

DIRECTORATE OF ADVANCED STUDIES
EVENT CATALOGUE
2021

4TH SEMINAR OF DAS EVENTS CALENDAR – 2021

**ENHANCEMENT OF TREE SEED GERMINATION AND
GROWTH UNDER CLIMATE CHANGE BY USING NOVEL
REGENERATION TECHNIQUES**



4th Seminar of DAS Event Calendar - 2021

**ENHANCEMENT OF TREE SEED GERMINATION AND GROWTH UNDER
CLIMATE CHANGE BY USING NOVEL REGENERATION TECHNIQUES**

Thursday, 4th March, 2021 at 02:00 pm

Venue: Agriculture Seminar Room & ZOOM - Meeting ID: 955 408 3170

Speaker:

Dr. Lubna Ansari
Assistant Professor
Department of Forestry & Range Management

Organizer:

Directorate of Advanced Studies
PMAS-AAUR

ACTIVITIES



Rational

- Total area of Pakistan.....
- Overall climate.....
- Forestry sector
- Climatic limitations
- Mostly area of country is arid/semi arid with average rainfall 300mm
- Sub humid and humid area is very less
- Limited towards North and North East
- Natural Forests only concentrated towards North and North West including KPK, AJK and parts of GB

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Albizzia lebbek

- Multi purpose tree
 - In Pakistan in grows in Sialkot to Hazara, Bajur, Buner and Malakand
- Fodder, fuel wood, Agricultural implements



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Albizzia procera

- Multipurpose tree specie
- The tree is native to Central and Southern India, Bangladesh and Burma
- In Pakistan it has been planted in Punjab and KPK



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Acacia modesta

- Multi purpose tree specie
- It is native to Pakistan, Afghanistan and India
- Slow growing
- Fodder, fuel, apiculture and gum



Acacia modesta

- Multi purpose tree specie
- It is native to Pakistan, Afghanistan and India
- Slow growing
- Fodder, fuel, apiculture and gum



Acacia nilotica

- This tree is native to Pakistan
- Multipurpose
- Apiculture, Gum extraction, tannin, fencing. Medicinal (bark for diarrhoea)



16S rRNA as a Biological Marker

- Bacterial isolation and identification of PGPR based upon 16S rRNA gene sequences, which is used to study bacterial phylogeny and taxonomy
 - All living organisms contain the small (16S or 18S) and the large (23S or 28S) subunit rRNA. these subunits are essential for protein synthesis
 - 16S rRNA is the RNA component of the small subunit of prokaryotic ribosomes. It's used in phylogenetics because it's very highly conserved.
- OR
- 16S rRNA, is a useful evolutionary chronometer to estimate the relationship of organisms.



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EFFECTS OF PGPRs ON Tree Growth

PGPRS	Tree	Parameters	References
Rhizobium	<i>Albizzia lebbek</i>	Growth enhanced and enrichment of rhizosphere	Bird <i>et al.</i> , 1985
Rhizobium	<i>Albizzia odianthifolia</i> <i>A.Zygia</i> , <i>A.Ferruginea</i>	Nodules elongated and bifurcate, palmate and fan shaped with elongate respectively	Wester and Hogberg, 1989
Bradyrhizobium	<i>Leucenea leucocephala</i>	Inoculation and effectiveness to check rhizobial specificity under green house conditions	Turk and Keyser, 1992
<i>Rhizobium galgae</i>	<i>Sesbania herbacea</i>	Isolated the nitrogen fixing rhizobial symbionts	Wang <i>et al.</i> , 1998

ISOLATION OF PGPR STRAINS

Steps Involved

Collection & Selection of Roots & Rhizosphere Soil



Surface Sterilization



Isolation of PGPR from Roots & Rhizosphere Soil



Preparation of Serial Dilution



Media Preparation



Obtaining Pure Culture



Gram Staining

1. Isolation & Purification of Bacteria

2. Morphological Characterization of Microorganisms



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Isolation & Purification of Bacterial Strains

From soil

- **Serial Dilution plate technique,**
- **Grown on media (LB) agar in sterilized petri plates.**
- **Incubation at 28°C for at least 24 hours.**

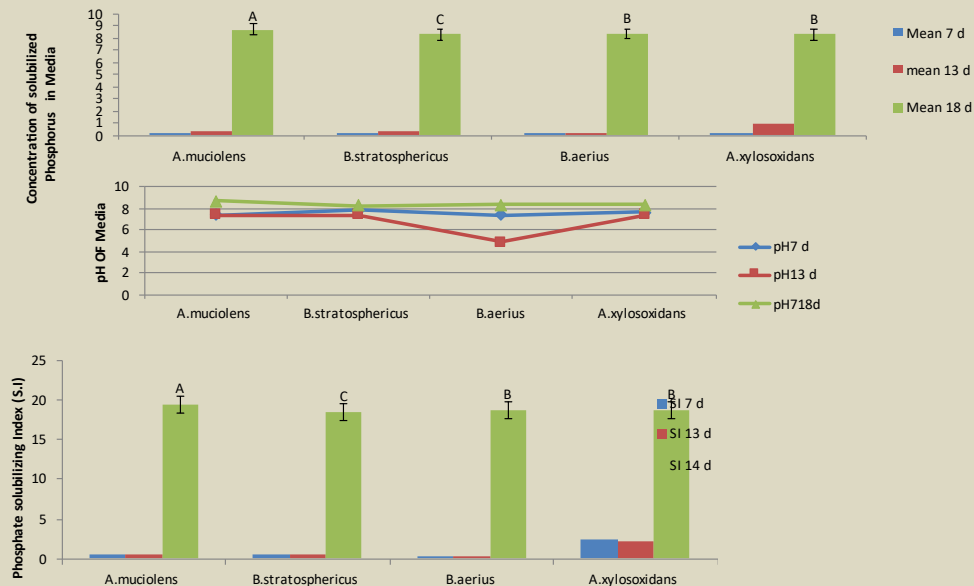
From Nodules

- **Healthy, unbroken and pink nodules were detached from roots.**
- **Surface sterilized 5% chlorox --95% ethanol for 5-10 seconds and washed with distilled water.**
- **Nodules were crushed, streaked on yeast extract mannitol (YEM) agar.**

Isolated strains were streaked and re-streaked several times till purified colonies were obtained.

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Phosphate Solubilization Index (SI), pH and amount of Phosphorous solubilized ($\mu\text{g/ml}$) by PGPR strains at 7, 13 and 18 d.



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HCN and Siderophore production

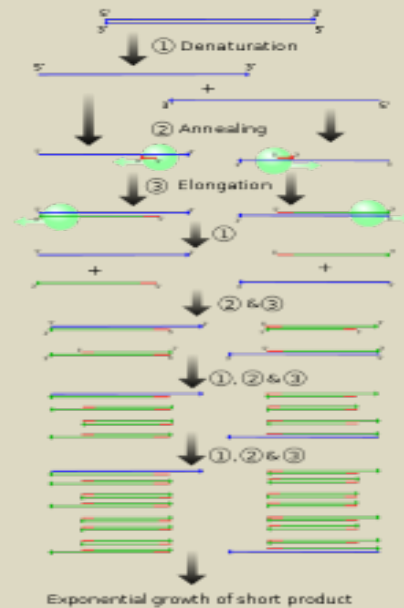


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DNA EXTRACTION AND AMPLIFICATION

DNA of all bacterial strains were extracted through PCR at 95 °C temperature for 10 min in thermal cycler. After this 50 µl PCR reaction volume was placed in the thermal cycler for DNA amplification

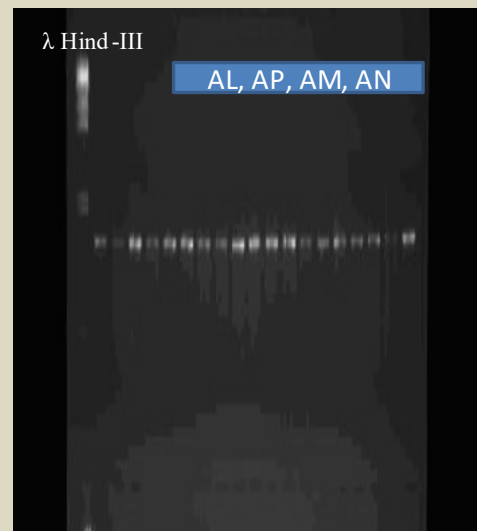
Polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence



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GEL ELECTROPHORESIS

- visualizing the amplification of DNA obtained from all strains.
- Figure confirmed the DNA amplification and purification
- DNA of all strains were carried out and stored for DNA sequencing.



GEL Electrophoresis of DNA strains isolated from tree rhizosphere

Molecular Characterization for Plant Growth Promoting Rhizobacteria

- nifH* Gene Amplification**

Identification using *nifH* primer and *nif* gene amplification by PCR (Poly et al. 2000)

Reverse primer (PoR ^b)	Forward primer (PoF ^b)
CCGTCAATTCCTTTGAGTTT	TGCGAYCCSAARGCBGACTC

- Production of Indole acetic acid (IAA)**

Assayed calorimetrically using ferric chloride-perchloric acid reagent (Brick et al. 1991)

- Oxidase, Catalase, Amylase, Protease & gram staining**

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Identification of Nodulated Bacteria

On the basis of PGP activities more than 50 potential strains were selected and identified by **16S rRNA gene sequencing**.

Polymerase chain reaction (PCR)

Genomic DNA extraction & amplification of 16S rRNA gene

Reverse primer	Forward primer
GGCTA CCTTGTTA CGA (1510R)	GA GTTTGA TCCTGGCTCAG (9F)
TACGGCTA CCTTGTTA CGA CTTCACCCC (P6)	AGA GTTTGA TCCTGGCTCAG AACGAACGCT (P1)

Gel Electrophoresis

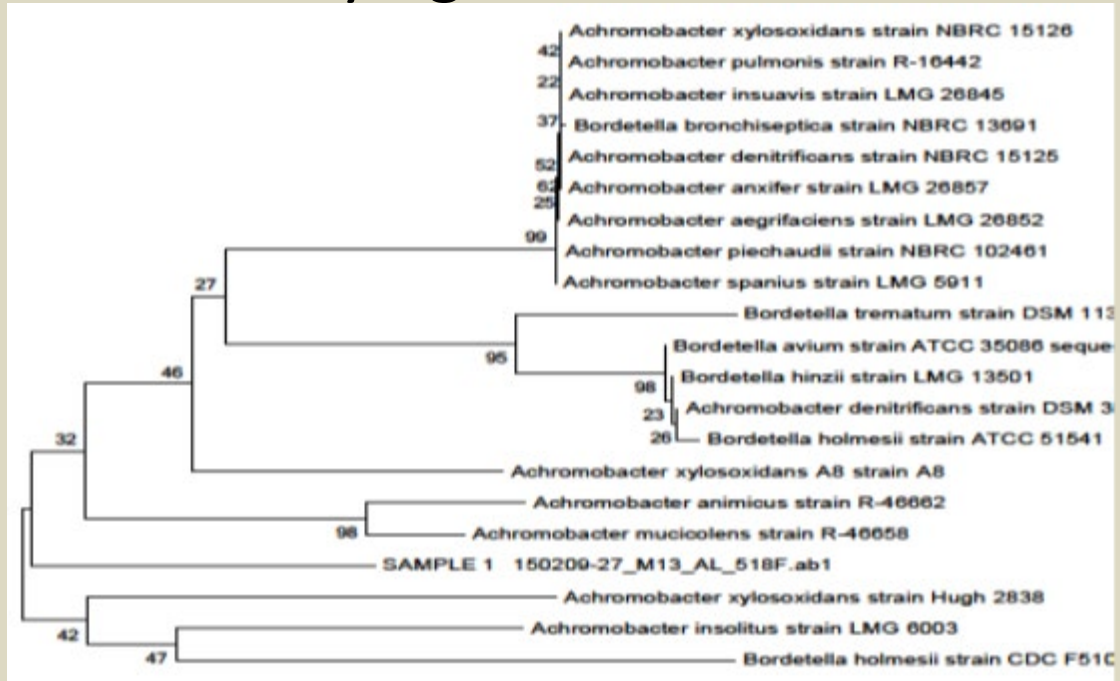
Confirmation of amplified PCR product

16S rRNA gene sequencing

Determine the order of the nucleotides for gene (MACROGEN, Korea)

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Phylogenetic Tree



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Nodules from field plants



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Leuceana leucocephala



- Isolation of Bacteria from rhizospheric soil of *Leuceana leucocephala*
- Promising fodder tree
- Leguminous nature nitrogen fixation
- Highly nutritive fodder, leaves CP 20-28%

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Zizyphus mauritiana



- Phytohormones production and FT-IR studies of bacteria Isolated from roots of *Zizyphus mauritiana*
- Most Important tree specie, fodder, fuel and honey production
- Govt. focusing on Zizyphus

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