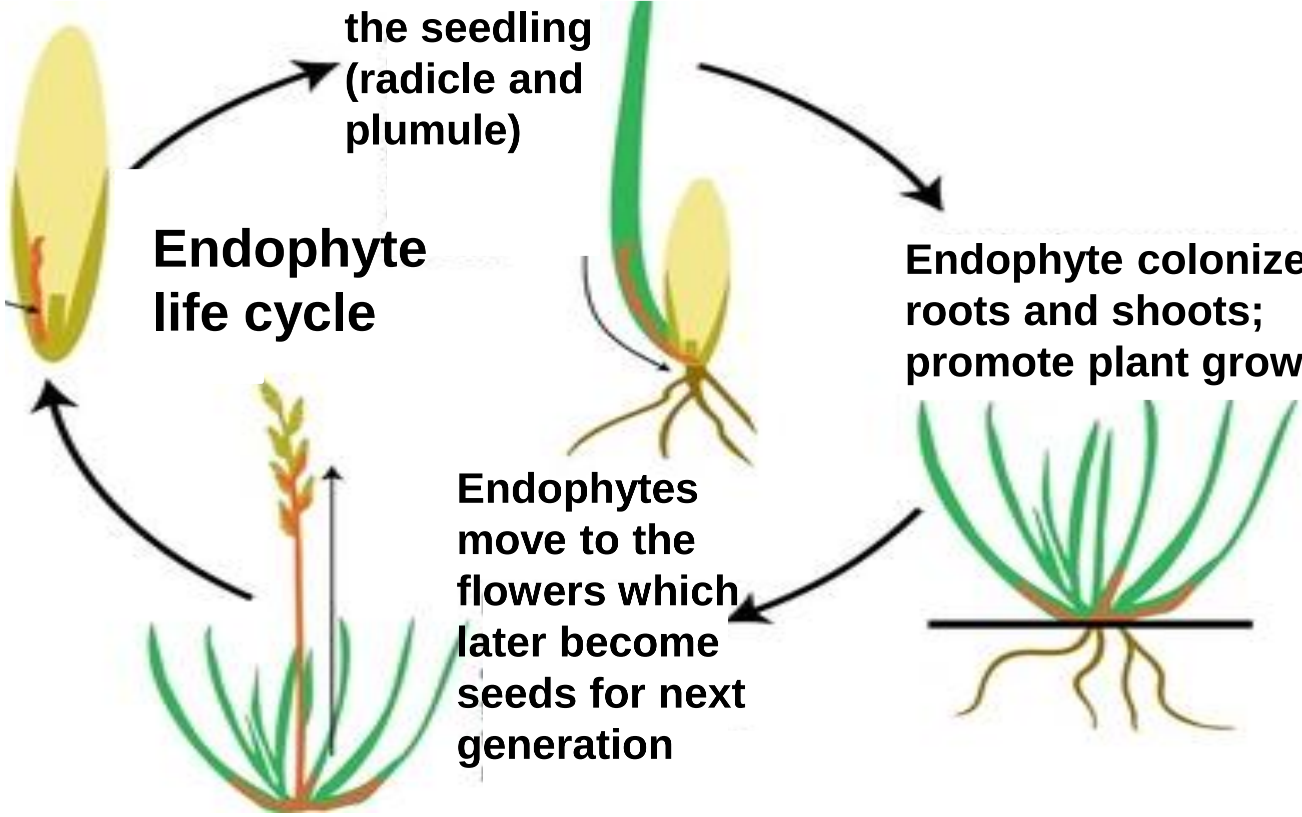
7th Annual Conference
Assam Forum of Great Britain (AFGB)

Seed Borne Bacterial Endophytes : Their transmission and Dynamics at Early Growth Stages of Rice

Jilmil Baruah
PhD Research Scholar
IASST,Guwahati, Assam, India
Newton Bhabha Fellow
School of Biological Sciences
University of Reading, United Kingdom

BACKGROUND

Endophytes: *“Microbes that colonize living internal tissues of plants without causing any negative effects”* .



Endophyte life cycle

Endophytes reside in seeds and get transmitted to the next generation by vertical transmission

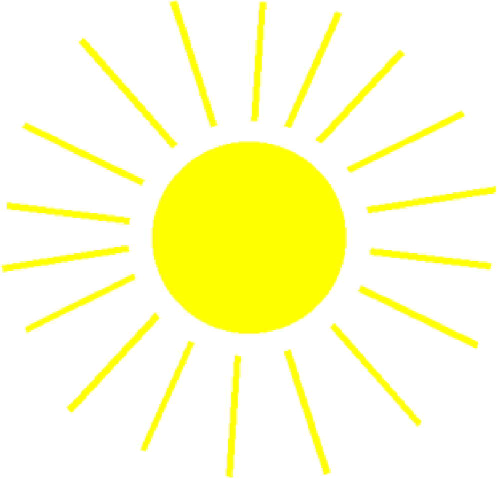
Endophyte moves into the growing parts of the seedling (radicle and plumule)

Endophyte colonizes roots and shoots; promote plant growth.

Endophytes move to the flowers which later become seeds for next generation

Source:http://www.utas.edu.au/

IMPORTANCE OF THE STUDY



Plant-microbe interaction

www.unisci24.com

OBJECTIVES

1

- **Isolation and identification of culturable seed-borne bacterial endophytes in paddy at different growth stages and nutrient variation.**

2

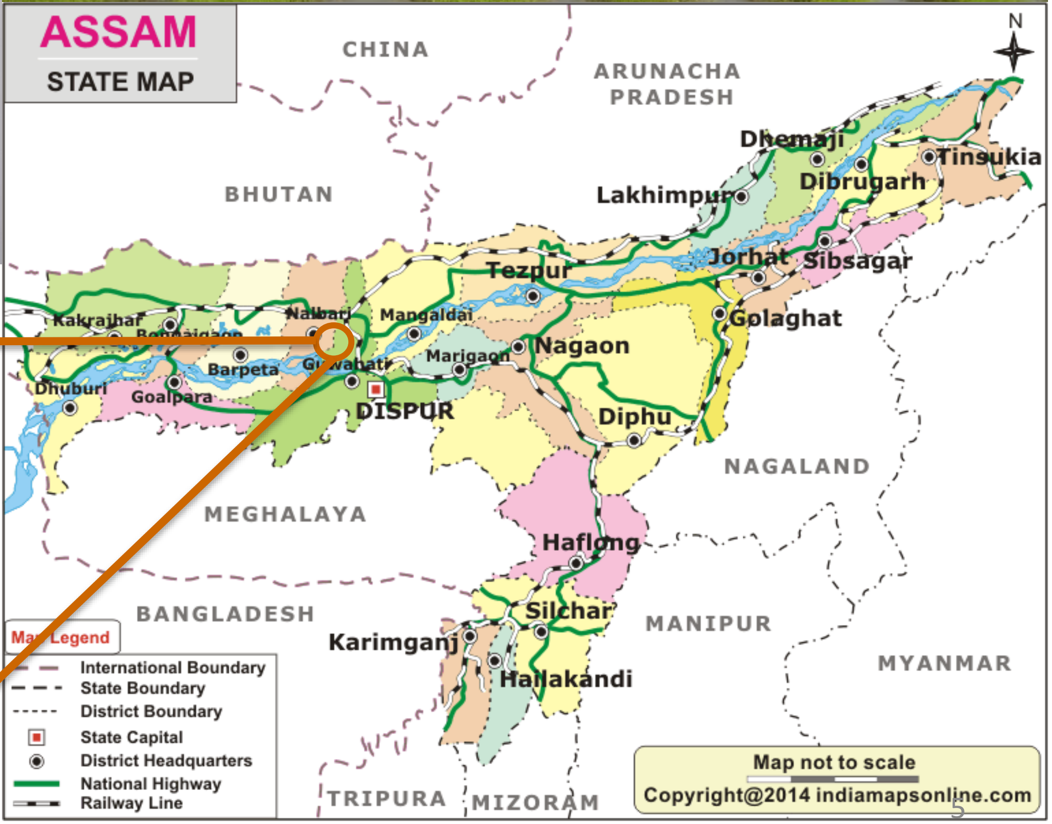
- **Study of diversity of endophytic bacteria in different growth stages and nutrient variation of paddy by culture-independent approach.**

3

- **Analysis of metabolic profile due to plant-endophyte interaction.**

MATERIALS AND METHODS

Sample Collection: *Kola Joha* rice seeds were collected from Regional Rainfed Lowland Rice Research Station (RRLRRS), Gerua, Kamrup, Assam on 27/01/2016.



Gerua

Lattitude:26.60

Longitude:91.59

MATERIALS AND METHODS

A. Surface sterilization of seeds

B. Detection of endophytes under Scanning Electron Microscope

OBJECTIVE 1:

Isolation and identification of culturable seed-borne bacterial endophytes in paddy at different growth stages and nutrient variation.

C. Isolation of culturable endophytes in Nutrient Agar, Pikovskayas Agar, Jensens Medium

D. 16S rDNA Sequencing for Identification of bacteria

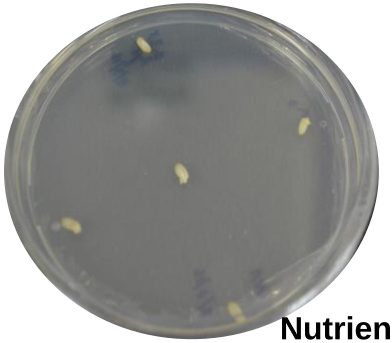
MATERIALS AND METHODS

Optimization of Surface sterilization

Surface sterilization

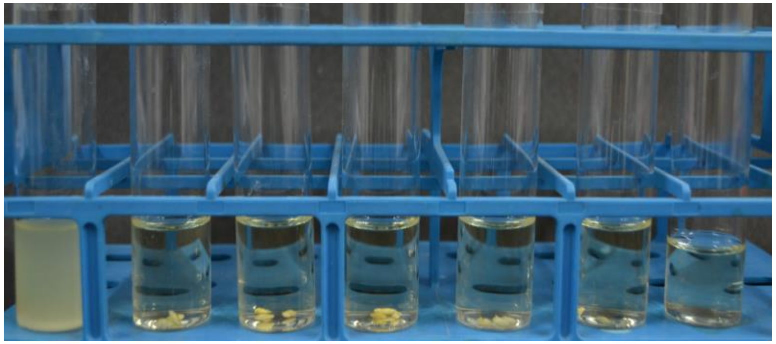
(Hardoim *et al.* 2012)

Sterility check



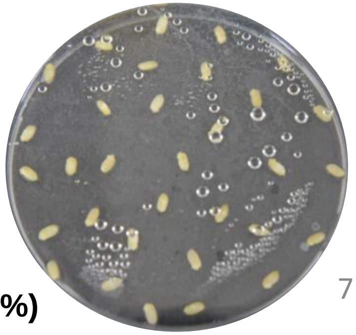
Nutrient Agar plate

Treatment time of hydrogen peroxide (at 30^0C, 150rpm) (in minutes)	Concentration of Sodium hypochlorite (%) in sterilizing solution (at 30^0C, 150rpm, for 20mins)	Bacterial Growth
5	0.2	+
10	0.5	+
15	0.8	+
15	1	-
15	1.5	-



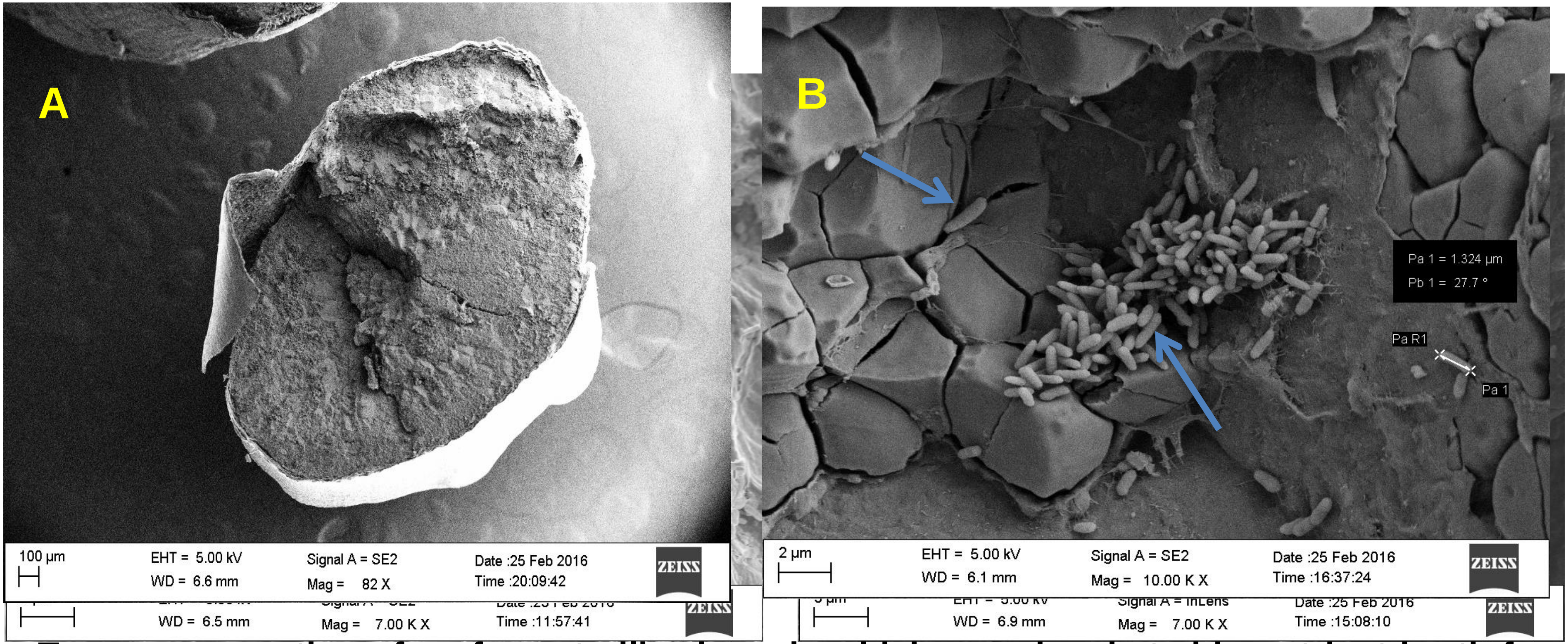
Nutrient Broth

Seeds germinated



Water Agar plate (0.4%)

Detection of bacteria in *Kola Joha* rice seed under SEM:



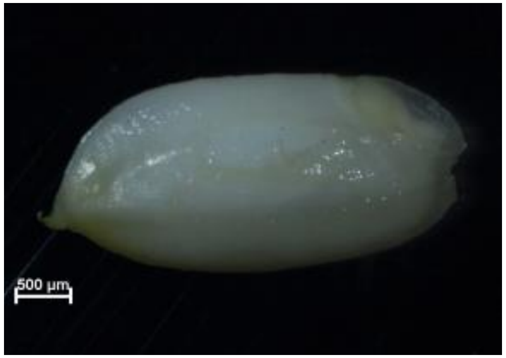
Without surface sterilization (Mag=7K X)

After surface sterilization (Mag=7K X)

Transverse section of surface sterilized seeds which were incubated in nutrient broth for 12 hours, (A) cut portion of the seed (Mag=82X)
(B) magnified image of cut surface (Mag=10K X)

Growth Stage selection for endophyte isolation

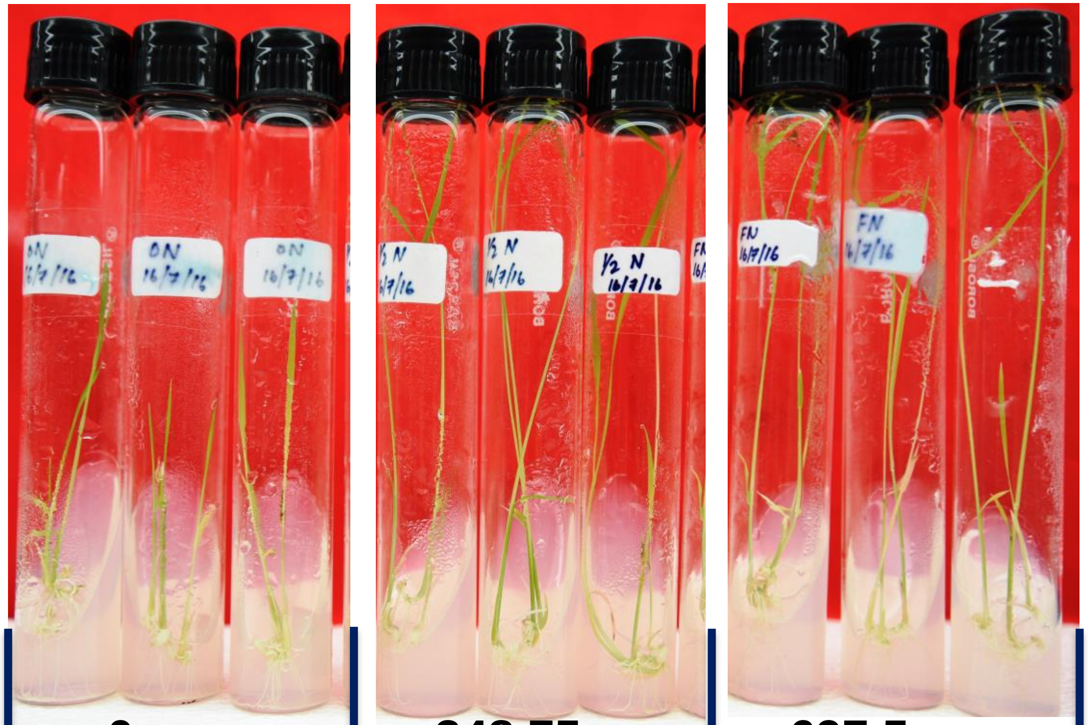
1) Surface sterilized
Kola Joha **rice seed (0 Day old)**



3 stages were selected

2) 5 days old germinated
Kola Joha **rice seed (GS)**

3) 15 days old plantlets
grown in Hoagland media



0ppm **348.75ppm** **697.5ppm**

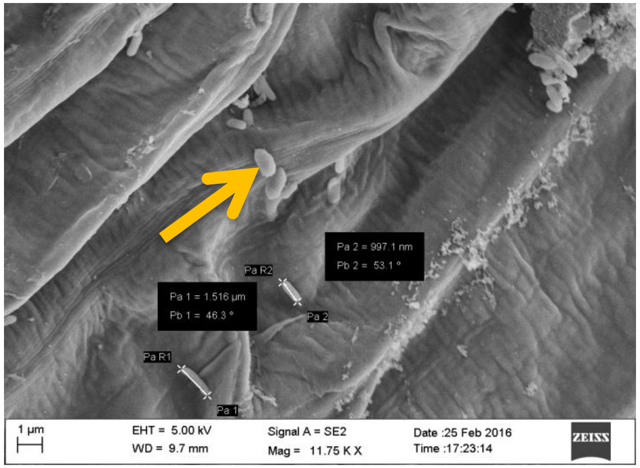
RESULTS

OBJECTIVE 1:

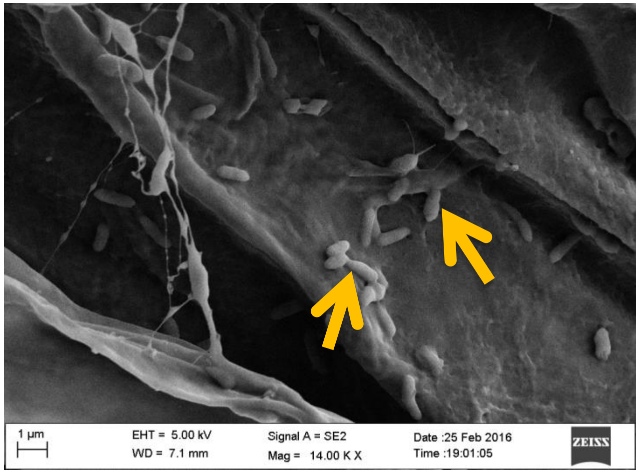
Isolation and identification of culturable seed-borne bacterial endophytes in paddy at different growth stages and nutrient variation.

Detection of bacteria in *Kola Joha* rice seedling under SEM:

Germinating seed's radicle and plumule

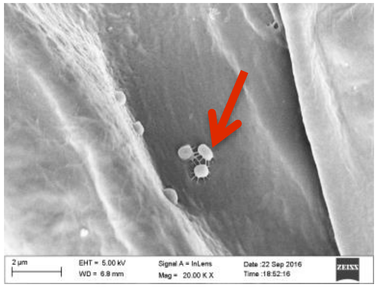


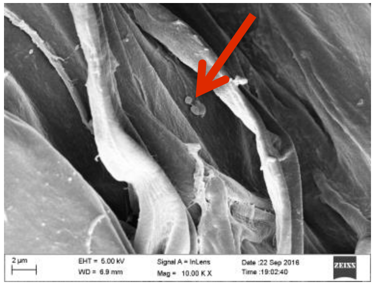
Plumule interior (Mag=11.75K X)

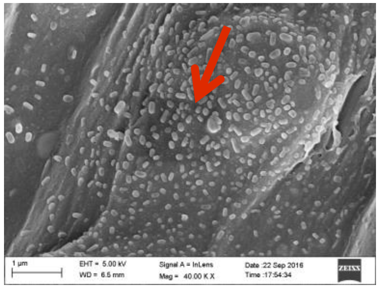


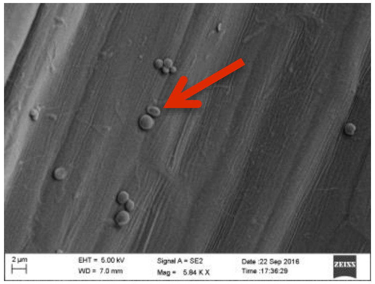
Radicle surface (Mag=14K X)

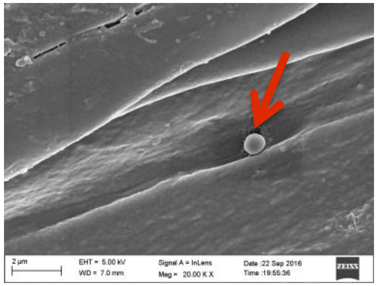
Detection of bacteria in *Kola Joha* rice plants (15 days old) under SEM:

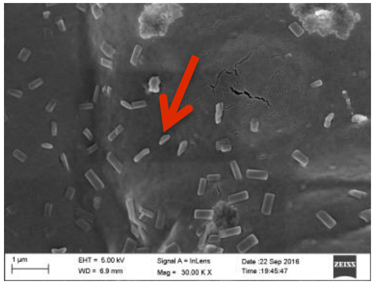

(A) 0NR S

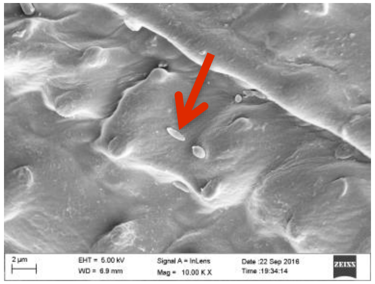

(B) 0NR Int

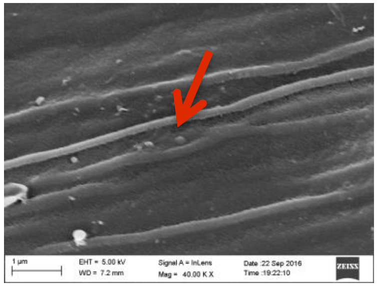

(C) ONS S

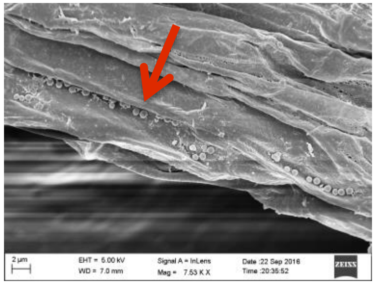

(D) ONS Int

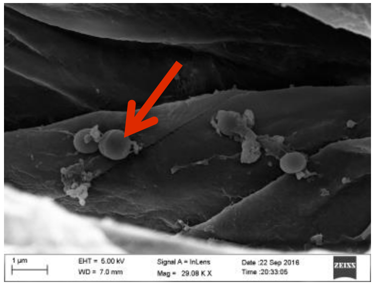

(E) HNR S

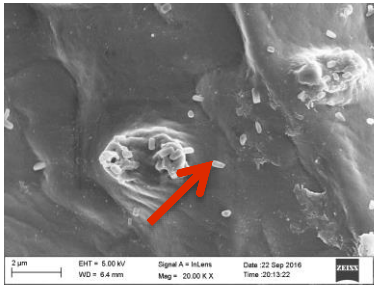

(F) HNR Int

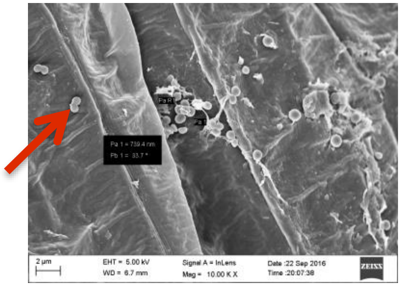

(G) HNS S


(H) HNS Int

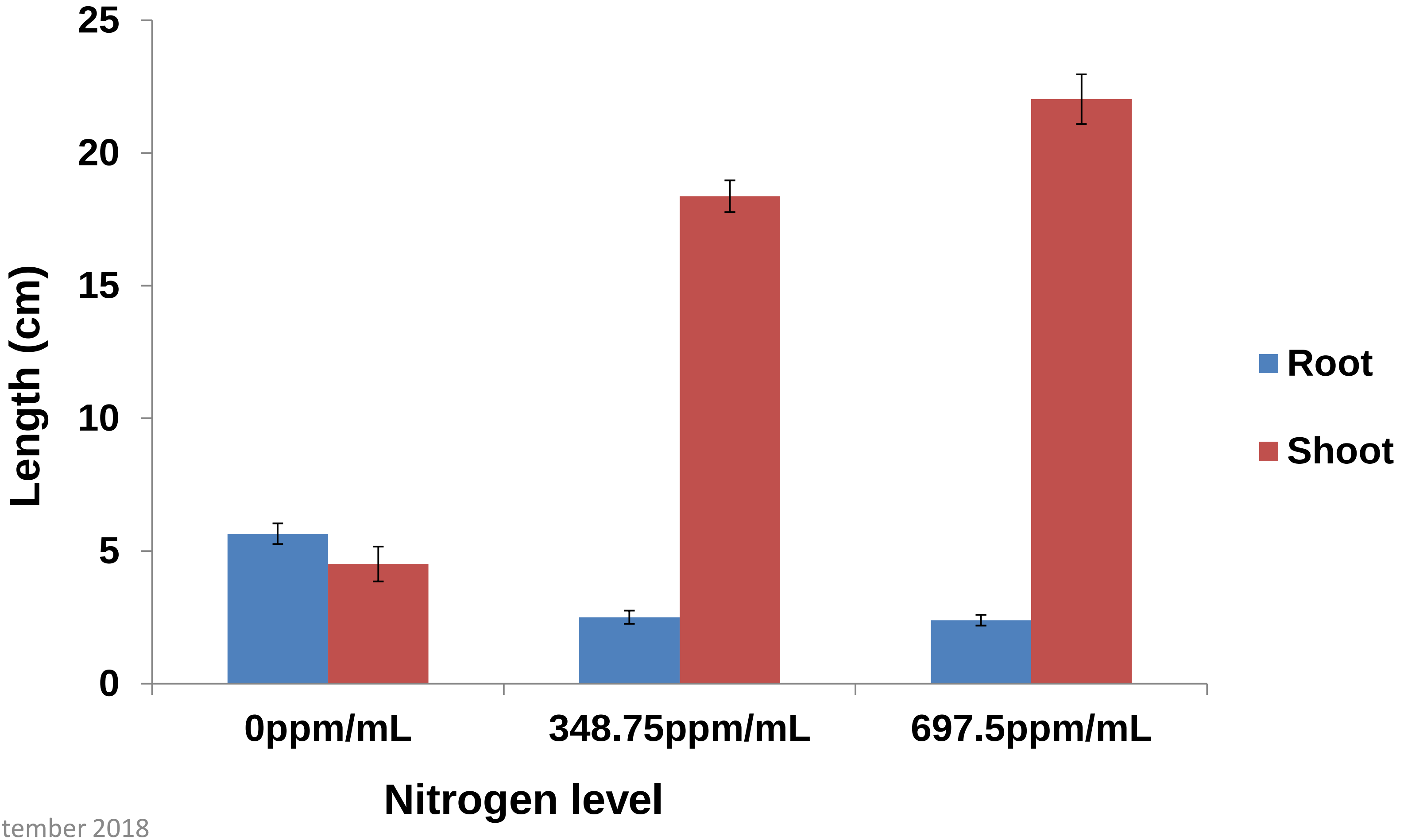

(I) FNR S


(J) FNR Int

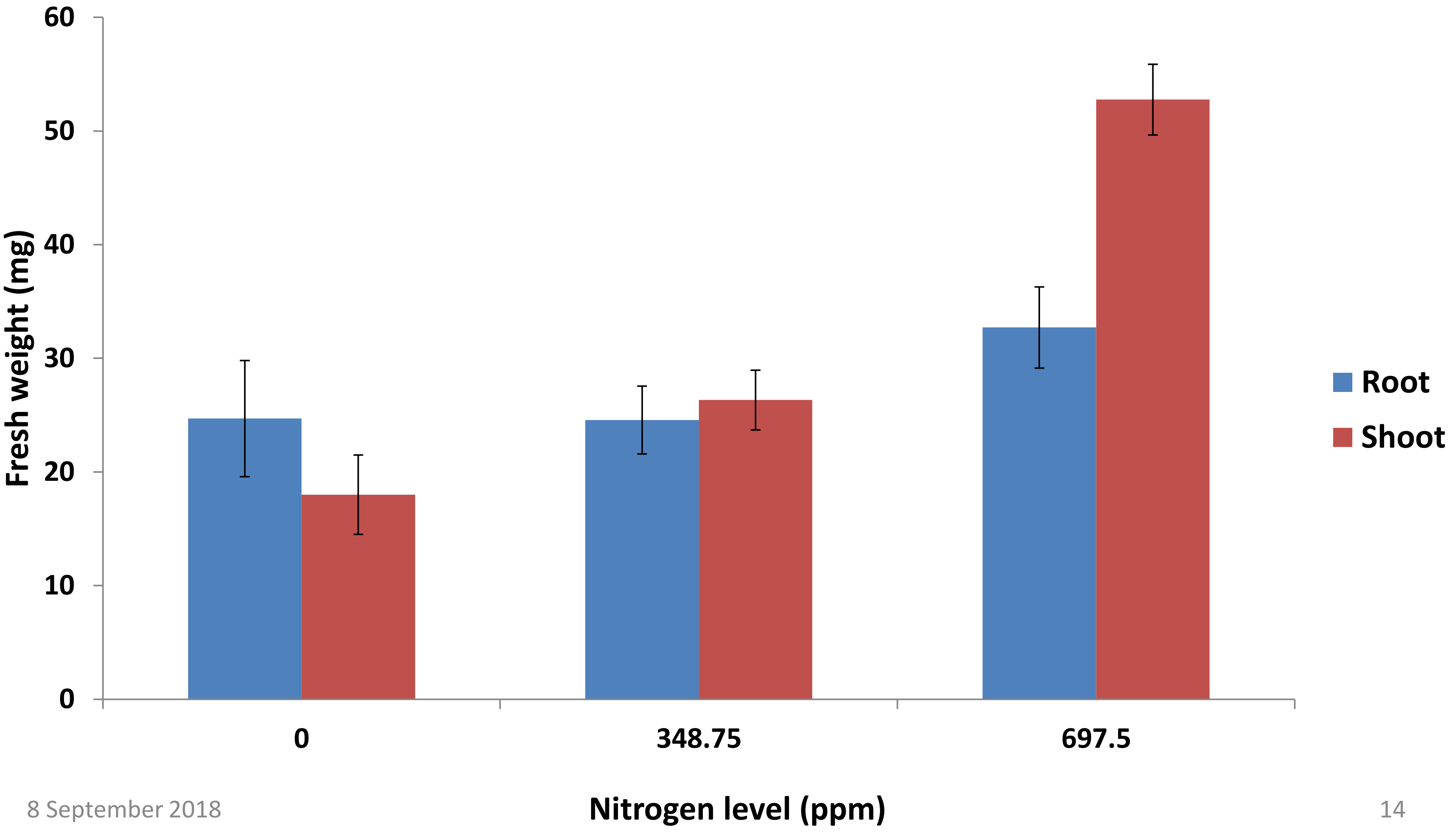

(K) FNS S


(L) FNS Int

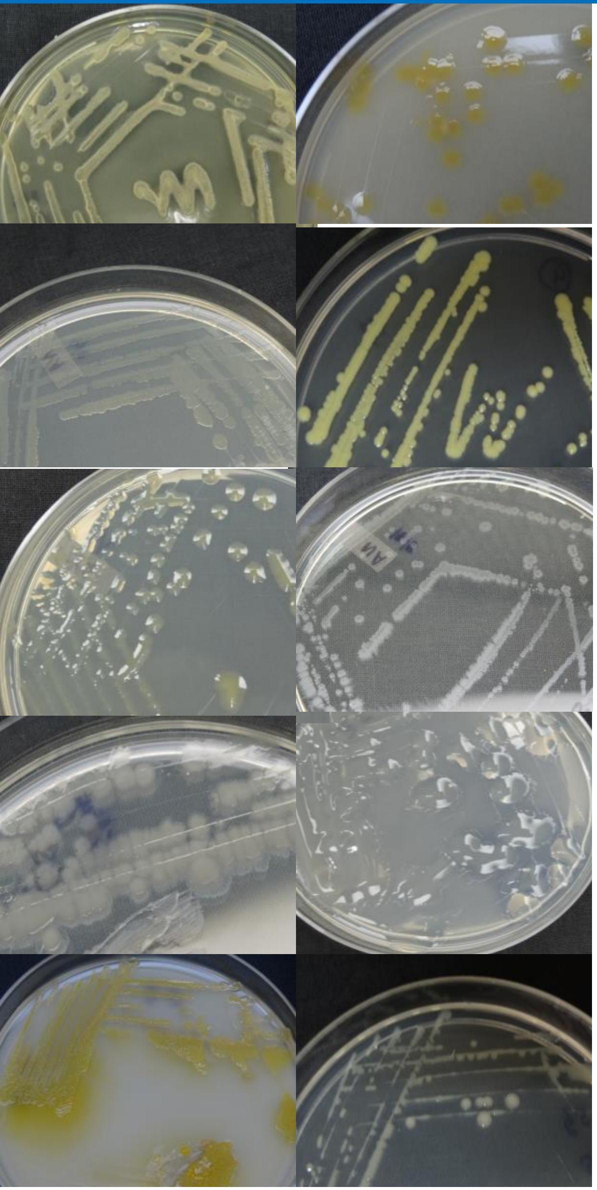
Variation in root and shoot length of 15 days old plants with respect to nitrogen in growth media



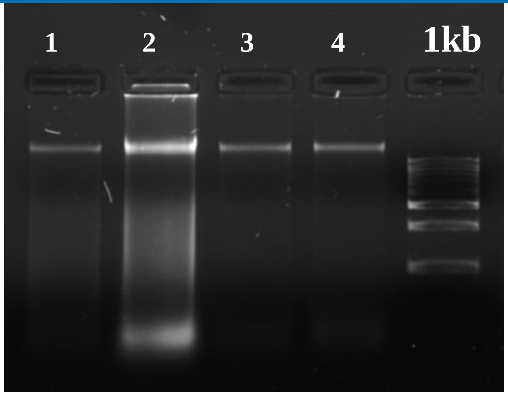
Variation in root and shoot fresh weight of 15 days old plants with respect to nitrogen in growth media



Identification of Culturable isolates

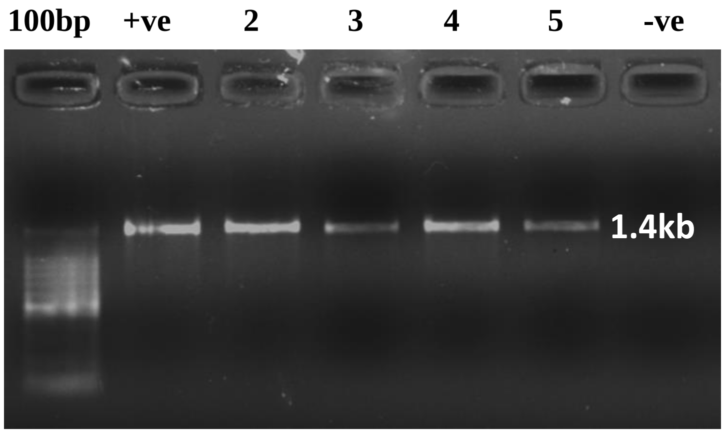


Culturable diversity (70 isolates)



Bacterial genomic DNA

Culture dependent approach

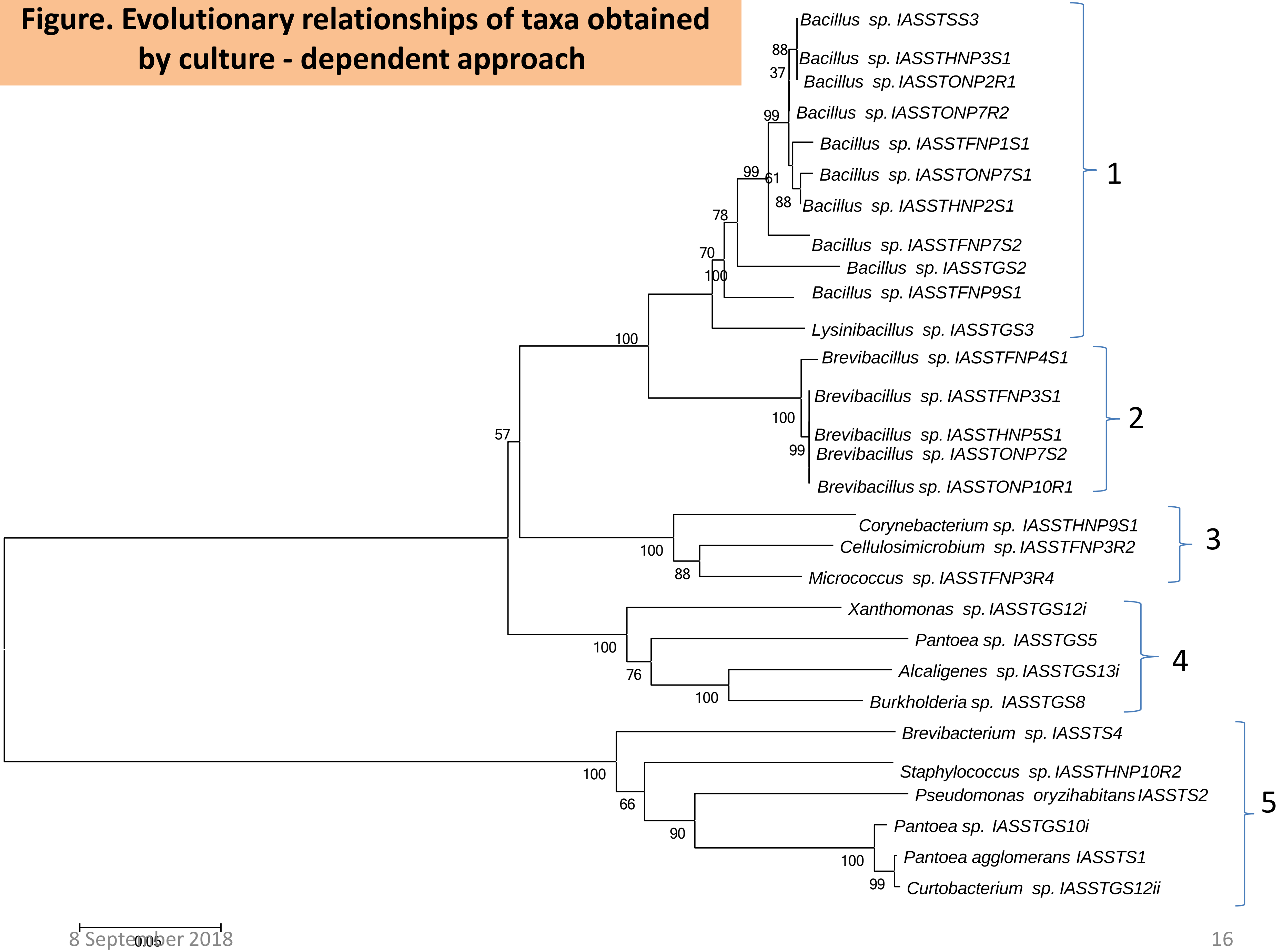


16S rRNA gene amplification

Glycerol stock

Sanger Sequencing

Figure. Evolutionary relationships of taxa obtained by culture - dependent approach



Culturable bacterial endophytes from different parts of the plant

Organism (Accession Number)	Closest relative	Presence/absence of bacteria in different parts of the plant				
		Seed (0th day)	Germinating seed (5th day)	Plant (15th day)		
				0ppm	348.75 ppm	697.5 ppm
Bacillus sp. IASSTSS3 (MF375106)	*Bacillus safensis strain NBRC 100820*	+	-	+	+	-
Bacillus sp. IASSTGS2(MF383409)	*Bacillus cereus ATCC 14579*	-	+	-	-	-
Bacillus sp. IASSTFP7S1 (MF465554)	*Bacillus aerius strain 24K*	-	-	+	+	+
Bacillus sp. IASSTFP9S1 (MF465562)	*Bacillus megaterium strain ATCC 14581*	-	-	-	-	+
Bacillus sp. IASSTFP7S2 (MF465561)	*Bacillus subtilis strain JCM 1465*	-	-	-	-	+
Pantoea agglomerans IASSTS1 (MF375048)	*Pantoea agglomerans strain DSM 3493*	+	+	-	-	-
Pseudomonas oryzihabitans IASSTS2(MF375049)	*Pseudomonas oryzihabitans strain IAM 1568*	+	-	-	-	-
Brevibacterium sp. IASSTS6 (MF417475)	*Brevibacterium casei strain DSM 20657*	+	-	-	-	-
Pantoea sp. IASSTGS10i (MF383415)	*Pantoea brenneri strain LMG 5343*	-	+	-	-	-
Alcaligenes sp. IASSTGS13i (MF383414)	*Alcaligenes faecalis strain NBRC 13111*	-	+	-	-	-

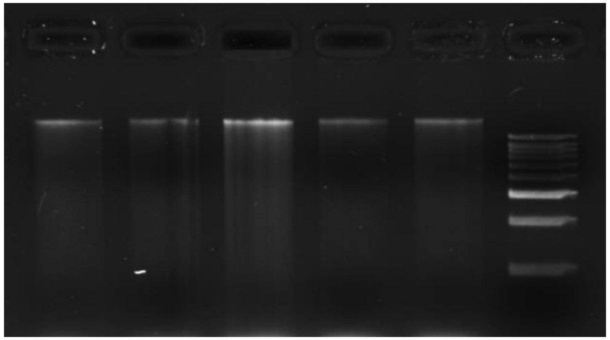
Organism (Accession Number)	Closest relative	Presence or absence of the organism in different parts of the plant				
		Seed (0th day)	Germinating seed (5th day)	Plant (15th day)		
				0ppm	348.75 ppm	697.5 ppm
Xanthomonas sp. IASSTGS12i (MF383413)	*Xanthomonas sacchari strain LMG 471*	-	+	-	-	-
Curtobacterium sp. IASSTGS12ii (MF383416)	*Curtobacterium plantarum strain CL63*	-	+	-	-	-
Burkholderia sp. IASSTGS8 (MF383417)	*Burkholderia gladioli strain NBRC 13700*	-	+	-	-	-
Lysinibacillus sp. IASSTGS3 (MF383410)	*Lysinibacillus macroides strain LMG 18474*	-	+	-	-	-
Brevibacillus sp. IASSTONP7S2 (MF461193)	*Brevibacillus nitrificans strain DA2*	-	-	+	+	+
Brevibacillus sp. IASSTFP4S1 (MF465558)	*Brevibacillus agri strain DSM 6348*	-	-	-	-	+
Corynebacterium sp. IASSTHNP9S1 (MF465543)	*Corynebacterium simulans strain UCL553*	-	-	-	+	-
Staphylococcus sp. IASSTHNP10R2 (MF465552)	*Staphylococcus auricularis strain WK 811M*	-	-	-	+	-
Cellulosimicrobium sp. IASSTFP3R2 (MF465565)	*Cellulosimicrobium funkei strain W6122*	-	-	-	-	+
Micrococcus sp. IASSTFP3R4 (MF465566)	*Micrococcus yunnanensis strain YIM 65004*	-	-	-	-	+
Total number of different bacteria		**4**	**8**	**3**	**5**	**7**

MATERIALS AND METHODS

OBJECTIVE 2:

Study of diversity of endophytic bacteria in different growth stages and nutrient variation of paddy by culture-independent approach

Culture-Independent Approach



Metagenomic DNA extracted from all the 3 stages

NGS Sequencing

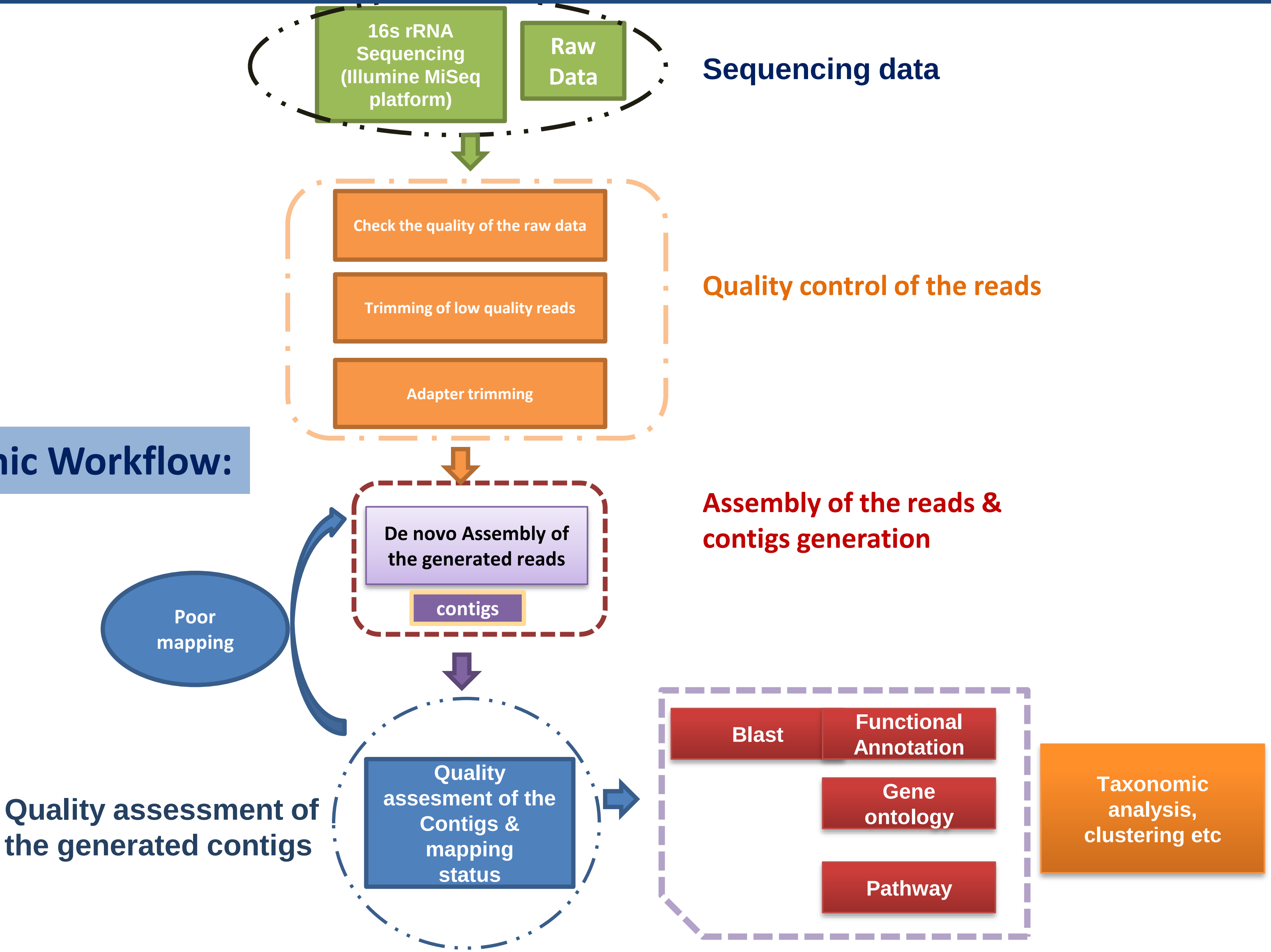
Sequencing Technology:

- **The sequencing libraries is prepared according to the Illumina 16S Metagenomic Sequencing Library**
- **16s rRNA sequencing by using Illumina Miseq platform**
- **pair-end sequencing (seq length 301bp)**

Data Analysis (QIIME, QUAST, UPARSE)

Culture-Independent Approach

Metagenomic Workflow:



16s rRNA Sequencing (Illumine MiSeq platform)

Raw Data

Sequencing data

Check the quality of the raw data

Trimming of low quality reads

Adapter trimming

Quality control of the reads

De novo Assembly of the generated reads

contigs

Assembly of the reads & contigs generation

Poor mapping

Quality assessment of the Contigs & mapping status

Quality assessment of the generated contigs

Blast

Functional Annotation

Gene ontology

Pathway

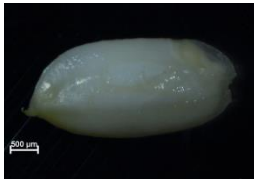
Taxonomic analysis, clustering etc

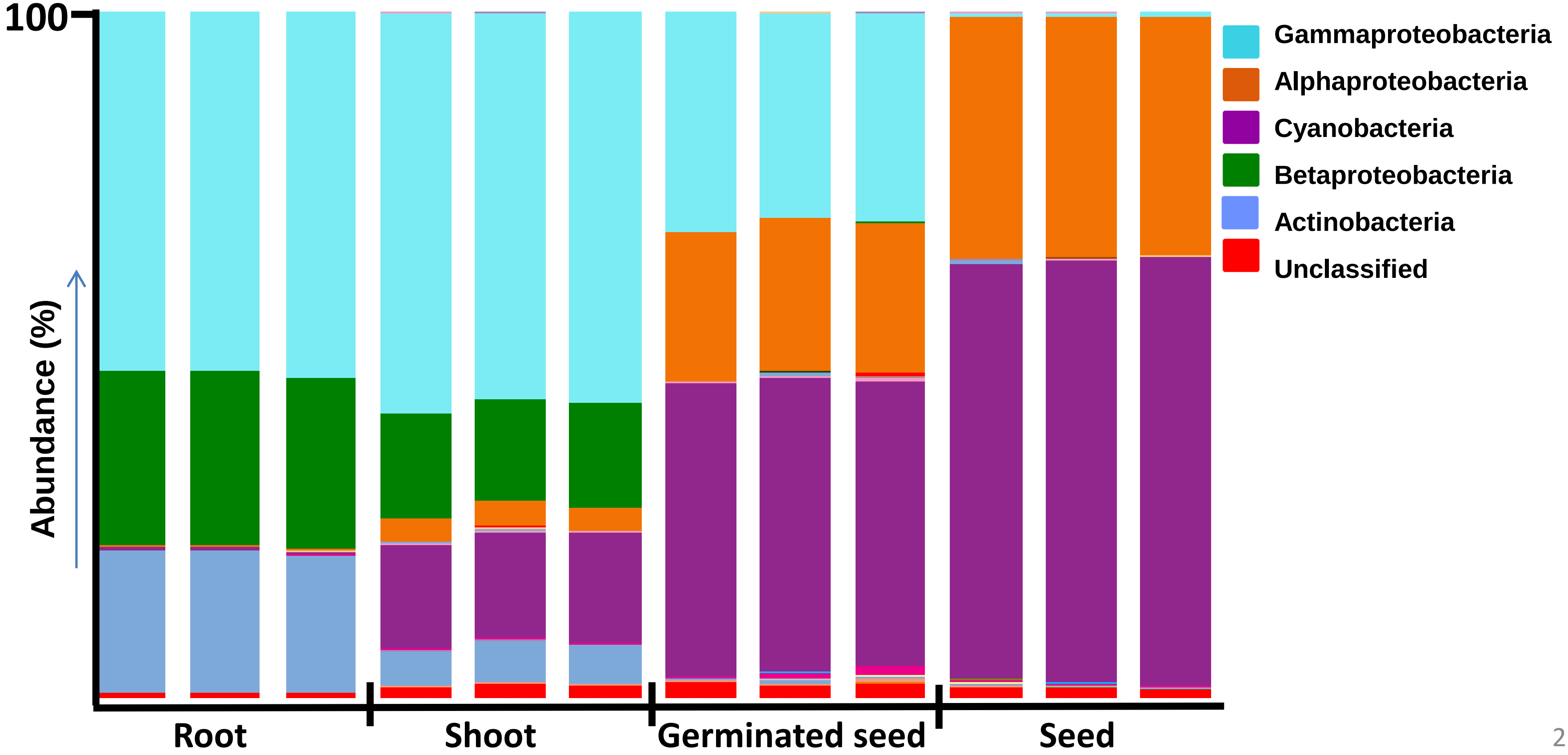
Taxonomic relationship

RESULTS

OBJECTIVE 2:
Study of diversity of endophytic bacteria with respect to different growth stages and nutrient variation of the host plant

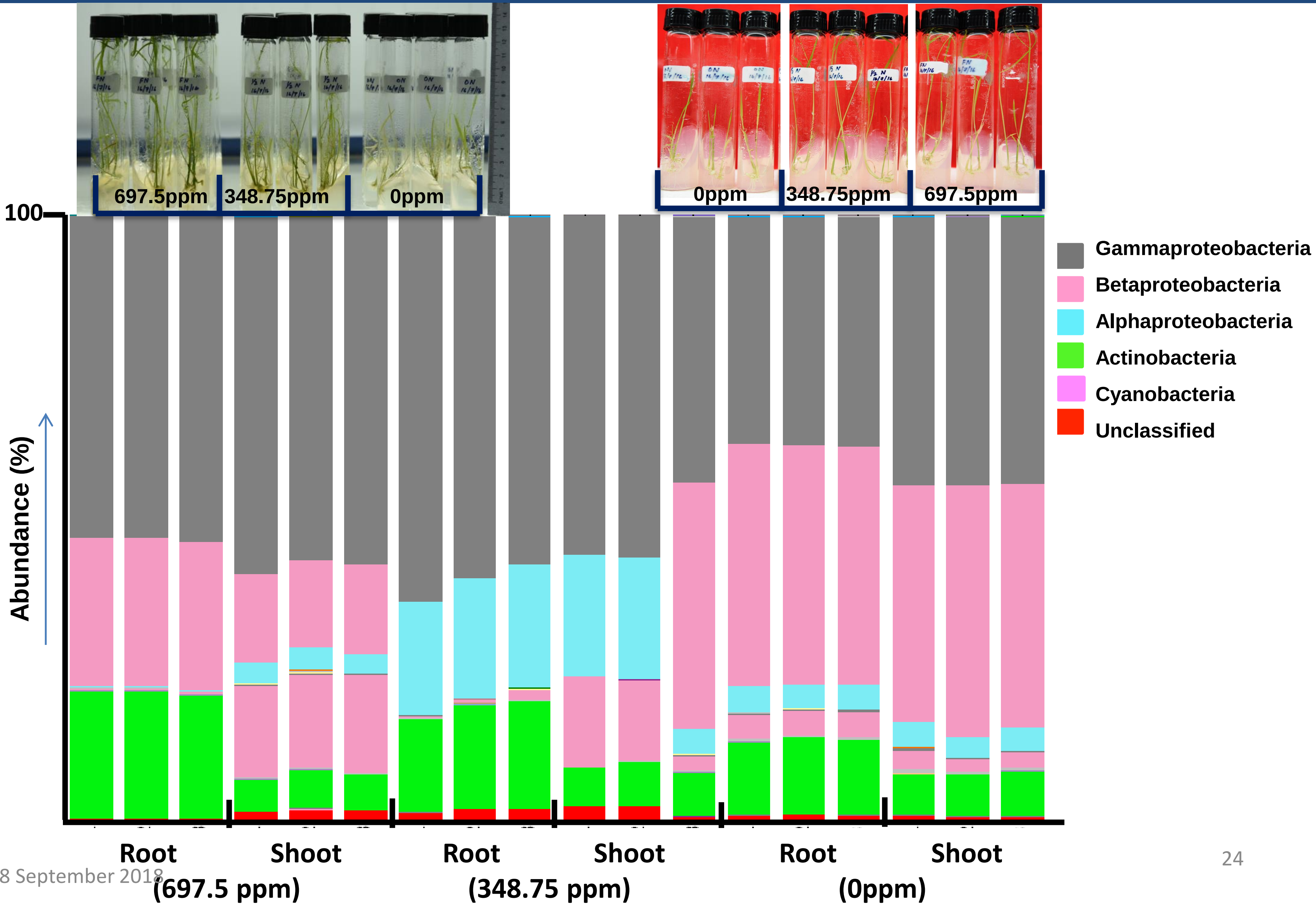
Bacterial genera abundance in different parts of *Kola Joha* rice plantlets during early growth stages





15 Days old plant

Bacterial genera abundance in different parts of *Kola Joha* rice plantlets at 15 days due to change in nitrogen level during growth



Bacterial genera abundance in 15 Days old plantlets(Unculturable Approach-NGS)

FN S1 (697.5ppm)

Genus	Contig %
Pantoea	69.754
Burkholderia	13.601
Pseudomonas	8.51
Xanthomonas	7.987
Ralstonia	0.104
Delftia	0.02
Lactobacillus	0.011
Bacillus	0.008
Enterococcus	0.005

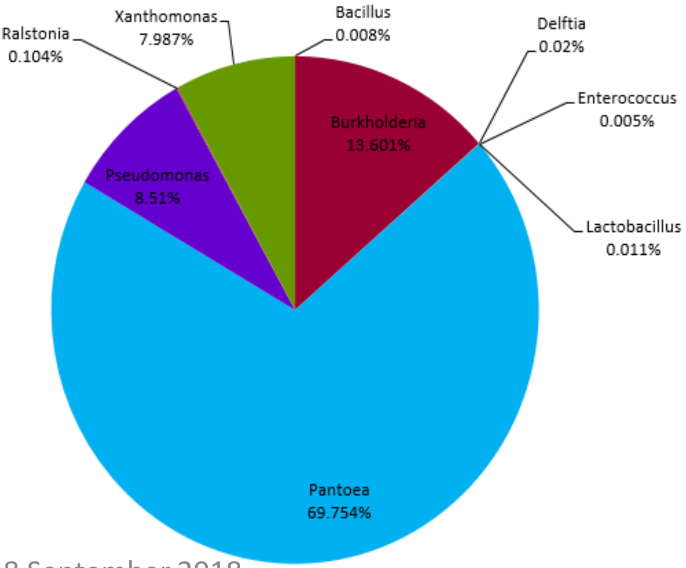
HN S1 (348.75ppm)

Genus	contig %
Pantoea	74.087
Pseudomonas	12.767
Xanthomonas	10.605
Cronobacter	2.514
Burkholderia	0.023
Staphylococcus	0.001
Streptococcus	0.001
Lactobacillus	0.001

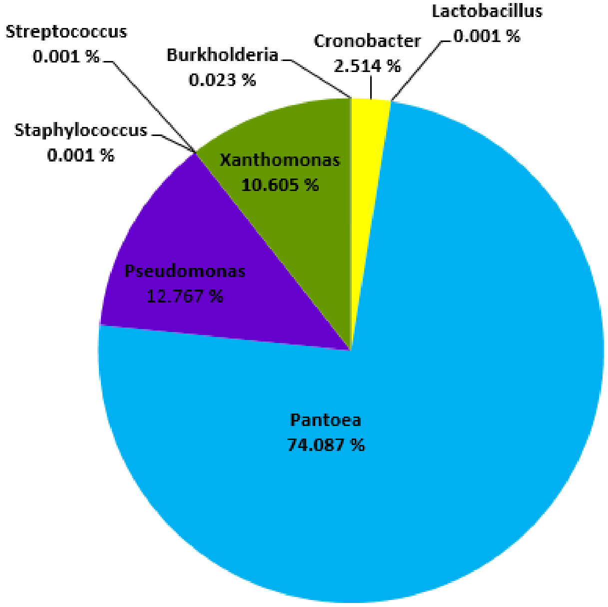
0N S1 (0ppm)

Genus	Contig %
Pantoea	62.87799
Burkholderia	34.62167
Xanthomonas	1.855318
Pseudomonas	0.613221

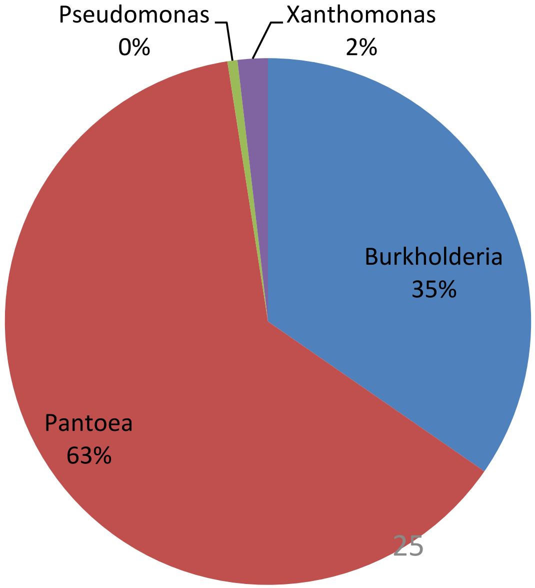
FN S1



HN S1



0N S1



Bacterial genera abundance in 15 Days old plantlets(Unculturable Approach)

FN R1 (697.5ppm)

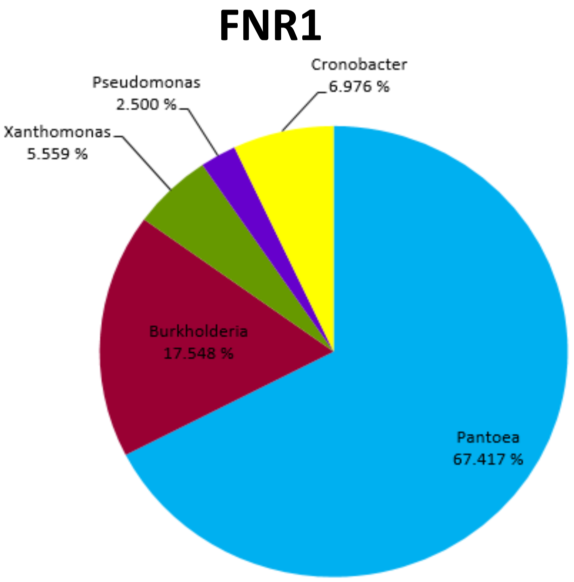
Genus	Contigs %
Pantoea	67.417
Burkholderia	17.548
Cronobacter	6.976
Xanthomonas	5.559
Pseudomonas	2.500

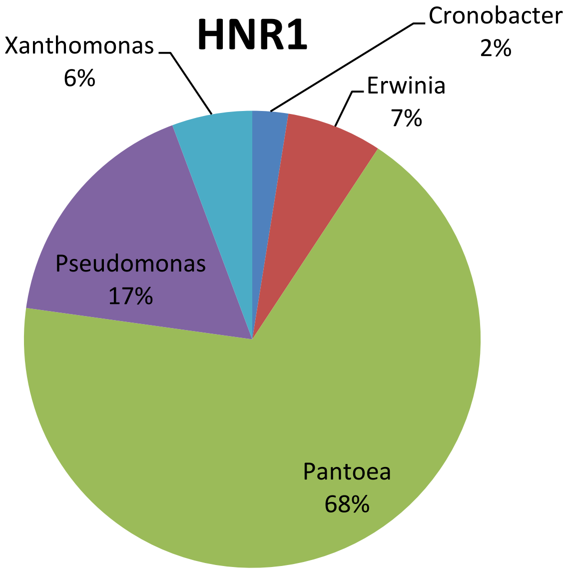
HN R1 (348.75ppm)

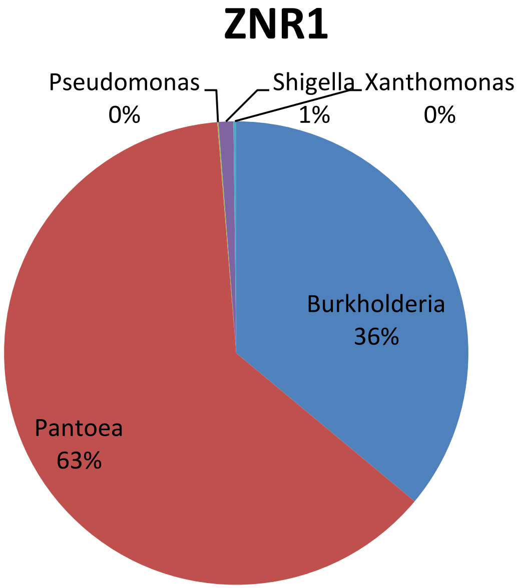
Genus	Contig %
Pantoea	67.89
Pseudomonas	17.11
Erwinia	6.75
Xanthomonas	5.69
Cronobacter	2.53

ZN R1 (0ppm)

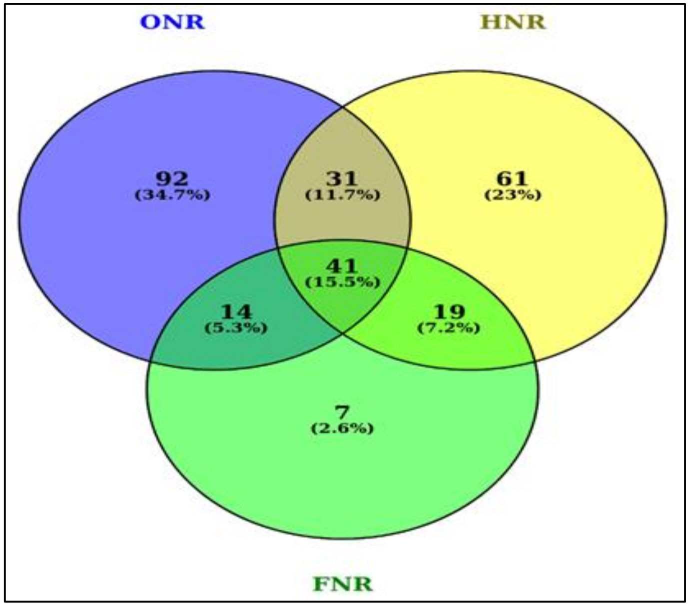
Genus	Contig %
Pantoea	62.63157
Burkholderia	36.04036
Shigella	1.03152
Xanthomonas	0.1924
Pseudomonas	0.075677



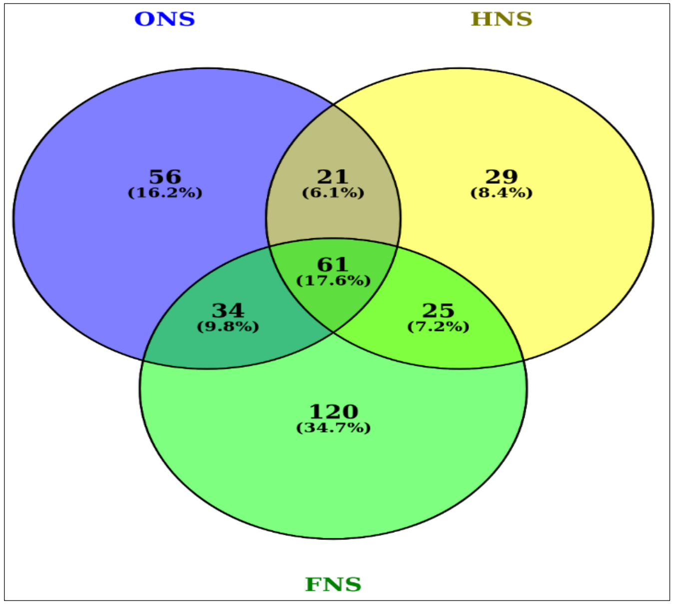




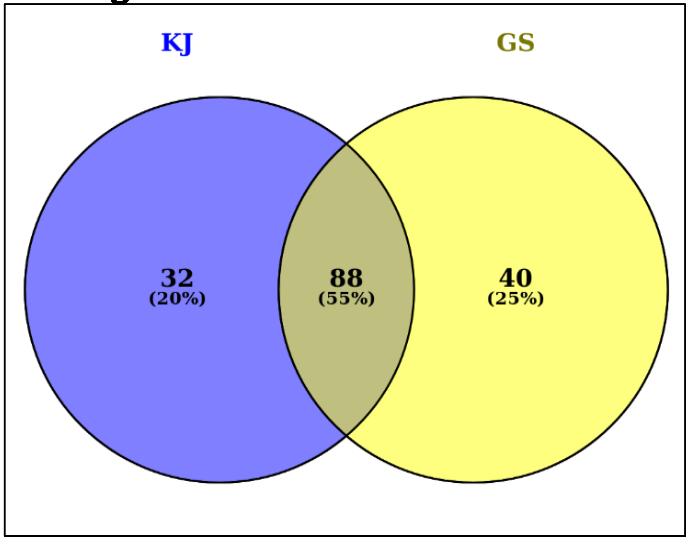
Shared OTUs in different parts of the host plant



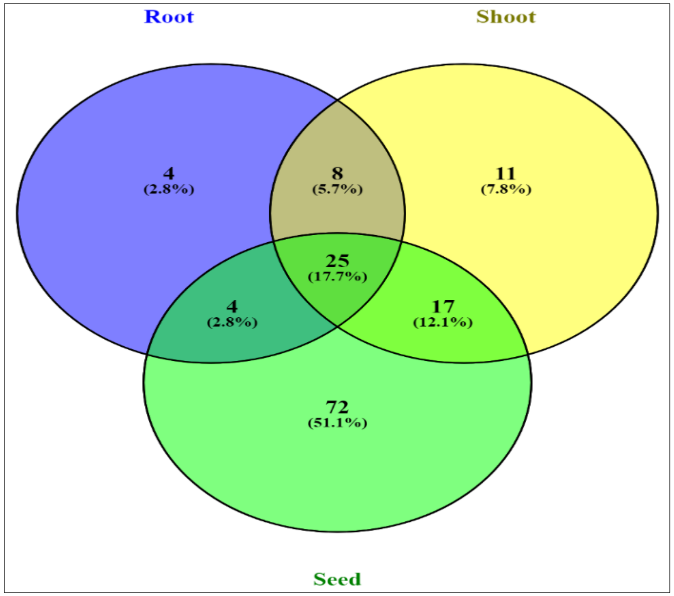
(A) Among roots with respect to nitrogen level



(B) Among shoots with respect to nitrogen level

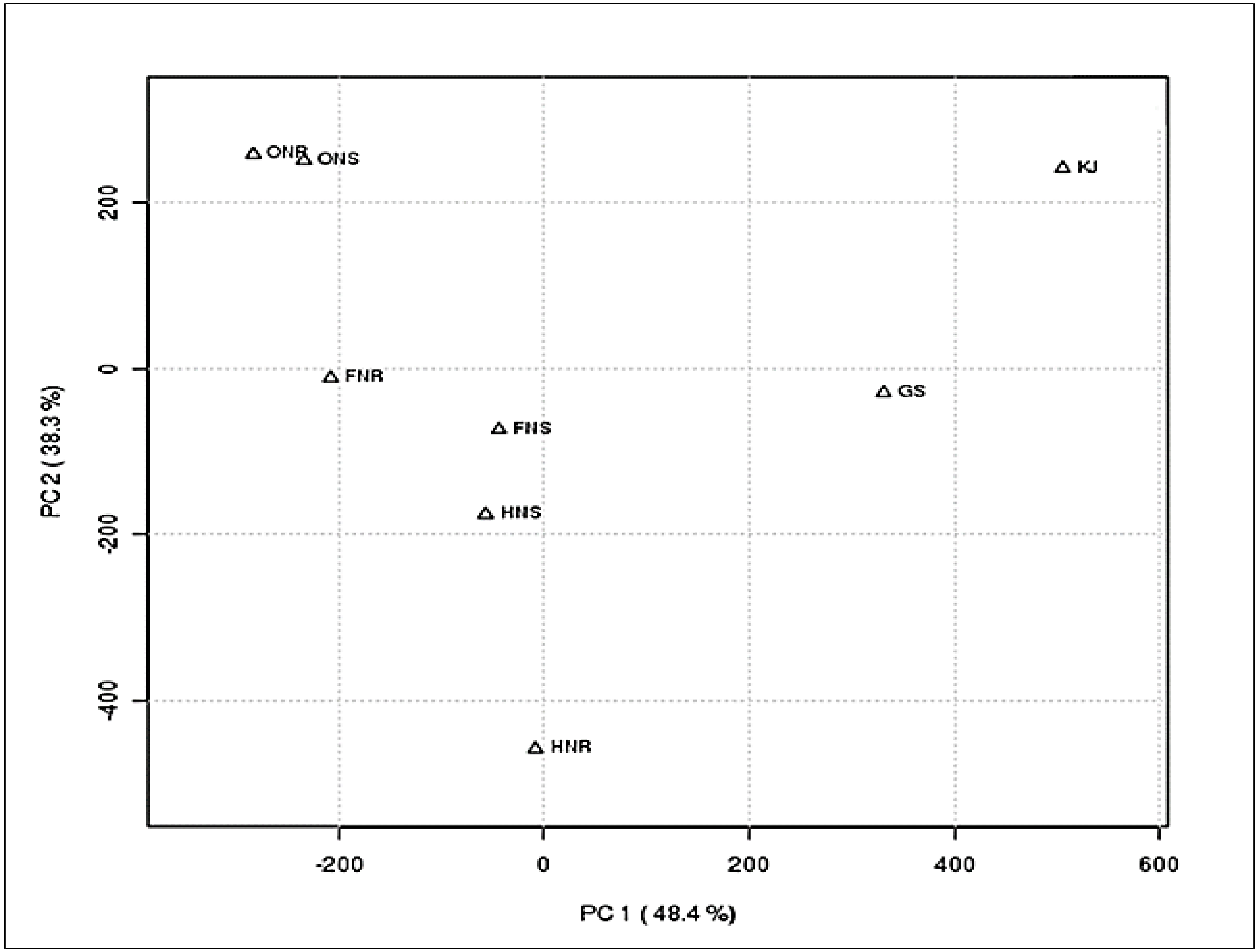


(C) Among seed and germinating seedling



(D) Among seed, root and shoot

PCA Plot Analysis



KJ- Seed
GS- Germinated seedling
0NR-Roots of 15 days plant grown without nitrogen
0NS- Shoots of 15 days plant grown without nitrogen
HNR-Roots of 15 days plant grown 348.75ppm nitrogen
HNS- Shoots of 15 days plant grown in 348.75ppm nitrogen
FNR-Roots of 15 days plant grown in 697.5ppm nitrogen
FNS- Shoots of 15 days plant grown in 697.5ppm nitrogen

Principal Component analysis illustrates differences between the bacterial communities in root, shoot and seed. Two first components (PC1 and PC2) were plotted and represented 86.7% of whole inertia

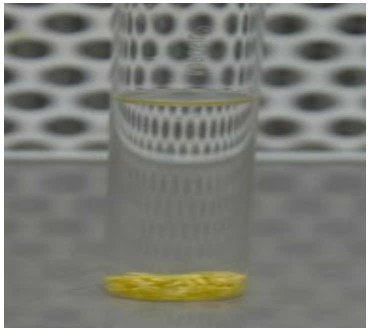
MATERIALS AND METHODS

OBJECTIVE 3:

Analysis of metabolic profile due to plant-endophyte interaction

Metabolic profile determination using GCMS-MS

Unsterile seeds



Surface sterilized seeds

Germinating seeds

Ground into fine powder

Workflow

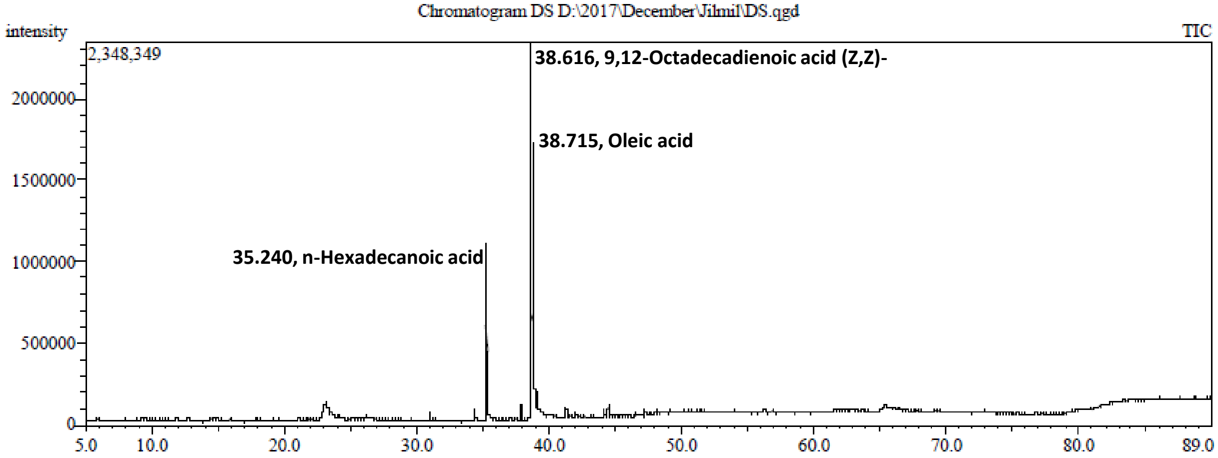
Solvent extraction in n-Hexane (for 72 hrs)

GCMS Analysis

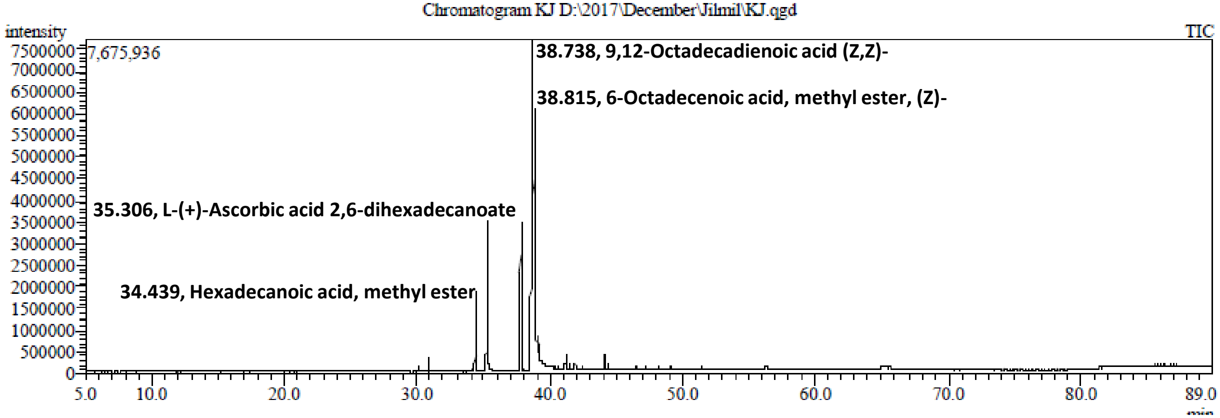
RESULTS

OBJECTIVE 3:
Analysis of metabolic profile due to plant-endophyte interaction

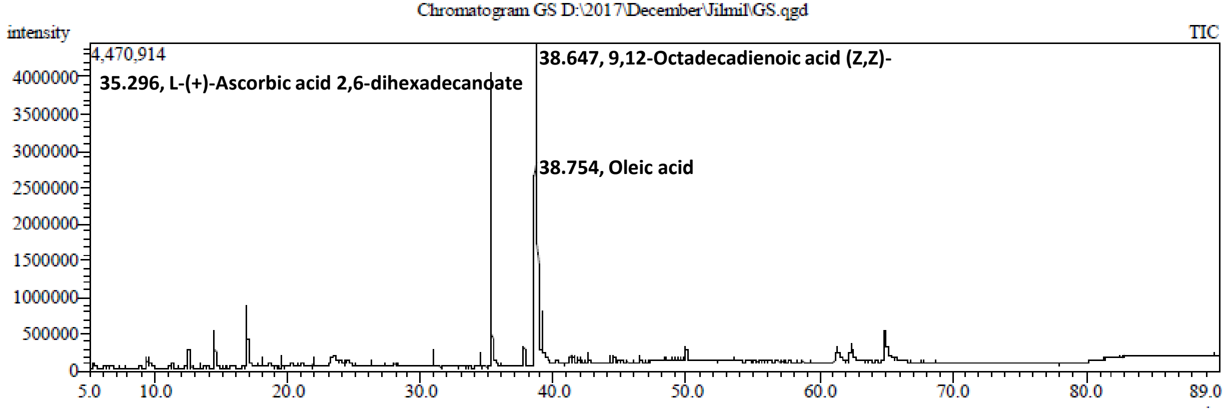
Chromatographic Analysis of Metabolites



Unsterilized dehusked dry rice seed

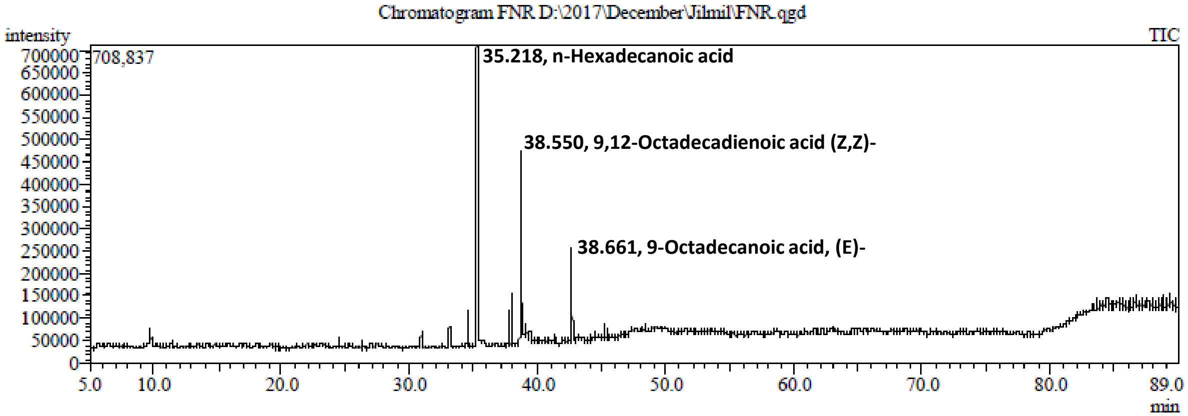


Dehusked Surface-sterilized rice seed

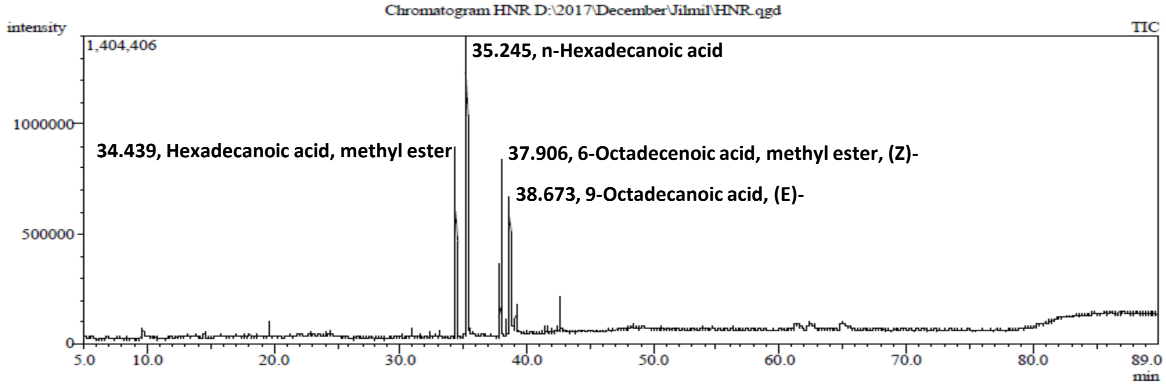


Germinated Surface-sterilized rice seed

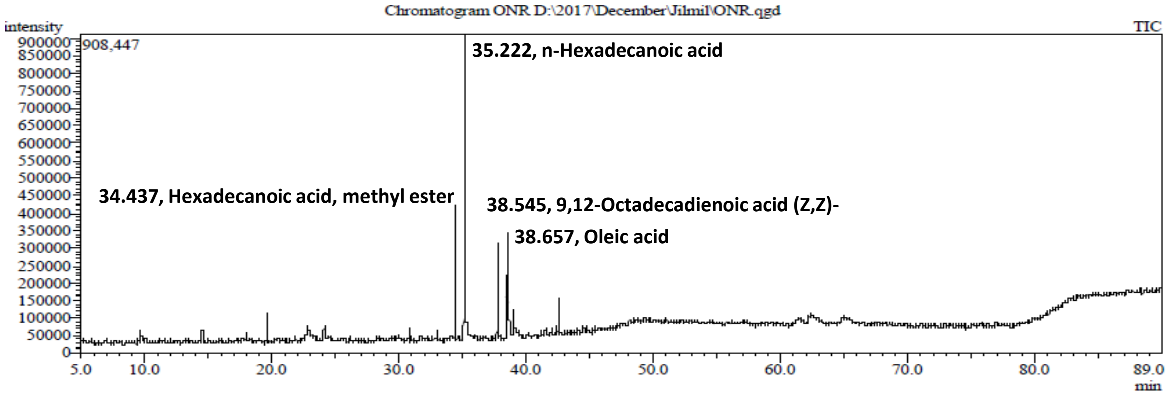
Chromatographic Analysis of Metabolites



Roots of 15 Days old plants grown in media with 697.5ppm nitrogen



Roots of 15 Days old plants grown in media with 348.75ppm nitrogen



Roots of 15 Days old plants grown in media with no nitrogen

Conclusion

Bacterial population showed distinct variation with respect to different organs (roots and shoots) and nitrogen concentration gradient during growth

γ-proteobacteria population was comparatively more in shoots than in roots and β-proteobacteria population was higher in roots as compared to shoots irrespective of nitrogen level (exception in HN- 348.75ppm/mL)

When growth media was supplemented with increasing concentration of nitrogen, population of cyanobacteria increased proportionately in shoots whereas, population of actinobacteria increased progressively in roots implying a positive correlation

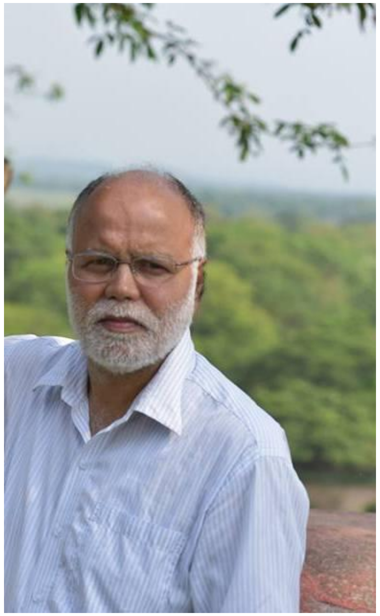
α-proteobacteria was found to be distinctly high in plants grown in intermediate level of nitrogen (348.75ppm/mL)

β-proteobacteria was found to be highest in plants where no nitrogen was provided

Conclusion

- **Distinct variation in few metabolites were found to change in seeds after surface sterilization and germination, for example: oleic acid, 9,12-Octadecadienoic acid (Z,Z)-, L-(+)-Ascorbic acid 2,6-dihexadecanoate**

- **Also some metabolites were found to vary in amount in roots of 15 days old plants with respect to gradient in nitrogen, for instance: oleic acid, 9,12-Octadecadienoic acid (Z,Z)-, 9-Octadecenoic acid, (E)-, n-Hexadecanoic acid**



Prof. N.C. Talukdar

Dr. Glyn Barrett and Prof. Robert Jackson

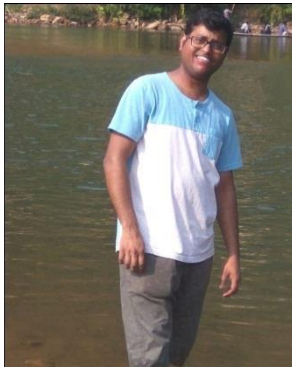
Garima Raj

Manashi Das

Thank You

Sujata Deka

Tamali Sinha



Mohd Shadab

Institute of Advanced Study in Science and Technology
Guwahati
1979

University of Gauhati

सत्यमेव जयते
Department of Science and Technology
Ministry of Science and Technology
Government of India

Newton-Bhabha Fund

University of Reading