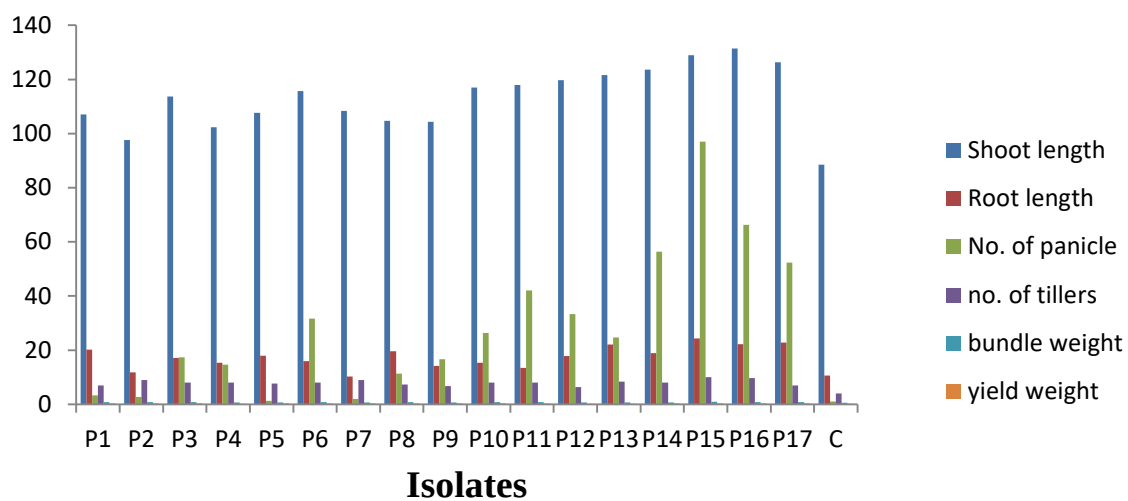


Fig 4.13 Plant growth promoting response in rice (B-15) by fluorescent *Pseudomonas* isolates.

4.8.2 PGPA in Wheat crop by fluorescent pseudomonas

Large scale trial was conducted for the wheat and screened all 17 isolates for the growth promotion in wheat plants (var.-Ratan). The panicle emergence was 5 days earlier than the control and plants number of panicle in the treatment ranged from 60 to 114 and in control only 40. In case of length of the panicle only 10 treatments showed prominent increase over control. The highest panicle length observed was 8.733cm (P7). Shoot length and root length also showed difference of 28 and 21cm respectively. That is 43.75% and 77.7% increase over the control plants. The plant growth promoting rhizobacteria were isolated and biochemically characterized and identified the potential isolates which gave positive result for nitrogen fixation and phosphorus solubilisation.

These PGPR increased the plant height, grain yield, straw yield and test weight in wheat by 17.5%, 79.8%, 78.6%, and 26.7% respectively in pot condition. Also 29.4%, 27.5%, 29.5% and 17.6% under the field condition. (A.Kumar et al., 2014). *Pseudomonas* sp. including the *Pseudomonas fluorescens* 153, *P.fluorescens* 169, *P.putida* 4 and *P.putida* 108 were tested for their IAA production ACC deaminase production and screened potential isolates on wheat under field and green house condition significantly increased the growth and yield. The yield increased by 58 and 37% under greenhouse and field conditions respectively (H.R, Zabihi, 2011).

Table: 4.8 Plant growth promoting response of wheat following seed bacterization with fluorescent *Pseudomonas* isolates

Isolate	Shoot length	Root length	No.panicle	panicle length
P1	84.667±0.581	42.2±0.173	81.667±0.882	8.433±0.12
P2	90.433±0.441	48.1±0.264	88.333±4.41	8±0.12
P3	88.067±0.636	41.867±0.203	92.333±1.764	8.107±0.052
P4	87.667±1.213	36.5±0.289	70±2.517	8.8±0.153
P5	86.733±0.742	43.233±0.145	53.333±1.202	7.833±0.12
P6	90.133±1.877	39.6±0.321	82.333±0.882	8.667±0.41
P7	82.933±0.533	30.867±0.448	61±1.155	8.733±0.318
P8	86.067±1.156	32.233±0.285	100±0.577	8.2±0.153
P9	81.4±0.199	40.633±0.376	60.333±0.882	7.4±0.12
P10	76.133±1.313	29.867±0.448	64.333±2.603	7.38±0.061
P11	78.067±0.769	37.1±0.153	66.667±0.882	7.047±0.224
P12	78.8±1.007	44.067±0.26	83.333±2.404	7.32±0.204
P13	83.5±1.041	22.633±0.41	64.667±2.906	8.1±0.208
P14	84.133±0.405	42.833±0.273	76.667±5.696	7.867±0.033
P15	92.067±0.371	41±0.289	101.333±1.856	8.647±0.079
P16	88.4±0.643	36.967±0.549	108±4.041	8.553±0.202
P17	86.267±0.941	43.833±0.841	114.333±1.453	8.467±0.088
CONTROL	64±2.082	27.767±0.219	49.667±5.044	7.167±0.601
CD(0.05)	17.333	8.117	7.990	0.67
CV	12.673	12.866	6.112	5.026

Treatments found Significant at 5% level of significance

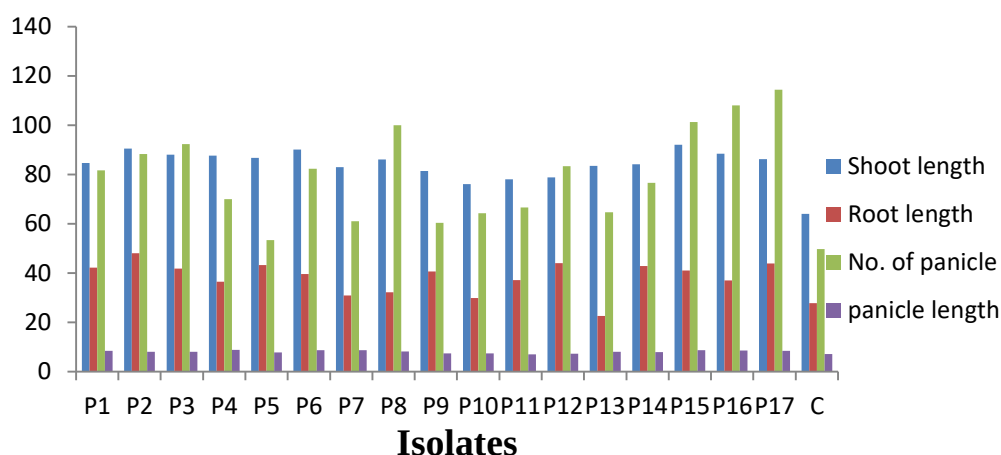


Fig 4.14 Plant growth promoting response in wheat by fluorescent *Pseudomonas* isolates

4.8.3 PGPA in Tomato and Brinjal by fluorescent *pseudomonas*

The vegetable crops tomato and brinjal were screened by the collected 17 fluorescent *pseudomonas* isolates for the Plant Growth Promoting Activity. The experiments were conducted as pot trial. For each crops three replications of treatments and control were made. The treatments were superior to the control in all aspects. In tomato trail some of the treatments even showed early flowering as compared to the control. There was distinct difference in root length, shoot length and root volume between the treated plants and control plants. In case of the length of the roots it varied from 22.5 to 33.16 cm and volume from 1.57 to 4.09cm. The shoot length increased 84.4% over the control. The trial carried for the brinjal to evaluate the PGPA by these collected isolates and the result showed a superior result as compared to the control. The shoot length, root length increased 23.5 and 18.26cm respectively from the control plants. The highest root volume was 15.0.27cm³ while the control was only 2.21cm³. The seed bacterization of tomato by *Pseudomonas fluorescence* CHA0 promoted the germination and plant growth (Z.O.Ardebili, 2011). The plant growth promoting activity of *Pseudomonas* sp. DW1 was evaluated on eggplant



Fig 4.15 Increase in the root length of rice plants (derived from seed treatment) as compared to control plants.



Fig 4.16 Increase in the root length of wheat plants (derived from seed treatments) as compared to the control plants.

(*Solanum melongana* L.). The inoculated plants as compared to the control plants grew to a significantly greater extent (Q. Fu, 2010).

Fluorescent *Pseudomonas* are abundant in rhizosphere of many plants. It have large capacity for the production of phytohormones, mainly auxins (Pattern and Glick 2002) and many secondary metabolites like antibiotics (Bergsma-Vlami et al.,2005), thus they are able to improve plant growth and plant health (Belimov et al.,2009).

Table: 4.9 Plant growth promoting response of tomato following seed bacterization with fluorescent *Pseudomonas* isolates (pot experiment)

Isolate	Shoot length	Root length	Root volume
P1	21.333±1.764	22.733±1.362	2.203±0.313
P2	39.667±2.848	22.5±0.5	2.203±0.313
P3	43±0.577	24.5±1.155	2.203±0.313
P4	48±1.155	29±0.866	3.464±0.634
P5	49±2.887	28.1±1.815	2.203±0.313
P6	50±2	25.5±1.362	4.097±0.317
P7	50.667±1.202	24.567±1.462	1.577±0.313
P8	35±2.646	29.433±2.034	2.203±0.313
P9	37±0.577	26.9±1.595	3.147±0.317
P10	40.667±1.764	25.933±0.745	3.147±0.317
P11	46±2.309	28.467±2.034	3.147±0.317
P12	45±1.732	23.5±2.082	2.203±0.317
P13	35±1.732	24.033±1.417	1.89±0.313
P14	29.667±1.202	29.2±1.528	1.577±0.313
P15	41.667±0.882	33.167±1.453	4.097±0.317
P16	39.333±0.333	24.467±1.184	2.203±0.313
P17	37.333±1.764	22.267±0.906	3.463±0.313
CONTROL	27±0.577	11.1±0.458	0.63±0.313
CD(0.01)	6.007	5.66	1.197
CD(0.05)	4.475	4.216	0.892
CV	6.786	10.044	21.188

Treatments found Significant at 1% and 5% level of significance

Table: 4.10 Plant growth promoting response in
brinjal by fluorescent *Pseudomonas*
isolates. (pot experiment)

Isolate	Shoot length	Root length	Root volume
P1	32.333±0.882	21±0.289	6.183±0.883
P2	34±1.528	22.267±0.96	6.183±0.883
P3	36±0.577	19.433±0.581	11.493±0.883
P4	38.833±0.441	22.333±1.014	8.837±0.887
P5	40.333±1.453	21.167±0.726	7.067±0.883
P6	44.333±1.202	23.5±0.764	6.183±0.883
P7	36.333±0.882	20.667±0.601	6.183±0.883
P8	40±1.528	31.167±1.202	9.723±0.887
P9	41±1.528	22.833±0.726	6.183±0.883
P10	35±0.577	23.433±0.933	6.183±0.883
P11	40±1.528	21.667±0.726	4.417±0.883
P12	41±1.528	30.167±1.764	6.183±0.883
P13	35±0.577	25.433±1.213	15.027±0.883
P14	39±0.577	31.467±1.55	9.723±0.887
P15	36.333±0.577	23.667±1.302	15.027±0.883
P16	46.333±0.882	26.567±1.551	9.723±0.887
P17	41±1.155	30±0.794	8.857±0.877
CONTROL	22.867±1.618	12.933±1.386	2.21±0.44
CD(0.01)	4.419	3.065	3.279
CD(0.05)	3.291	2.283	2.443
CV	5.253	5.765	19.284

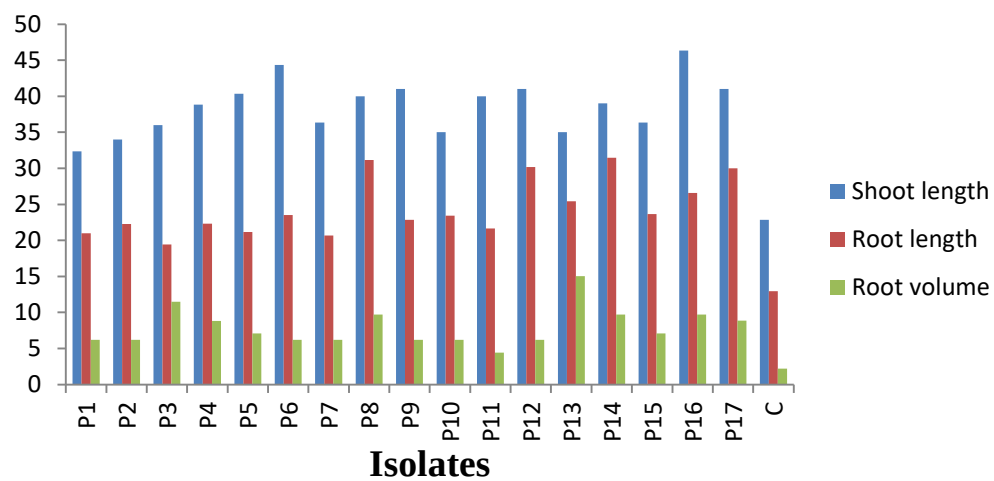


Fig 4.17 Plant growth promoting response in tomato by fluorescent *Pseudomonas* isolates.

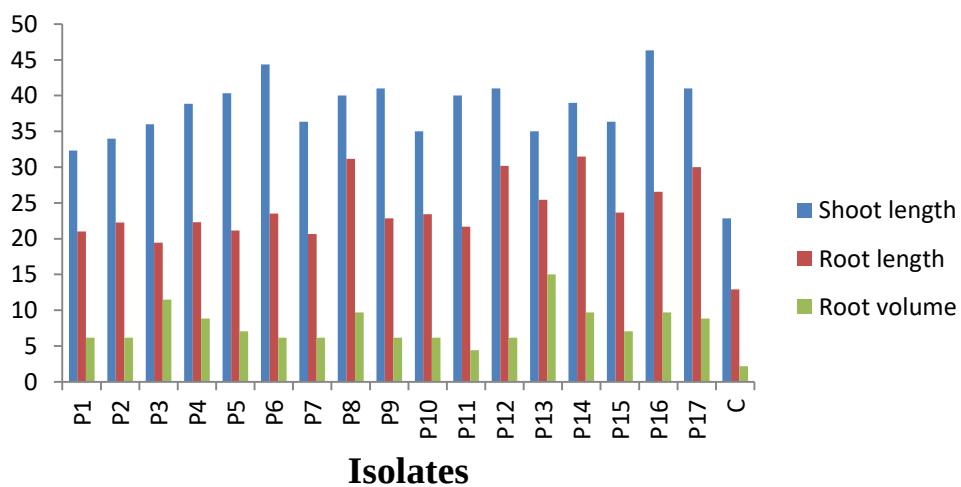


Fig 4.18 Plant growth promoting response in brinjal by fluorescent *Pseudomonas* isolates



Fig 4.19 Plant growth promoting effects of fluorescent *Pseudomonas* isolates on tomato seedlings.

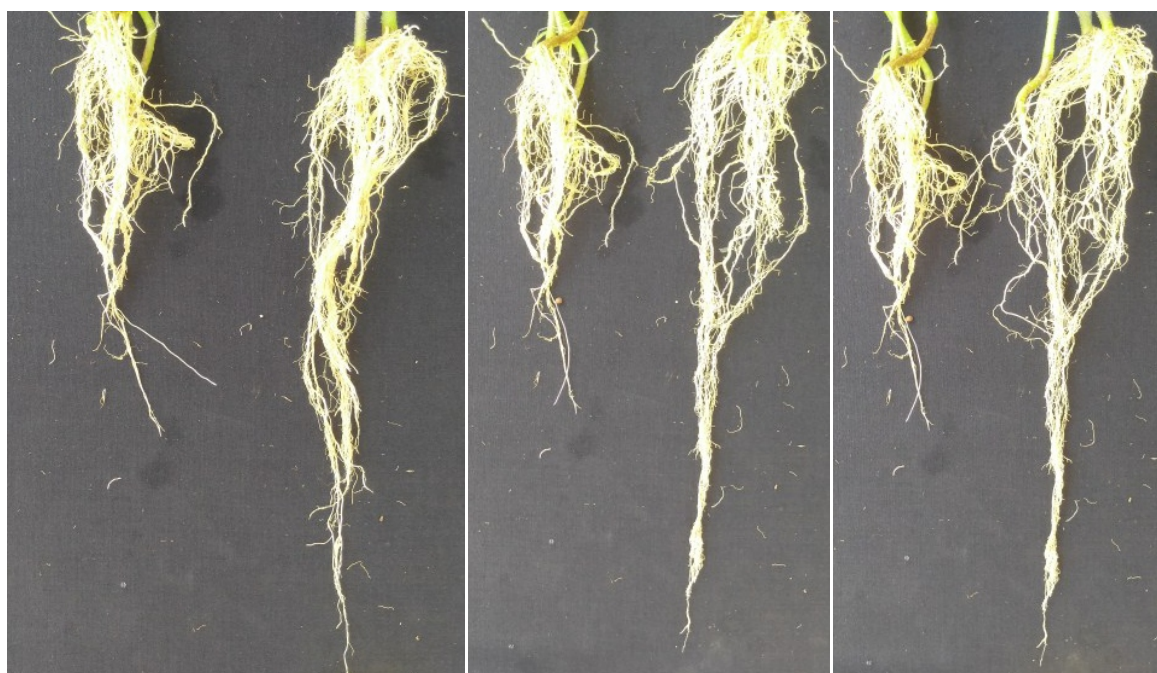


Fig 4.20: Seed bio-priming with fluorescent *Pseudomonas* isolates on tomato seedlings affecting root system of tomato plants.



Fig 4.21 Plant growth promoting effects of fluorescent *Pseudomonas* isolates on brinjal seedlings

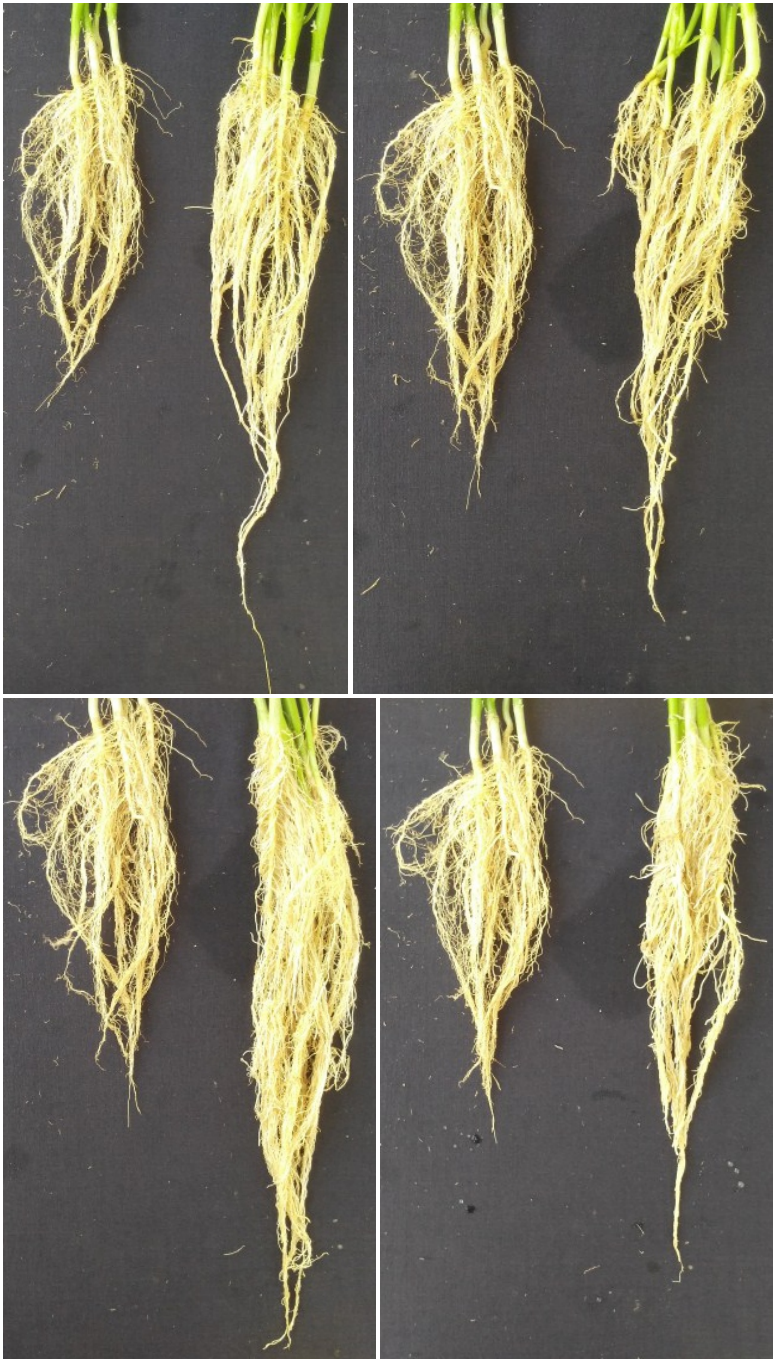


Fig 4.22 Seed bio-priming with fluorescent *Pseudomonas* isolates on brinjal seedlings affecting root system of brinjal plants.

4.9 Molecular characterization of fluorescent *pseudomonas*

16S rRNA gene amplification and REP, BOX and ERIC based genotypic analysis

To confirm the strains as fluorescent *pseudomonas*, rRNA intervening sequence specific primers 27F and 1492 R were used to get an amplicon size 1500bp. The PCR with ERIC, REP and BOX primers showed amplification and high polymorphism was seen for these primers. This polymorphism in the bands obtained indicated the diversity of the collected isolates.

Based on the similarity in sequence the isolates were designated as *Pseudomonas aeruginosa*, *P. monteilii*, *P.plecoglossicida*, *P.mosselii*, *P.libaniensis*, *P.aeuriginosa*. Through the different genomic DNA fingerprinting techniques the isolates belonging to same group were differentiated. These techniques includes repetitive extra genic palindrome (REP), randomly amplified polymorphic DNA (RAPD), enterobacterial repetitive intergenic consensus (ERIC), amplified ribosomal DNA restriction analysis (ARDRA), bacterial repetitive BOX elements (BOX). The rep- PCR generated fingerprinting pattern and the genetic diversity observed among the isolates revealed that the fluorescent *pseudomonads* associated with the rhizosphere of rice was *P.plecoglossicida* (Rameshkumar et al., 2014).

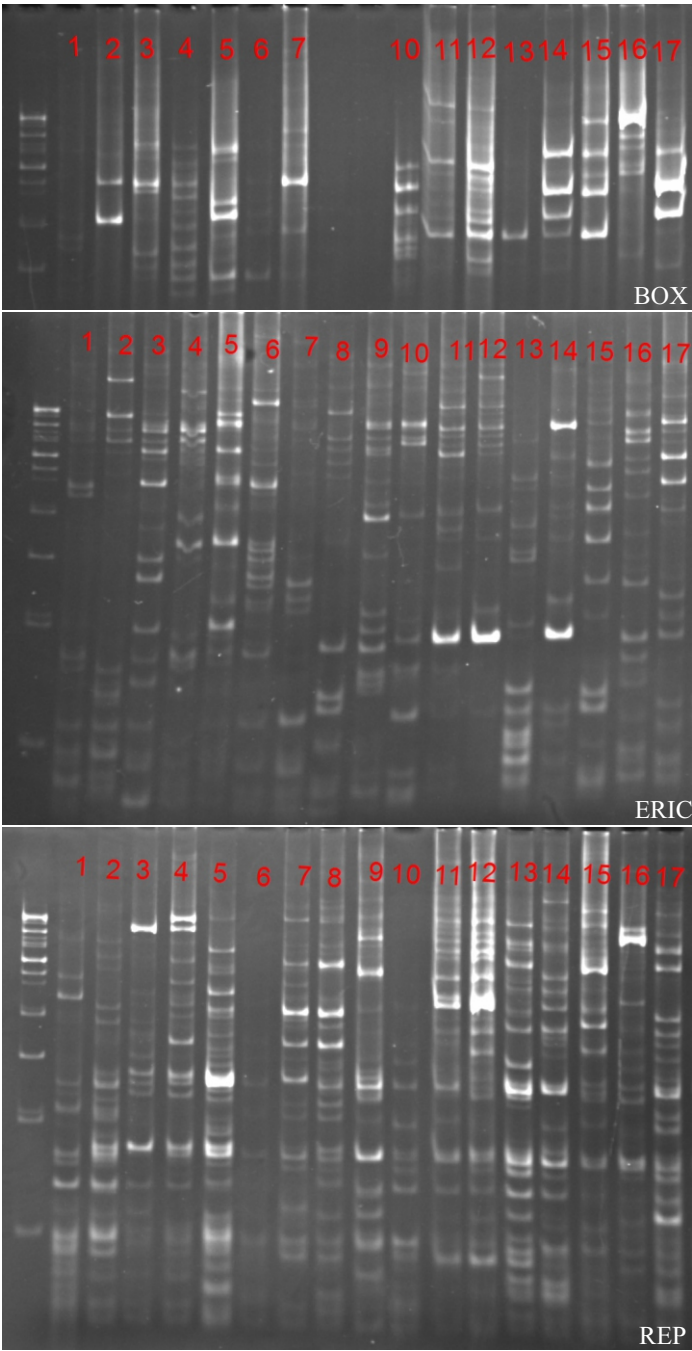


Fig 4.22: PCR analysis of 17 fluorescent *Pseudomonas* isolates generated through REP, ERIC and BOX primer's

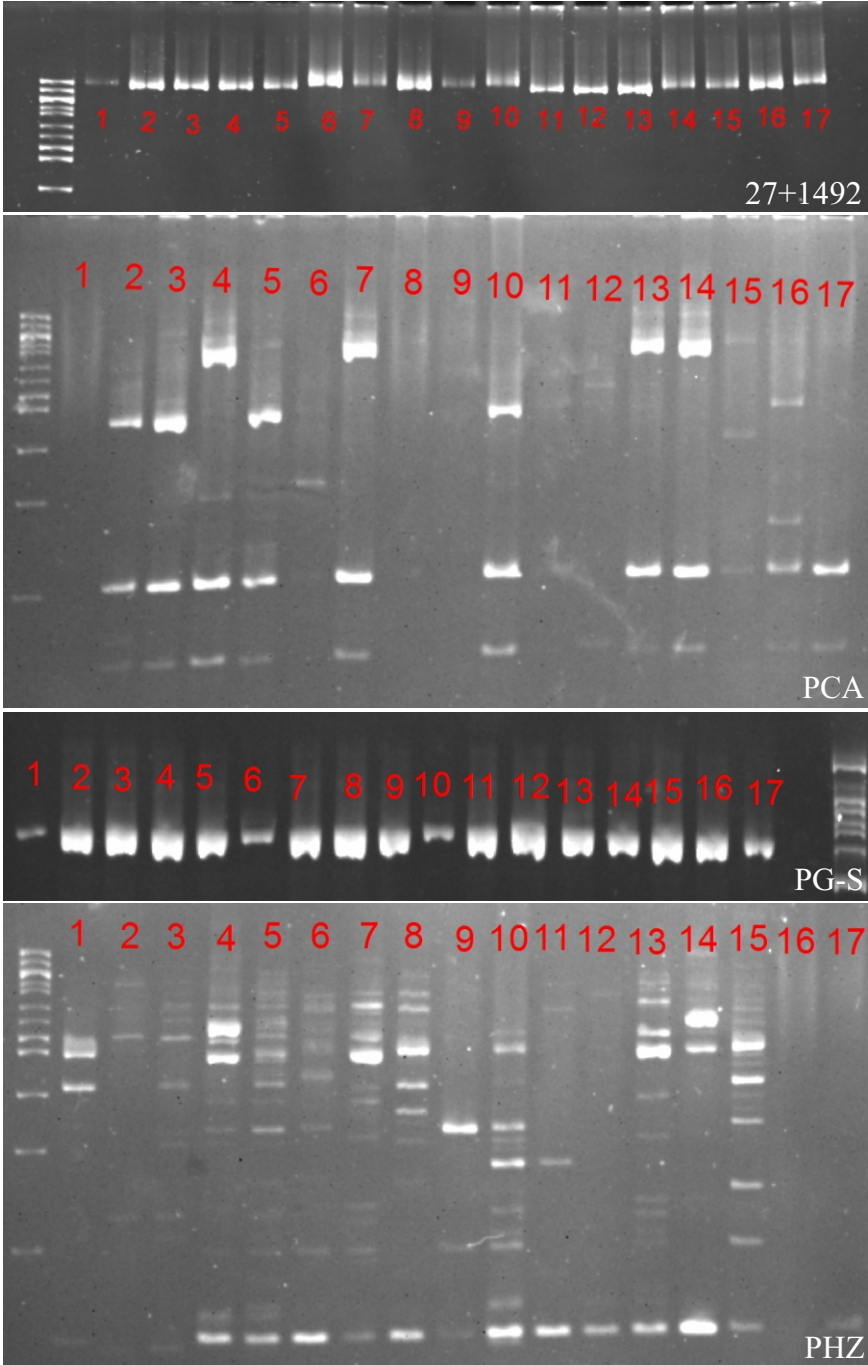


Fig 4.23: PCR analysis of 17 fluorescent *Pseudomonas* isolates generated through 27+1492 , PG-S, PCA and PHZ primers

4.10 Efficacy of bio-control activity fluorescent *pseudomonas* *in vitro*

The efficacy of bio-control activity of collected fluorescent *pseudomonas* was studied against the fungal pathogens *Sclerotium rolfsii* and *Rhizoctonia solani*. For this dual culture equal quantity of KMB and PDA was mixed and poured in the plates. The pathogens were inoculated at the center of the plates and incubated at $25\pm 2^{\circ}\text{C}$ for 3 days. The bipartite interactions were done using the method of confrontation assay in which glass the funnel technique was made use of (Kotasthane, Agrawal, Zaidi, & Singh, 2017). Around the pre-inoculated fungal pathogen the bio-agent (fluorescent *pseudomonas*) was deposited by the edge of the glass funnel. After incubation for 3 day at $28\pm 2^{\circ}\text{C}$ the inhibition zone was measured. There was variation in the efficacy of bio-control activity within the isolates. All the isolates showed antagonistic activity towards *S.rolfsii* in varying range. P1, P2, P3, P12, P14, P15 showed inhibition percentage above 50 and varied range from 37.7 to 52%. In this highest inhibition percentage was shown by P14 (62%). In case of *R.solani* P1, P2, P10, P15, P16 showed above 50% inhibition and the range varied from 22.1 to 64.4%. P2 showed highest percentage of inhibition.

The application of the fluorescent *Pseudomonas* to the soil or seed provides excellent control against plant pathogens (Lagzian et al. 2013; De La Fuente et al. 2006). *Pseudomonas* spp. produce antimicrobials, phenazines, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, siderophores and 2,4-diacetylphloroglucinol (DAPG) (Weller 2007; Thomashow and Weller 1991). The common antibiotic compounds which secreted by the microbes includes hydrogen cyanide, oomycin A, 2,4- diacetylphloroglucinol (DAPG) and phenazine (Loon et al.) These antibiotics are important for the suppression of pathogens in soils (Raaijmakers et al., 2012), e.g. phenazines produced by *Pseudomonas chlororaphis* against *Fusarium oxysporum* (Davide Bulgarelli et al., 2013; Pierson et al., 2010, n.d.; Thomashow et al.). For instance some microbes are able to suppress the soil borne

pathogen *Rhizoctonia solani* (Mendes et al., 2011), including *Pseudomonas* spp. which secrete phenazine-1-carboxylic acid and 2,4-DAPG (Raaijmakers et al., 1997). The production of lipoproteins by *Pseudomonas* and *Bacillus* spp. can also inhibit growth of a wide range of pathogens (Raaijmakers et al., 2010, n.d.; Watrous et al., 2012, Zachow et al., 2015). Several lines of evidence also indicate that plant growth promoting bacteria have inhibitory effects against phytopathogens.

Table: 4.11 Inhibitory effect of fluorescent *Pseudomonas* isolates against *R. solani*

Sr.NO.	Isolate number	Inhibition %
1	P1	63.15
2	P2	64.4
3	P3	42
4	P4	37
5	P5	22.3
6	P6	24
7	P7	22.2
8	P8	22.4
9	P9	22.1
10	P10	51
11	P11	22
12	P12	23
13	P13	43
14	P14	22.2
15	P15	52
16	P16	50
17	P17	22.2

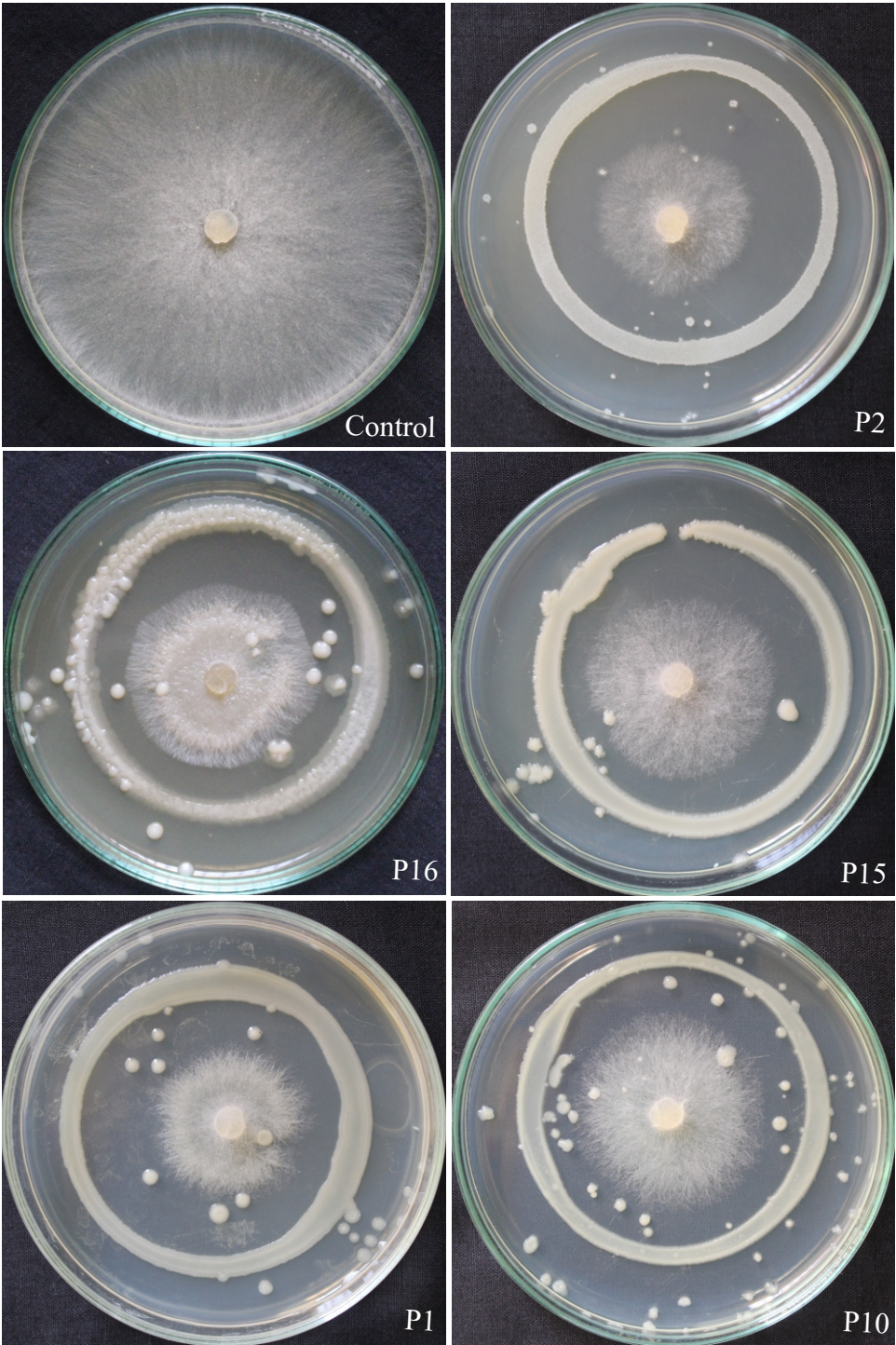


Fig 4.25: Confrontation assay to evaluate the efficacy of different fluorescent *Pseudomonas* to suppress the growth of *R. solani*

Table: 4.12 Inhibitory effect of fluorescent *Pseudomonas* isolates against *S. rolfsii*

Sr.NO.	Isolate number	Inhibition %
1	P1	55.55
2	P2	56
3	P3	55.55
4	P4	45.5
5	P5	46.66
6	P6	47.5
7	P7	48
8	P8	48.88
9	P9	47.88
10	P10	37.77
11	P11	38
12	P12	53.33
13	P13	46
14	P14	62
15	P15	51.11
16	P16	40
17	P17	44.44

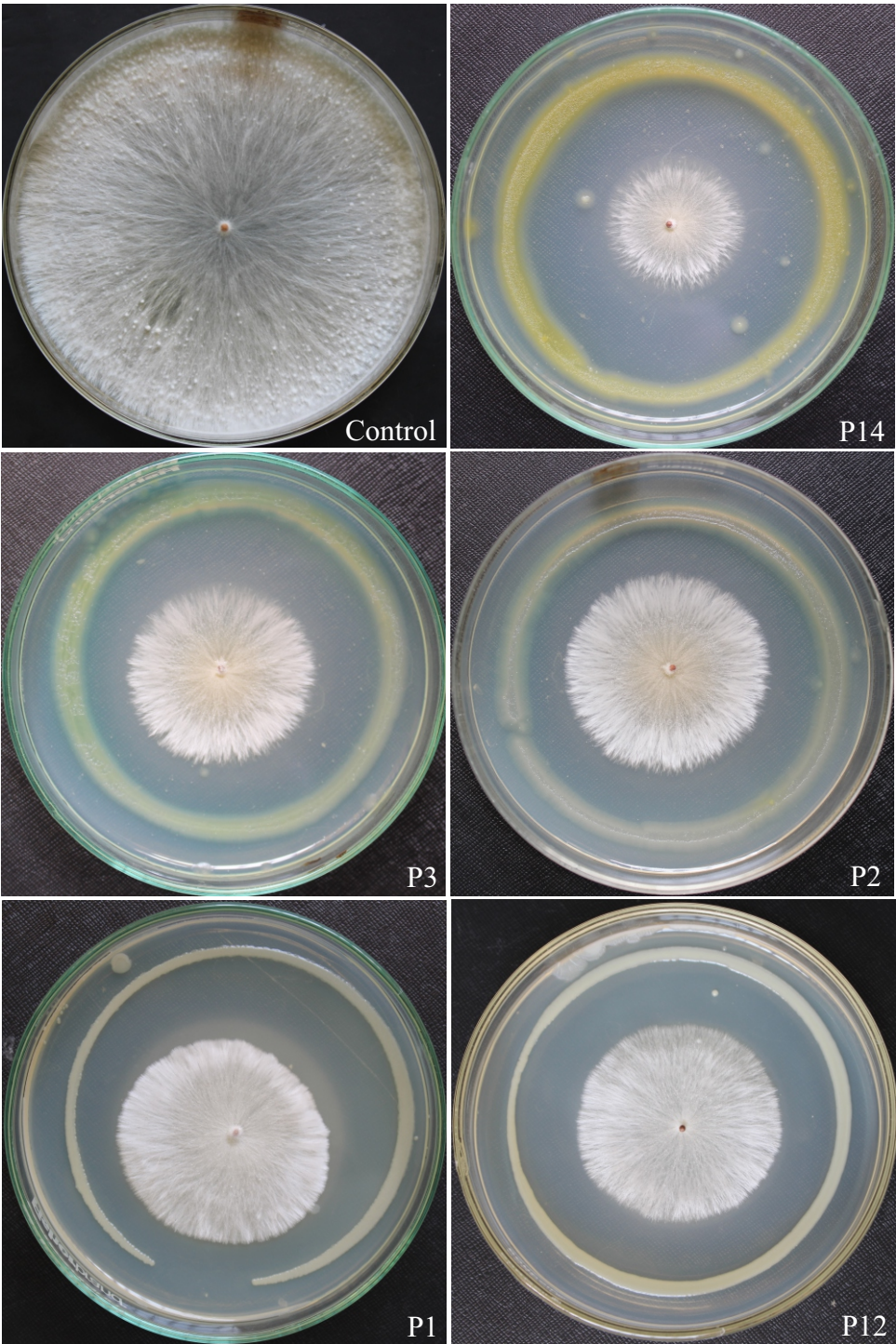


Fig 4.26: Confrontation assay to evaluate the efficacy of different fluorescent *Pseudomonas* to suppress the growth of *S. rolfsii*

CHAPTER –V

SUMMARY AND CONCLUSIONS

Fluorescent pseudomonades are aerobic gram negative bacteria that are ubiquitous in the cultivated soils. They are colonised in the rhizospheric region and are well adapted to growing in the rhizosphere. They have wide range of mechanisms make them well suited for as plant growth promoting and bio-control agents. These includes the ability to 1) utilise the seed and root exudates rapidly, 2) rapidly grow *in vitro* 3) produce large number of bioactive metabolites (plant growth promoting substances, antibiotics, siderophores and volatiles), 4) compete very aggressively with other microbes 5) adapt to environmental stresses. Also these pseudomonads are responsible for the natural suppressiveness of some soil borne pathogens (Weller et al., 2002). Because of the catabolic versatility, capacity to produce a wide range of enzymes and metabolites that helps the plant to withstand biotic and abiotic stresses fluorescent *pseudomonas* have received global attention (Vivekananthan et al, 2004). These are also having the ability to produce ACC deaminase (Shah et al., 1998) that cleaves the ACC (precursor of ethylene) and thereby it lowers the level of the ethylene in the stressed plant (Mayak et al., 2004). The ACC deaminase by these growth promoting bacteria induces the formation of longer roots and it enable the plant seedling to survive under various biotic as well as abiotic stresses (Glick and Grichko 2001). The deleterious effect of ethylene produced because of flood stress can be tolerated by the plants which are treated with PGPR (Glick and Grichko 2001). About PGPR, very little is known regarding the role of in mediating drought resistance in field crops. In view of this the present investigation was undertaken to isolate, characterize and identify candidate ACC deaminase, IAA, siderophore phosphatase-producing fluorescent *Pseudomonas* isolates which were derived from plants which naturally survived water stressed red lateritic soils. We focused to identify potential fluorescent *Pseudomonas* isolate among the 17 collected that could ameliorate abiotic stress tolerance in rice and wheat. All the fluorescent

Pseudomonas isolates were screened for the plant growth promoting activity following seed biopriming of Rice, Wheat Tomato and Brinjal. Conforntation assays (*in-vitro*) against *S. rolfsii* and *R. solani* identified potential isolates with mycoparasitic abilities. Biochemical characterization,DNA fingerprinting using gene specific markers and sequencing helped us to resolve species identity of the collected isolate.

Isolation of fluorescent *Pseudomonas* sp. were attempted from the rhizosphere of 65 plants which naturally survived in water stressed red lateritic soil. Rhizosphere and rhizoplane soils inoculated on KMB medium expressed the bacterial colonies with strong fluorescence. Seventeen plant samples expressed the association of fluorescent *pseudomonas and* were isolated and maintained on KMB medium.

- Fifty four different biochemical tests were performed against 17 isolates (Carbohydrate utilization, Gelatin liquefaction test, Nitrate reduction test TSI (acid butt/alkaline slant), TSI (bubbles/cracks), TSI (black precipitate), cetrimide agar base test,. Oxidase test, Hugh leifson's test, Egg yolk test, Fluorescein test, Pyocyanin test, Kanamycin sensitivity test, Carbenicillin test) which resolved diversity among the collected isolates.
- Capacity to solubilize fixed forms of phosphates through the release of organic acids is another characteristic feature of fluorescent *Pseudomonas*. All the isolates derived from red lateritic soil had the potential to solubilize unavailable form of "P" from the sol and was qualitatively estimated on PVK agar media and quantitatively estimated on NBRIP NBRIY and PVK broth medium. One potential fluorescent *Pseudomonas* isolates P11 was identified as strong phosphate solubilizer.

- Using DF salt minimal media which was supplemented by tryptophan (1.02 g/L from 5mM stock solution.) was used as biochemical screen. All the isolates produced varying levels of IAA out of which P15 (72.905 $\mu\text{g/ml}$) was identified as highest producer of IAA. Fluorescent *Pseudomonas* spp. produces siderophore which are strong iron chelators of low molecular weight facilitates the biocontrol activity by making the iron deficient for the pathogens. Ferric-siderophore is transported inside the bacterial cell via active transport system through specific membrane receptors. The potential isolates of fluorescent pseudomonas which produces strong siderophores can be used as constituent of biological products for the plant disease control and can be a good substitute for chemical pesticides. Siderophore producing ability was analyzed using four different biochemical tests. Tetrazolium test for hydroxamate type of siderophore gave positive result for all the isolates. Arnow's assay for catechol type of siderophore where P2 and P14 showed positive result. Hydroxyquinoline test identified 10 fluorescent *Pseudomonas* isolates (P2, P3, P4, P5, P8, P11, P12, P13, P15, and P16) isolates. Isolates identified following Hydroxyquinoline test are usually identified as strong iron chelators.
- The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase produced by some fluorescent pseudomonas strains provides stress tolerance against abiotic stresses in the plants. This enzyme cleaves ACC which is a precursor of plant ethylene into ammonia and alpha keto butyrate and decreases the amount of ethylene and thereby decreases the stress. The high concentration of ethylene may lead to death of the plants. This unique feature of fluorescent *Pseudomonas* prompted us to screen our collected isolates for ACC deaminase production in ACC supplemented modified DF salt minimal media. Ten isolates expressed ACC deaminase +ve reaction. The amount of ACC deaminase produced ranged from 0.765 $\mu\text{M/ml}$ to 82.235 $\mu\text{M/ml}$. Isolate # P15 produced maximum amount of ACC deaminase (82.235 $\mu\text{M/ml}$).

- The collected isolates were screened for imparting water stress tolerance in rice and wheat. Water stress tolerance to rice variety IR-64 was imparted by 13 different isolates of fluorescent *Pseudomonas* spp. and was therefore potential isolates. Isolates of fluorescent *Pseudomonas* spp (P4, P5, P6, P7, P17 (29.4%)) imparted water stress tolerance to both the rice variety B15 and IR 64.. Similarly isolates # P7, P8, P13, P14, P15, P16 (35.3%) imparted water stress tolerance to RRF-75. This set had some fluorescent *Pseudomonas* in common (P7, P13, P15) which also imparted water stress tolerance to rice variety IR64. Rice variety IR-64 was more responsive in sustaining water stress following seed treatment with fluorescent *Pseudomonas* as compared to rice varieties B15 and RRF75 which contained gene(s) / QTL's for water stress / drought tolerance. In wheat The treatments that were identified as potential ones were P7, P8, P13, P14, P15, P16 (35.29%). Fluorescent *Pseudomonas* spp employs a variety of mechanisms (phosphate solubilization, IAA production, siderophore production, production of 1-Aminocyclopropane-1carboxylate deaminase (ACC)) to enhance plant growth. Our present investigation also reports herein the plant growth promoting effects induced by fluorescent *Pseudomonas* spp. following seed biopriming. Seed biopriming of different crops i.e., rice, wheat, tomato and brinjal using 17 fluorescent *Pseudomonas* spp produced variety of plant growth promoting effects which were significantly superior as compared to control. On rice variety seed bio-priming with isolate P15 induced highest tiller number, Shoot bundle weight. In rice variety B-15 the panicle emergence was early as compared to the control plants. Most commonly reported effects of fluorescent *Pseudomonas* sp on plants is the increase in shoot and root length. We also report here in similar effects on Tomato seedlings / plants derived following seed treatment where the increase in shoot and root length was 43.75% and 77.7% increase over the control plants. Similar effects were observed on brinjal. Significantly higher (15.0.27cm³) root volume was observed in seedlings derived from seed

bioprimered with fluorescent *Pseudomonas* spp. while the untreated control expressed poor root volume (2.21cm³).

- The molecular characterization of the 17 isolates was carried out. PCR amplification of 16S ribosomal RNA with primers 27+1492 resulted in amplification of all the 17 isolates.

Amplification with the primers ERIC, REP and BOX primers showed high polymorphism ranging from 100 to 1500 bp. Amplification with some antibiotic primers like PHz, PCA showed positive result in some isolates. Majority of the isolates showed amplification for primers.

- Bipartite interactions were performed following a simple confrontation assay developed by Kotasthane et al 2017 to identify prospective bio-agent. There were differences in the antagonistic abilities of all of the 17 isolates of fluorescent *Pseudomonas* against the two soil borne pathogens *R. solani* and *S. rolfsii*. Confrontation assays revealed isolates # P1, P2, P3, P12, P14, P15 as potential antagonists against *S. rolfsii* and P1, P2, P10, P15, P16 against *R. solani*. These isolates exerted strong inhibitory effects (> 60%) on the mycelia growth against the two soil borne pathogens *R. solani* and *S. rolfsii*.

Conclusions

1. Among the fluorescent *pseudomonas* isolates derived from the water stressed red lateritic soil some potential isolates were identified which solubilize fixed form of phosphates, capable for the production of indole acetic acid, strong siderophores and ACCd enzyme
2. The seed bacterization with these isolates imparted drought tolerance as well as plant growth promoting activity in rice and wheat. PGPR activity was also expressed in tomato and brinjal.

3. Biochemical as well as molecular characterization revealed the diversity within the collected isolates.
4. Some of the isolates were identified to have antagonistic activity against phytopathogens *R.solani* and *S.rolfsii*.

Suggestions for future work

- The identified potential isolates can be screened for imparting resistance for any other abiotic stresses.
- The isolates identified as efficient bio-control agents *in- vitro* against *S. rolfsii* and *R. solani* can be screened for the similar result in the field conditions also.
- These identified potential isolates can be tested with the combination of different dose of fertilizers.

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