Mitigation of drought stress in plants using PGPB

ARESEARCHPROJECT

Submittedby

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"DisseminationofeducationforKnowledge,Scienceandculture"

-ShikshanmaharshiDr.BapujiSalunkhe

ShriSwamiVivekanandShikshanSanstha`s

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OF

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Dr. Komal K. Bhise

Research Project Guide

Examiner

For

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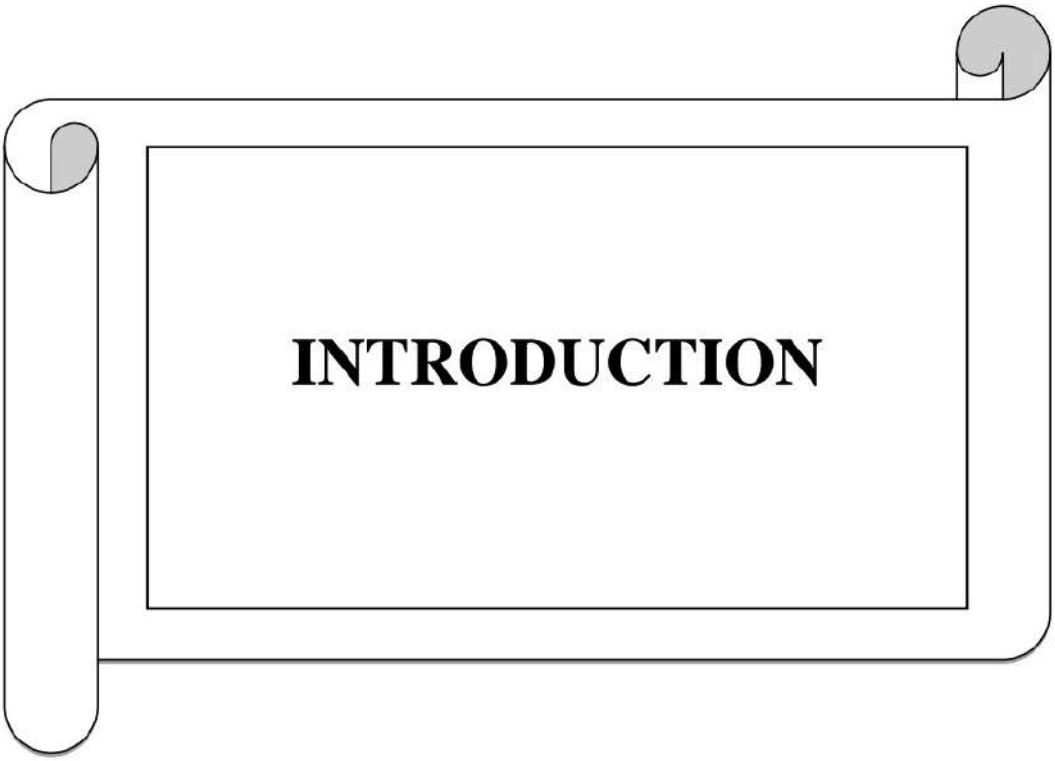


INTRODUCTION

Mitigation of drought stress in plants using PGPB

Introduction

Agriculture is fundamental to human survival and the global economy. As the primary source of food, it ensures food security for billions around the world. Beyond nourishment, agriculture supplies essential raw materials for industries such as textiles, pharmaceuticals, and biofuels, playing a key role in driving economic growth.

In many developing countries, agriculture serves as the backbone of employment—not only in farming but also in allied sectors like transportation, food processing, and retail. It supports rural economies, fosters development, and helps reduce urban migration by creating local job opportunities.

Sustainable agricultural practices are vital for preserving biodiversity and protecting natural resources, contributing to long-term environmental health. Moreover, agricultural exports significantly impact global trade, generating foreign exchange and helping stabilize national economies.

1. Soil Fertility and Nutrient Cycling

Microorganisms play a vital role in maintaining soil fertility by breaking down organic matter and recycling nutrients essential for plant growth. Their activities contribute to a healthier and more productive soil environment. Key ways in which microorganisms support soil health include:

Decomposition: Microbes break down plant residues, animal waste, and other organic materials into simpler compounds. This process releases essential nutrients—such as nitrogen, phosphorus, and potassium—back into the soil, enriching it naturally.

Nitrogen Fixation: Certain bacteria, including *Rhizobium and Azotobacter*, convert atmospheric nitrogen into a form that plants can absorb. Since nitrogen is a critical nutrient for plant development but is inaccessible in its atmospheric form, this microbial process is essential for plant nutrition.

Phosphorus Solubilization: Microorganisms such as *Penicillium and Pseudomonas* help make phosphorus more available to plants by breaking down complex, insoluble forms of this nutrient in the soil.Through these processes, microorganisms not only enhance soil fertility but also promote sustainable and eco-friendly agricultural practices.

2. Plant Growth Promotion

Certain microorganisms actively promote plant growth by producing substances that improve root development and enhance nutrient uptake. Key contributors to this process include:

Plant Growth-Promoting Rhizobacteria (PGPR): Beneficial bacteria such as Bacillus and Pseudomonas colonize plant roots and support growth by producing natural plant hormones like auxins, which stimulate root elongation. They also release siderophores—compounds that bind and transport iron—making it more accessible to plants.

Mycorrhizal Fungi: These fungi form symbiotic associations with plant roots, significantly extending the root system's reach. This expanded network improves water and nutrient absorption, particularly phosphorus, while also enhancing the plant's tolerance to environmental stress.

3. Disease and Pest Control

Microorganisms play a significant role in protecting crops from diseases and pests, offering a natural alternative to chemical pesticides. They help maintain plant health through various mechanisms, including:

Biocontrol Agents: Certain beneficial bacteria and fungi naturally suppress plant pathogens. For example, Trichoderma fungi combat root rot and other fungal diseases by either outcompeting harmful microbes or directly attacking them.

Endophytes: These are microorganisms—bacteria or fungi—that live within plant tissues without causing harm. They enhance the plant's resistance to diseases and pests by producing protective compounds that deter pathogens and herbivores.

Insect Pathogens: Some microbes, like Bacillus thuringiensis (Bt), produce toxins that are lethal to specific insect pests but safe for humans and animals. Bt is widely used in organic farming as a natural and effective bioinsecticide.

By offering natural protection, these microorganisms reduce the reliance on synthetic chemicals, contributing to safer and more sustainable agricultural practices.

4. Enhancing Soil Structure

Microorganisms, particularly bacteria and fungi, play a key role in improving soil structure by producing substances that bind soil particles into aggregates. These aggregates enhance soil aeration, water infiltration, and root penetration, creating a healthier environment for plant growth. For example, fungal hyphae—long, thread-like structures—act like natural fibers that weave through the soil, binding particles together and promoting a loose, crumbly texture that benefits root development.

5. Biofertilizers and Biopesticides

Microorganisms are increasingly being used to develop biofertilizers and biopesticides—sustainable and eco-friendly alternatives to chemical inputs in agriculture.Biofertilizers: These contain living microorganisms that enhance soil fertility by improving nutrient availability. For instance, *Azospirillum* bacteria in biofertilizers fix atmospheric nitrogen, making it accessible to plants, while phosphate-solubilizing bacteria enhance the uptake of phosphorus—both essential nutrients for plant growth.Biopesticides: Derived from beneficial microorganisms, these products specifically target harmful pests without affecting beneficial organisms or the environment.

6. Mechanisms of PGPB in Drought Mitigation

PGPB help plants cope with drought through several beneficial mechanisms:

- Phytohormone Production: PGPB produce hormones like IAA, gibberellins, and cytokinins that enhance root growth and water uptake, aiding plant survival in dry soils (Spaepen et al., 2007; Ali et al., 2014).
- ACC Deaminase Activity: By breaking down ACC, a precursor to ethylene, PGPB reduce stress-induced ethylene levels, promoting healthier root development under drought (Glick, 2014).
- Siderophore Production: These iron-binding compounds improve iron availability, essential for photosynthesis and stress tolerance (Ma et al., 2011).
- Exopolysaccharide (EPS) Production: EPS-producing bacteria improve soil aggregation and water retention, creating a moisture-rich zone around plant roots (Sandhya et al., 2009).
- Nutrient Solubilization: PGPB enhance the availability of key nutrients like phosphorus and potassium, supporting plant nutrition and growth under water-limited conditions (Rodriguez et al., 2004)
- Together, these mechanisms strengthen plant resilience to drought while

enriching soil fertility and health.

7. Importance of PGPB in Drought Management

- PGPB play a vital role in sustainable drought management through the following benefits:Environmental Sustainability: PGPB reduce dependence on chemical fertilizers and excessive irrigation, conserving natural resources and minimizing agriculture's environmental impact (Adesemoye et al., 2009).
- Improved Soil Health: By supporting microbial diversity and enhancing nutrient cycling, PGPB improve soil structure and fertility, promotinglong-term soil sustainability (Beneduzi et al., 2012).
- Enhanced Crop Resilience: PGPB boost water-use efficiency and nutrient uptake, helping plants withstand drought stress and maintain stable yields under challenging climate conditions (Nadeem et al., 2014).

8.Biofertilizer Production Using Our Potent Organisms

We have developed a biofertilizer using potent microorganisms with plant growth-promoting properties. The process involved the following steps:

1. Selection of Potent Organisms: We selected microorganisms known for their ability to fix nitrogen and solubilize phosphorus which are vital for enhancing soil fertility and plant growth.

2. Culture Preparation: The microorganisms were cultured in nutrient-rich media under sterile conditions to achieve a high population density, ensuring their effectiveness

3. Formulation of the Biofertilizer: The cultured microorganisms were concentrated.We Can mixit with a carrier material like peat, which stabilized the organisms for soil application.

9.Seed Germination and Pot Trials

We conducted both seed germination and pot trials to evaluate the effectiveness of the biofertilizer.

1. Seed Germination Trial:

To assess the biofertilizer's effect on seed Germination. Seeds were soaked in the biofertilizer suspension before planting, and we compared the germination rate, root and shoot length, and seedling vigor index with a control group.

2. Pot Trials:

To evaluate the biofertilizer's impact on plant growth and drought tolerance. We planted crops in pots, applying the biofertilizer to some while leaving others as controls. Drought stress was induced by withholding water, and we monitored plant health, root development, chlorophyll content, and overall growth.

REVIEW OF LITERATURE

Review of Literature

1. Drought Stress in Agricultural Soils

Drought stress is one of the primary abiotic factors limiting agricultural productivity globally. Reduced water availability in soils leads to stunted plant growth, nutrient deficiencies, and diminished crop yields. According to recent studies, drought conditions not only affect plant physiology but also degrade soil structure and diminish microbial biodiversity, further aggravating the challenge of achieving sustainable crop yields under water scarcity (Farooq et al., 2009). This has led to a growing interest in identifying sustainable, cost-effective solutions to mitigate drought effects on crops, especially in light of climate change, which is expected to increase the frequency and intensity of droughts.

2. PGPB:

Plant Growth-Promoting Bacteria (PGPB) are a group of naturally occurring, beneficial microorganisms known for their ability to enhance plant growth and stress tolerance. Researchers have classified PGPB into free-living, symbiotic, and endophytic categories based on their relationship with plants (Glick, 2012). PGPB support plant growth through various mechanisms, including nutrient solubilization, hormone modulation, and pathogen inhibition. Over recent decades, PGPB have gained recognition as an eco-friendly alternative to synthetic inputs in agriculture, offering sustainable benefits such as improved crop resilience to abiotic stresses like drought (Vejan et al., 2016).

❖ ACC Deaminase Activity:

ACC deaminase is an enzyme produced by certain soil microorganisms that plays an important role in helping plants cope with various environmental stresses, including drought, salinity, and heavy metal toxicity. Here's a detailed overview of ACC deaminase activity and its significance:

1. Role and Mechanism of ACC Deaminase

ACC deaminase catalyses the breakdown of ACC, which is the immediate precursor of the plant hormone ethylene. Ethylene is produced in plants as a response to stress and has various functions in growth and development. However, high ethylene levels, especially under stress conditions, can inhibit root growth and compromise plant health.

The reaction catalysed by ACC deaminase is as follows:

$$\text{ACC} \longrightarrow \text{Ammonia } (NH_3) + \text{alpha-ketobutyrate}$$

$$C_4H_7NO_2 \longrightarrow C_4H_6O_3 + NH_3$$

Through this reaction, ACC deaminase-producing bacteria can lower ACC levels, reducing ethylene synthesis in plants. This, in turn, alleviates stress and allows the plant to maintain normal growth patterns.

2. Sources of ACC Deaminase

ACC deaminase is found in several PGPB, particularly those associated with the rhizosphere (the root zone), where they interact with plant roots. Common genera of bacteria that produce ACC deaminase include:

Pseudomonas, Rhizobium,Azospirillum, Burkholderia,Bacillus,Enterobacter

These bacteria can form beneficial associations with plants, often attaching to or colonizing root surfaces where they can interact directly with root-produced ACC.

3. Impact on Plant Growth Under Stress Conditions

Ethylene, while essential in small amounts, can inhibit root growth when produced in excess. This is particularly problematic under stress conditions, such as drought, salinity, or flooding, where ethylene levels tend to rise. High ethylene can lead to:

- Reduced root elongation
- Premature leaf senescence (aging)
- Decreased chlorophyll content, affecting photosynthesis
- Increased susceptibility to further environmental stresses

By lowering ethylene levels, ACC deaminase helps plants to:

Promote Root Elongation: With lower ethylene, roots can grow deeper into the soil, which is especially beneficial under drought conditions.Enhance Stress Tolerance: Plants with lower ethylene levels tend to be more resilient to stress conditions, as they can allocate resources to growth rather than just stress responses.Improve Nutrient and Water Uptake: A healthy root system enhances the plant's ability to absorb nutrients and water, even under challenging environmental conditions.

4. Application in Agriculture

ACC deaminase-producing bacteria have become a valuable tool in sustainable agriculture. These microbes are often used in biofertilizers and bio stimulants aimed at improving crop productivity, especially in stress-prone areas. Some key agricultural benefits include:Drought Resistance: In arid or semi-arid regions, ACC deaminase-producing bacteria can support plants by minimizing the adverse effects of drought on growth.Soil Health and Reduced Chemical Inputs: By promoting plant growth naturally, these bacteria reduce the need for synthetic fertilizers and other chemicals, supporting soil health.

Yield Stabilization: In conditions of environmental stress, such as high salinity or heavy metal toxicity, these bacteria help stabilize crop yields by maintaining plant health.

5. Research and Advancements

Recent research has focused on isolating and genetically improving ACC deaminase-producing strains to enhance their effectiveness in stress management. Advances include: Genetic Engineering: Creating microbial strains with enhanced ACC deaminase activity to better support plant growth under extreme stress.Formulating Consortia: Combining ACC deaminase-producing bacteria with other beneficial microbes, such as phosphate-solubilizing bacteria, to create more effective biofertilizers.

❖ Qualitative & Quantitative analysis of PhosphateSolubilisation :-

Phosphate solubilization refers to the process by which certain microorganisms, mainly bacteria and fungi, convert insoluble forms of phosphate into soluble forms that plants can absorb. This ability is particularly significant for agriculture, as phosphate is a crucial nutrient for plant growth, yet is often present in soils in forms that plants cannot readily access. Here are some details about the process and its significance:

➢ Mechanism of Phosphate Solubilization

1. Organic Acid Production: Many phosphate-solubilizing microorganisms (PSMs) produce organic acids, such as gluconic acid, citric acid, and lactic acid, which acidify the soil microenvironment. These acids chelate cations bound to phosphate, thereby releasing the phosphate into a soluble form.
2. Enzyme Secretion: Certain enzymes, like phosphatases, are secreted by these microbes, which helps in breaking down organic forms of phosphate into inorganic phosphate that plants can take up.
3. Acidification: PSMs often lower the pH of their surroundings by producing protons, further aiding the dissolution of insoluble phosphate minerals.

➢ Types of Phosphate-Solubilizing Microorganisms

Bacteria: Genera such as *Pseudomonas, Bacillus,* and *Rhizobium* are known for their phosphate-solubilizing abilities. Fungi: Fungi like *Aspergillus* and *Penicillium* are also recognized for their effectiveness in phosphate solubilization. *Actinomycetes*: Though less common, *Actinomycetes* also contribute to phosphate solubilization.

➢ Importance in Agriculture

Improved Crop Yield: By making phosphate more accessible to plants, PSMs enhance plant growth and productivity.Reduced Fertilizer Usage: Phosphate-solubilizing microbes can reduce the dependency on chemical fertilizers, promoting more sustainable agricultural practices.

Soil Health: These microbes also contribute to soil biodiversity and improve soil health over time.

❖ Quantitative Estimation of Phosphate

Methods for Quantitative Phosphate Analysis

The most common methods for quantitative phosphate analysis include colorimetric, spectrophotometric, and ion chromatography techniques.

Colorimetric Method: This method involves the reaction of phosphate with a molybdate reagent to form a blue complex, which is measured spectrophotometrically. This method is widely used for analyzing soil and plant tissue extracts (Murphy and Riley, 1962).

Spectrophotometric Method: In this method, the concentration of phosphate in plant tissues or soil extracts is determined by measuring absorbance at a specific wavelength, typically 880 nm, after the formation of a molybdenum-blue complex (Van Veldhoven and Mannaerts, 1987).

❖ Nitrogen fixation Ability

Nitrogen fixation is a crucial biological process in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3), making nitrogen accessible to plants in a form they can use for growth. Plants need nitrogen as a nutrient, but they cannot directly absorb atmospheric nitrogen. Therefore, certain microorganisms, mainly bacteria, perform this fixation process and significantly contribute to soil fertility and sustainable agriculture.

➢ Mechanism of Nitrogen Fixation

Enzyme Nitrogenase: Nitrogen-fixing bacteria produce the enzyme nitrogenase, which catalyses the conversion of nitrogen gas to ammonia. This enzyme functions under anaerobic conditions, as it is highly sensitive to oxygen.

Symbiotic Nitrogen Fixation: In this type, bacteria, particularly *Rhizobium* and *Bradyrhizobium*, form symbiotic relationships with leguminous plants. They infect the root nodules, where they fix nitrogen in exchange for carbohydrates from the plant.

Free-Living Nitrogen Fixers: Some bacteria, like *Azotobacter* and *Clostridium*, do not require a host plant to fix nitrogen and instead function independently in the soil.

Associative Nitrogen Fixation: Other bacteria, such as *Azospirillum*, form loose associations with the roots of non-leguminous plants, enhancing nitrogen fixation in the rhizosphere.

➢ Importance in Agriculture

Enhanced Crop Yield: By increasing the nitrogen available to plants, nitrogen-fixing organisms promote plant growth and productivity.Reduction in Nitrogen Fertilizers: With the help of nitrogen-fixing bacteria, farmers can reduce the amount of nitrogen fertilizer required, which lowers costs and minimizes environmental pollution.

Soil Health: Nitrogen-fixing bacteria improve soil structure and fertility, contributing to long-term soil health and sustainability.

❖ Production of Phytohormones:

Certain PGPB strains produce phytohormones like indole-3-acetic acid (IAA), gibberellins, and cytokinin, which play essential roles in root growth, water uptake, and overall plant development under stress. IAA, for instance, stimulates root elongation, allowing plants to access deeper water sources in drought-prone soils (Spaepen et al., 2007).

❖ IAA Production Ability

Indole-3-acetic acid (IAA) production is a key trait in plant growth-promoting microorganisms, especially bacteria and fungi associated with plant roots. IAA is a form of auxin, a plant hormone that plays an essential role in plant growth and development, including root elongation, cell differentiation, and stress response. The ability of microorganisms to produce IAA can significantly impact plant health, making it a valuable area of study for agricultural applications.

❖ Importance of IAA-Producing Microorganisms in Agriculture

Enhanced Root Growth: Microbial IAA can stimulate plant root elongation and branching, allowing plants to absorb nutrients and water more efficiently.Stress Tolerance: IAA-producing microbes can help plants endure abiotic stresses, such as drought and salinity, by enhancing root structure and function.Plant Growth Promotion: Microorganisms with IAA-producing capabilities are commonly used as biofertilizers to promote sustainable agriculture, reducing the need for synthetic chemicals.

❖ Examples of IAA-Producing Microorganisms

1)*Rhizobium spp*

2)*Azospirillum brasilense*

3)*Pseudomonas fluorescens*

❖ Gibberellins:-

A class of plant hormones, are crucial for regulating a wide range of growth and developmental processes. They were first discovered in the fungus *Gibberellafujikuroi*, which infected rice plants and caused exaggerated growth. Gibberellins (GAs) are found throughout the plant kingdom and play a key role in growth regulation, influencing processes from seed germination to flowering and fruit development.

2. Agricultural Applications of Gibberellins

- Gibberellins are used commercially to regulate growth and improve yield in various crops:Seedless Fruits: GAs are applied to promote the development of seedless grapes and other fruits.
- Growth Promotion: In crops like sugarcane, GAs are used to boost growth rates and sugar content.
- Malting in Barley: GAs are used to enhance enzyme production in barley, a key step in the beer-making process.
- Overcoming Dormancy: Applied to seeds, bulbs, and tubers to stimulate germination and sprouting in commercial agriculture.

❖ Siderophore Production:

Microorganisms: Many bacteria and fungi produce siderophores to survive in iron-limited environments. Pathogens like *Escherichia coli, Pseudomonas aeruginosa, and Aspergillus fumigatus* are well-known for their siderophore production, which helps them to acquire iron and thrive in hostile environments, including within host organisms.

Mechanism: Siderophores chelate iron with high affinity and transport it into the cells through specific receptors. This mechanism not only aids in iron acquisition but also contributes to an organism's survival and virulence, particularly in pathogenic microbes.

Applications:

Agriculture: Beneficial bacteria like *Pseudomonas fluorescens* produce siderophores that promote plant growth by making iron more bioavailable in the rhizosphere.Biomedicine: The inhibition of siderophore synthesis or uptake in pathogenic bacteria is being explored for its potential in new antimicrobial therapies.

❖ Exopolysaccharide (EPS) Production:

Exopolysaccharides (EPS) are high-molecular-weight polysaccharides secreted by microorganisms, including bacteria, fungi, and algae, into their surrounding environment. EPS play a crucial role in microbial interactions with plants, soil, and other organisms. They contribute to microbial adhesion, biofilm formation, and environmental stress tolerance, such as in drought conditions. In the context of PGPB,EPS are particularly beneficial for plant health, especially under stress.

1. Role of EPS in Plant Stress Tolerance

Drought Tolerance: Under drought conditions, EPS-producing PGPB are particularly beneficial: Moisture Retention in Rhizosphere: EPS increase the water-holding capacity of the soil around plant roots, maintaining moisture availability and creating a more stable microenvironment. Reduction of Hydraulic Conductance: EPS form a viscous layer in the rhizosphere that minimizes rapid water loss, slowing down water evaporation from the soil. Salt Stress Alleviation: EPS can help plants tolerate salinity by binding and sequestering ions in the soil, reducing the uptake of toxic ions by plants.

2.Influence of EPS on Soil Structure and Fertility

Soil Aggregation and Stability: EPS secreted by soil microbes, particularly in the rhizosphere, bind soil particles and contribute to the formation of stable soil aggregates.

Improved Aeration and Root Growth: By stabilizing soil structure, EPS help improve soil aeration and allow better root growth, which is essential for healthy plant development.

Organic Matter Accumulation: EPS contribute to the accumulation of organic matter in soils, enhancing soil fertility and microbial activity.

Carbon Sequestration: EPS are carbon-rich compounds, which contribute to the organic carbon pool in soil, potentially aiding in carbon sequestration.

3. Agricultural Applications of EPS-Producing Bacteria

Drought-Resistant Biofertilizers: EPS-producing bacteria are being explored for use as biofertilizers, especially in drought-prone areas. These bacteria enhance soil water retention, promote root growth, and improve plant resilience to water scarcity.

Soil Stabilizers: EPS-producing bacteria may be used in soil stabilization projects to improve soil structure in degraded or sandy soils, making them more suitable for agriculture.

Phytoremediation: EPS-producing bacteria can be applied in phytoremediation, particularly in contaminated soils with heavy metals or high salt levels. By immobilizing toxins, they help plants grow in adverse conditions.

❖ Mechanisms of Ammonia Production by PGPB under Drought Stress

1. Biological Nitrogen Fixation (BNF):

Many PGPB, particularly nitrogen-fixing bacteria such as Azospirillum, Rhizobium, and Bacillus species, possess the ability to fix atmospheric nitrogen and convert it into ammonia, which plants can utilize for growth. This process is highly beneficial under drought stress as the availability of nitrogen becomes a limiting factor in plant growth. Ammonia produced by these bacteria can be directly utilized by plants or further processed into other nitrogen forms. Under drought stress, this nitrogen provision is critical for maintaining plant metabolism and growth, especially when other sources of nitrogen are limited (Glick, 2012).

2. Ammonia as a Stress Mitigator:

Ammonia produced by PGPB can serve as a signaling molecule, modulating plant responses to stress. In drought conditions, ammonia may help to alleviate oxidative stress by reducing the accumulation of reactive oxygen species (ROS). Additionally, ammonia may stimulate the synthesis of plant hormones like cytokinins, which are known to improve plant tolerance to water deficit (Bhattacharyya and Jha, 2012). Moreover, the production of ammonia by PGPB can enhance plant water retention and root development, aiding in drought tolerance (Vessey, 2003).

3. Ammonia and Root System Development:

Under drought conditions, plants often exhibit stunted root growth due to water stress. Ammonia, when produced by PGPB, can help alleviate this issue by promoting root development and lateral root formation, ensuring better access to water and nutrients. Studies have shown that PGPB like Bacillus and Pseudomonas species can increase root mass and depth in drought-stressed plants by releasing ammonia, which stimulates root growth (Rao et al., 2010).

4. Mechanisms of Ammonia Production:

The ammonia production by PGPB under drought conditions is facilitated by the nitrogenase enzyme complex in nitrogen-fixing bacteria, which converts nitrogen gas into ammonia. In non-leguminous plants, PGPB with nitrogenase activity can provide a continuous supply of ammonia. Other PGPB, such as Bacillus and Pseudomonas, produce ammonia via the enzymatic conversion of amino acids or from atmospheric nitrogen, especially when environmental stress factors like drought enhance the need for alternative nitrogen sources (Khan et al., 2014).

❖ Mechanisms of HCN Production under Drought Stress

1. Plant Production of HCN:

In plants, HCN is primarily produced by the enzymatic conversion of cyanogenic glucosides, such as linamarin and lotaustralin, through the action of cyanogenic enzymes like β-glucosidases and hydroxy nitrile lyases (Møller et al., 2001). During drought stress, plants might increase the production of these compounds as part of their defensive strategy against herbivores and pathogens, as HCN is toxic to many organisms (Katrin et al., 2004). The breakdown of cyanogenic compounds into HCN could also be triggered by oxidative stress, which is more prevalent under drought conditions (Pilon-Smits et al., 2009).

2. Microbial Production of HCN:

Several microorganisms, particularly certain strains of Pseudomonas and Aspergillus, produce HCN under drought stress. This production is usually facilitated by the activity of cyanide hydratase enzymes, which catalyze the conversion of nitriles into HCN (Haug et al., 2005). In drought-stressed environments, microbial strains often exhibit increased HCN production, potentially as a mechanism for adapting to limited water availability or as a way to suppress competing microbes through toxicity (Becerra et al., 2011).

3. Environmental and Physiological Impacts:

The production of HCN under drought conditions has significant physiological implications for both plants and microorganisms. In plants, elevated HCN levels can lead to reduced growth and impaired photosynthesis, especially if water is not replenished (Haug et al., 2005). Additionally, HCN production may interfere with the normal functioning of enzymes and cellular structures, leading to further stress and possible cell death (Hochschild et al., 2006).

4. Role of HCN in Stress Tolerance:

Interestingly, HCN production can also play a role in increasing tolerance to drought. Some studies have shown that plants with higher levels of HCN production exhibit improved tolerance to environmental stresses, such as drought and salinity, possibly by enhancing the plant's ability to manage oxidative stress (Pilon-Smits et al., 2009). Microorganisms producing HCN can also promote plant growth under drought conditions by enhancing nutrient availability or by inhibiting pathogens that might otherwise cause disease (Becerra et al., 2011).

Plant growth-promoting bacteria (PGPB) are known for their beneficial role in enhancing plant growth under various abiotic stresses, including drought. One of the key ways in which PGPB can aid in plant survival under drought conditions is through the production of ammonia (NH_3). Ammonia plays a significant role in promoting plant growth by acting as a nitrogen source and influencing plant metabolic processes.

❖ Seed Germination in Petri Plate

Seed germination is a fundamental process in plant development and is commonly studied in laboratory settings using Petri plates to ensure controlled conditions. In this method, seeds are sterilized (usually with 0.1% $HgCl_2$ or 70% ethanol) to prevent microbial contamination, then placed on moistened filter paper inside a sterile Petri plate. The plates are incubated in a growth chamber or at an optimal temperature (typically between 22–28°C) for several days. Water or nutrient solution is added regularly to maintain moisture. Germination is monitored daily, and parameters such as radicle emergence, shoot length, and germination rate are recorded for analysis. This setup allows researchers to observe the direct effects of various treatments on germination under controlled, replicable conditions (Bewley et al., 2013; ISTA, 2020; Taiz et al., 2015).

❖ Pot Trials

Pot trials are commonly used in drought stress research to simulate controlled water-deficit conditions and evaluate plant responses under such stress. These trials allow precise manipulation of environmental factors such as soil moisture, nutrient availability, and temperature. In drought-stressed pot experiments, researchers often limit water availability at specific growth stages to assess morphological, physiological, and biochemical adaptations of plants. Pot trials are particularly valuable in screening crop genotypes for drought tolerance due to their reproducibility, space efficiency, and cost-effectiveness (Blum, 2011; Passioura, 2006). They also facilitate close monitoring of root traits, water use efficiency, and osmotic adjustment, which are critical in understanding plant drought responses (Farooq et al., 2009). Despite their limitations in mimicking field conditions perfectly, pot trials remain an essential preliminary step before field evaluation under drought (Vadez et al., 2014).

❖ Chlorophyll :

1. Impact of Drought on Chlorophyll Biosynthesis

Drought stress inhibits chlorophyll biosynthesis by disrupting the uptake of essential nutrients such as nitrogen, magnesium, and iron, which are critical components of the chlorophyll molecule (Anjum et al., 2011). It also downregulates the activity of enzymes like aminolevulinic acid (ALA) synthase, which plays a central role in the chlorophyll biosynthetic pathway (Farooq et al., 2009).

2. Enhanced Chlorophyll Degradation

Under drought conditions, chlorophyll degradation is accelerated due to the upregulation of enzymes such as chlorophyllase, pheophytinase, and Mg-dechelatase, leading to the dismantling of the chlorophyll molecule (Khanna-Chopra &Selote, 2007). Additionally, drought stress leads to increased production of reactive oxygen species (ROS), which cause oxidative damage to chloroplast membranes and pigments (Gill & Tuteja, 2010).

3. Structural Damage to Chloroplasts

Drought-induced oxidative stress affects the ultrastructure of chloroplasts, especially thylakoid membranes where chlorophyll resides. This disrupts pigment–protein complexes and leads to leakage and photobleaching of chlorophyll (Ashraf & Harris, 2013).

4. Physiological Effects and Visible Symptoms

Chlorosis: Yellowing of leaves due to the breakdown of chlorophyll.

Reduction in Photosynthetic Efficiency: Lower chlorophyll content reduces light-harvesting capacity.

Stunted Growth: Diminished photosynthesis leads to reduced biomass and yield.

Early Senescence: Drought often triggers premature aging and leaf fall, reducing the photosynthetic period (Flexas et al., 2004).

5. Adaptive Responses in Plants

Plants activate several defense mechanisms to minimize chlorophyll loss:

Antioxidant enzyme systems (SOD, CAT, APX) reduce oxidative damage (Mittler, 2002).

Accumulation of Osmo protectants like proline and glycine betaine stabilize proteins and membranes (Ashraf &Foolad, 2007).

Gene expression: Drought-responsive genes such as stay-green (SGR) help retain chlorophyll longer under stress (Thomas & Howarth, 2000).

6. Measurement and Applications

Chlorophyll content can be measured using:

Spectrophotometry: Based on pigment extraction and absorbance at specific wavelengths (Arnon, 1949).Chlorophyll stability under drought is used as a selection trait in breeding programs for drought-tolerant cultivars (Blum, 2011).

❖ RhizosphericCompetency :-

Rhizospheric competency refers to the ability of introduced microorganisms, particularly Plant Growth-Promoting Bacteria (PGPB), to successfully colonize the rhizosphere, survive, and compete with indigenous microbial communities. This competency is essential for the establishment and effectiveness of PGPB in promoting plant growth, especially under abiotic stresses like drought (Compant et al., 2010). Effective colonization involves root surface attachment, biofilm formation, utilization of root exudates, and persistence under fluctuating soil conditions. PGPB with high rhizospheric competency can quickly adapt to environmental stresses, maintain stable populations, and consistently deliver beneficial effects such as phytohormone production, nutrient solubilization, and stress alleviation (Lugtenberg& Kamilova, 2009). Under drought conditions, the microbial colonization capacity may be reduced due to limited water availability and changes in root exudate composition. Hence, selecting drought-tolerant PGPB strains with superior rhizospheric competency ensures their survival and continued interaction with the plant root system, enhancing drought resilience through improved water uptake, root architecture

modulation, and osmotic adjustment (Timmusk et al., 2014). Rhizospheric competency is therefore a vital selection criterion in the development of effective bioinoculants for drought-prone environments.

❖ Suppression of Ethylene Production

ACC Deaminase Activity: Many PGPB produce ACC deaminase, an enzyme that breaks down ACC, the precursor of ethylene.

Reduced Ethylene Levels: High ethylene levels can inhibit root growth under stress. By lowering ethylene levels, PGPB allow roots to continue growing and exploring the soil for water, even under drought conditions.

Enhanced Root Growth and Stress Resilience: Lower ethylene levels lead to better root elongation, allowing plants to access water more effectively in drought-prone soils.

❖ PGPB-Plant Interactions in Drought Conditions

The interactions between PGPB and plants under drought stress have been well-documented. (Vurukonda et al. 2016) observed that PGPB inoculation in maize led to enhanced root architecture and biomass accumulation, helping plants survive prolonged water scarcity. Furthermore, studies indicate that PGPB can induce systemic resistance (ISR) in plants, enhancing their tolerance to various abiotic stresses, including drought. The ISR response, along with other PGPB-induced metabolic changes, makes plants more resilient by preparing them to better respond to water stress (Kang et al., 2014).

PGPB are beneficial microbes that form symbiotic or associative relationships with plants, enhancing plant growth and tolerance to various environmental stresses, including drought. Under drought conditions, PGPB help plants by improving water uptake, stimulating growth, enhancing root architecture, and modulating stress responses. The mechanisms through which PGPB aid plants under drought stress are diverse and complex, involving both direct and indirect pathways.

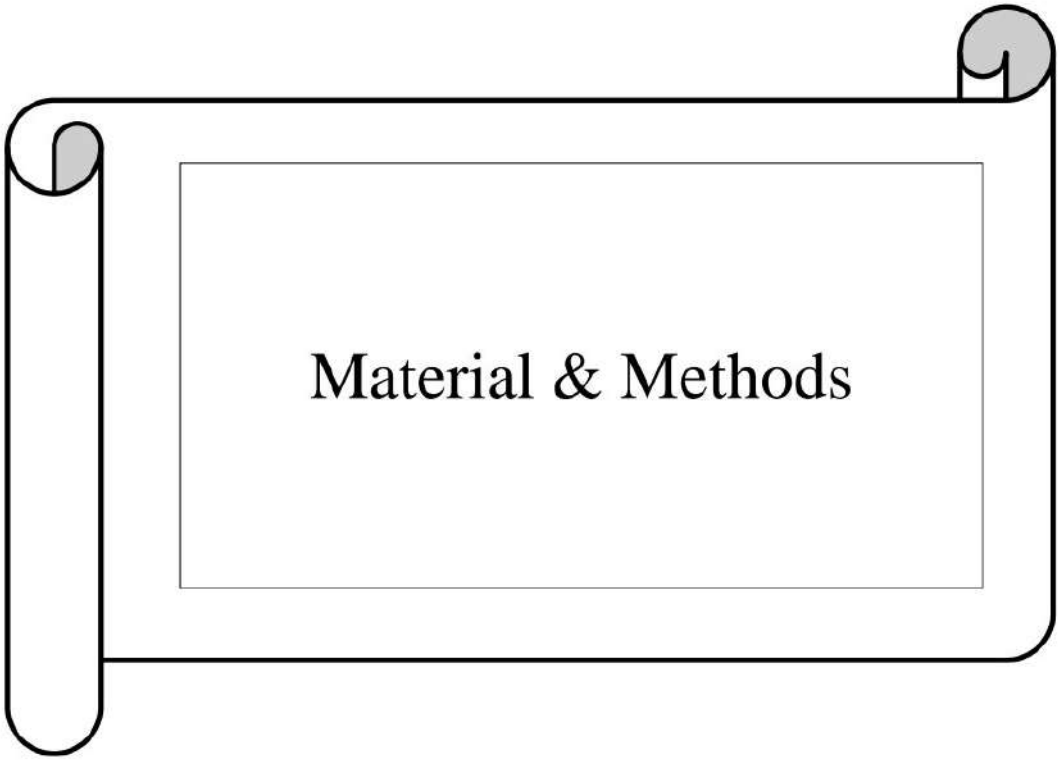
1. Mechanisms of Plant Growth-Promoting Bacteria in Drought Tolerance

Production of Phytohormones: PGPB produce phytohormones like auxins (IAA), cytokinin, gibberellins, and abscisic acid (ABA). These hormones play key roles in modulating plant responses to drought:

- Auxins: PGPB-produced IAA enhances root growth and branching, allowing plants to access deeper water reserves.
- Cytokinin: Help delay leaf senescenceand maintain cellular function under stress.
- Gibberellins: Stimulate growth processes, though their levels are often balanced with ABA during drought to prevent excessive growth.
- ABA: Acts as a stress hormone, regulating stomatal closure to reduce water loss and enhance drought tolerance. Some PGPB can increase ABA levels in plants, contributing to better water-use efficiency.
- Enhanced Root Architecture and Development: By producing auxins and other growth-promoting compounds, PGPB improve root growth and architecture. Enhanced root systems provide:
- Greater Root Surface Area: Increases water and nutrient absorption, helping plants survive in low-moisture soils.
- Deeper Root Penetration: Allows plants to reach water sources deeper in the soil profile, which is essential under drought.
- Production of EPS: PGPB can secrete exopolysaccharides, which help in:
  Soil Aggregation: EPS bind soil particles, improving soil structure and water retention around roots.
- Moisture Retention: EPS retain moisture near the root zone, creating a microenvironment that maintains humidity, which is critical for plant survival in drought conditions.
- Osmotic Adjustment and Production of Compatible Solutes: Some PGPB produce osmolytes, such as proline, glycine betaine, and trehalose, which help in:
- Maintaining Cellular Turgor: These osmolytes help maintain cell water balance and protect cellular structures from dehydration.
- Reducing Oxidative Stress: Osmolytes serve as antioxidants, neutralizing reactive oxygen species (ROS) generated under drought stress.
- Induction of Antioxidant Enzyme Activity: Drought stress causes oxidative damage in plants due to increased ROS production. PGPB can boost the plant's antioxidant

defence mechanisms by:

- Enhancing Enzymes Like Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD): These enzymes detoxify ROS, reducing cellular damage.
- Producing Antioxidants: PGPB can produce antioxidants such as glutathione, which helps protect plant cells from oxidative stress induced by drought.
- Nitrogen Fixation: Certain PGPB, especially those in the rhizobia group, can fix atmospheric nitrogen, converting it into a form that plants can use.
- Improved Nitrogen Availability: In drought-stressed soils, where nutrient availability is often limited, nitrogen-fixing bacteria provide a steady nitrogen supply, aiding in protein synthesis and stress tolerance.
- Reduced Dependency on Soil Nutrients: Nitrogen fixation by PGPB enables plants to maintain growth even when soil nitrogen is limited under drought conditions.
- Phosphate Solubilization and Nutrient Mobilization: Drought conditions often limit the availability of nutrients, particularly phosphates, in the soil.
- PGPB Solubilize Phosphate: Through the production of organic acids and phosphatases, PGPB can solubilize and mobilize phosphorus, making it available for plant uptake.
- Enhanced Nutrient Uptake: PGPB help in mobilizing other nutrients like potassium plant health.



Material & Methods

❖ Collection of Soil Sample

- The study Focused on soil sample collected from various Location Like Bambavade, Sangli, Shivaji University and Shenda Park.
- A systematic approach was employed. Sample was collected from drought area

A: Bambavade

B: Shenda Park

C: Shivaji University

D: Sangli

❖ Physical Analysis :-

Collected Soil sample needs to analysed for further study.

For analysis we determine various characteristic of Soil

- **Texture :-**

Collected soil sample number 1 was found to be moist, Sample number 2 was found to be loam, sample number 3 was found to be sandy & sample number 4 was found to be loam

- **Colour**

Soil colour is often and indicator of its composition and organic matter contain

Dark soil are typically rich in organic matter

Red soil are typically rich in iron oxide

Light colour areoften Sandy and low in organic matter

- **Temperature**

Soil temperature impact seed germination root growths and microbial activity

Dark side often warm up faster than light soil impacting the growing season for plants

3. Isolation of Drought-Tolerant Bacteria (PGPB)

Soil Dilution:

1 g of each soil sample was diluted serially up to 10^{-6} .

Inoculation:

Dilutions (10^{-3} to 10^{-6}) were spread on sterile nutrient agar plates using the spread plate technique.

Incubation:

Plates were incubated at room temperature for 24 hours.

Colony Selection:

Distinct colonies were selected and labeled accordingly.

Samples and dilutions were coded (e.g., Aa1, Bb1, Cc1, Da1 etc.)

❖ Screening

To study characterization of PGPB we need to perform some test for example.

- ACC Deaminase
- Qualitative & Quantitative analysis of phosphatesolubilisation
- Nitrogen fixing ability
- IAA production ability
- Siderophore.
- Exopolysaccharide production ability
- Ammonia Production
- Hydrogen Cyanide
- Preparation of bio Inoculum
- Seed germination
- Pot Trial
- Chlorophyll Estimation
- Rhizospheric competence study

❖ **ACC Deaminase :-**

➢ ACC deaminase activity of PGPB was assayed by evaluating the ability to grow on DF minimal medium (Dworkin and Foster 1958)

- DF minimal medium (Dwonkin and Foster) supplemented with ACC was prepared.
- DF minimal medium agar plates were spot inoculated with loop full of culture.
- plates were incubated at at room temperature
- Development of colony wasconsidered positive for ACC deaminase production

Table 1:- Composition of DF medium

Sr. No.	Media	Quantity
1	Glucose	0.2 gm
2	Gluconic acid	0.2 gm
3	Citric acid	0.2 gm
4	KH2PO4	0.4 gm
5	Na2HPO4	0.6 gm
6	MgSO4.7H2O	Trace
7	CaCl2	Trace
8	Na2MoO4	Trace
9	KI	Trace
10	NaBr	Trace
11	MnC12	Trace
12	CoC12	Trace
13	CuCl2	Trace
14	AIC13	Trace
15	NiSO4	Trace
16	Agar powder	1 gm
17	Distilled Water	100 ml

❖ Qualitative & Quantitative analysis of phosphate Solubilizing

➢ For determining the phosphate solubilizing ability we performed the following steps

- Phosphate solubilizing ability was studied using the method of Fiske & Subbarow (1925)We have prepared sterile Pikovskaya agar plats. Then each selected colony was inoculated by spot inoculation method. plates were incubated at room temperature for 3-4 days. After incubation we have observed clear zone around some Colonies. Composition of Pikovskaya agar Peptone-10 g, Dextrose-10 g, Potassium Chloride-0.5 g, Agar- 15 g, Distilled Water- 1 Lit

Quantitative analysis of phosphate

➢ For determining of quantity of phosphate content we perform following steps

- Phosphate content was studied by using the method of Fisk and Subbarow .
- Prepared 100 ml pikovskay's broth medium and inoculated potent or bacterial culture (cb1 & cb2) separately.
- Incubate under shaking condition at 30°c for 7 days
- After every 24 hrs 1 ml of culture was taken and centrifugated at 6000 rpm for 15 min
- 500 µl of supernatrint was separated and with500 µl of trichloroacetic acid
- Then 4 ml of color reagent was added which was prepared by mixing 3M H2SO4 ammonium molybdate, ascorbic acid & distilled water in 1:1:1:2 ratio
- Then the reaction mixture was incubated at R-T for 15 min
- Intensity of resulting blue color was measured at 820 rim using colorimeter

❖ Nitrogen fixation Ability

- Nitrogen fixation ability was studied using the method of sergeiwinogradsky (1888)
- We have prepared sterile Ashby's mannitol agar plates and add bromothymol blue Selected organism were inoculated on each separate plates. by using spot inoculation method. Plates was incubated at room temperature (25-28 °C) for 4-5 days&Observed the zone of inhibition around the colony
- Composition of Ashbey's mannitol agar Mannitol- 20.0 g, K_2HPO_4. 0.2 g, $MgSo_4$ $7H_2O$- 0.2 g, NaCl- 0.2 g, K_2So_4- 0.1 g, $CaCO_3$- 5.0 g, Agar- 20.0 g, D/W- 1000 ml, pH- 7.2.

❖ IAA production ability.

➢ For determination of IAA production abilityof we performed following steps

- IAA production ability was studied using the method of Gordan & weber (1951)We prepared 50ml nutrient broth for each isolates.Loopful of isolates were inoculated into the broth. Flask were incubated at room temperature for 5-6 days.After incubation 1ml Sample was collected for centrifugation.After centrifugation 1ml supernatant from each tubes was Collected. Then we added 2ml Salkowaski reagent & 2 drops of Orthophosphoric acid in each tube then we optioned pink colour.Optical density was checked using colorimeter at 530 nm filter.

❖ Siderophore Production Ability :-

➢ For Determination of siderophore production ability of we performedfollowing steps

- siderophore production ability was studied using the method of Schwyn &Neiland (1987). We prepared sterile CAS agar plate. Selected organism was inoculated on plates. by using spot inoculation method. Incubation was done at room temperature for 4-5 days & noted down the observation

  Preparation: Involved complex dye and minimal medium formulation.

❖ Exopolysaccharide production ability

➢ For Determination of siderophore production ability of we performed following steps

- Eps production ability was studied using the method of Nicolous(1999).
- we prepared 50 ml. Nicolous broth for each isolate. loopful of isolates were inoculated into the broth.
- Flasks were incubated at room temperature for 5-6 days. After incubation sample was collected for centrifugation.
- After centrifugation the 1 ml supernatant was collected and added with chilled ethanol.
- The white precipitate formed shows positive result.
- Yeast extract- 1gm, trisodium citrate- 0.3gm, KCL- 0.2gm $MgSo_4$ $7H_2O$- 2gm, $MnSo_4$ H_2O- 0.36gm, $FeSo_4 7H_2O$- 5gm.

❖ Ammonia Production

❖ For determining of Ammonia content we perform following steps

- Ammonia production ability was studied using Method of (Marques & others 2010)
- The culture of isolated organism where inoculated separately into 5ml peptone water
- It incubated at room temperature for 48hrs
- 0.3 ml nesslersreagent was added into eachtube
- Development of brown color indicates positive test

❖ **Hydrogen cyanide :-**

For determining of hydrogen cyanide content we perform following steps

- Hydrogen Cyanide content was studied by using the method of Kings and others(1954).
- Nutrient agar plates were prepared and spot inoculated. with the culture
- The petri plates were covered with lid containing piece of filter paper soaked with 1% picric and moistened with few drops of 10% NaCo3
- Plates were sealed with parafilm and incubated at room temperature
- change in color of filter paper from yellow to brown was considered positive

From the above tests we found 2 organisms Cb1 and Cb2 potent which showed positive results in all the above tests. Now some more tests were done for that organism.

❖ Preparation of bio inoculum

- ❖ To prepare the bacterial inoculums, we followed a modified version of the method described by Penrose and Glick (2003).
- ❖ The potent isolates were cultured in nutrient broth until it reached the logarithmic growth phase.
- ❖ The bacterial cells were then harvested via centrifugation, washed with sterile saline solution to remove any residual media, and finally resuspended in sterilized distilled water to achieve the desired cell density

❖ Seed germination

- Surface sterilization of *V. Aconitifolia* L., seeds were essential to ensure the efficacy of the bacterial inoculation.
- This was achieved by immersing the seeds in 70% ethanol for one minute, followed by four rinses with sterile distilled water.
- After treatment, the seeds were spread on petri plates .
- The Seed germination study was conducted, taking into account the different water condition like Drought, normal, Drought condition with bio inoculum etc . Ambient temperatures ranged from 30 to 35°C

❖ pot trial

- Surface sterilization of V. Aconitifolia L., seeds were essential to ensure the efficacy of the bacterial inoculation.
- This was achieved by immersing the seeds in 70% ethanol for one minute, followed by four rinses with sterile distilled water.
- Soil was sterilized then it transfers into pot then the treated seeds were sow in pots
- The Pot Trials was conducted, taking into account the different water condition like Drought, normal, Drought condition with bio inoculum etc. Ambient temperatures ranged from 30 to 35°C

❖ Growth Parameters:-

- After pot trials conducted for 14 days we measure seedling length, root length, fresh weight, dry weight of each well grown plant.

❖ Chlorophyll

➢ For determining of Chlorophyll content, we perform following steps

- Chlorophyll content was studied by using the method of Arnon's 1949Method.
- Fresh leaves of plants from the pot trails Haw taken randomly after 14 days of plantation
- Leaves were crushed and Acetone was added and stored overnight overnight under refrigeration
- The extract was filtrated
- The absorbance of filtrated way measured. was recorded by wing colorimeter at 645 nm

❖ Root Colonization Assay

- To quantify root colonization by Cb1 & Cb2
- root samples with adhering soil were dipped in a sterile saline solution (0.85% NaCl).
- Serial dilutions of bacterized roots were prepared in sterile saline and spread on nutrient agar plates. The plates were incubated at 30 °C for 24 h.
- The average number of bacterial colony-forming units was calculated and presented as mean cfu root biomass.

Result & Discussion

Result & Discussion

❖ Result

❖ Collection of Soil

After Collection of Soil Sample itis necessary to analyse collected soil for further studied. We have determined various characteristics of Soil for Example Texture, Colour& Temp

Table 4 :- Properties of collected soil samples

Sr. No	Location	Texture	Colour
1	Bambavade	Moist	Dark brown
2	Shenda Park	Loam	Brown
3	Shivaji University	Sandy	Light
4	Sangli	Loam	Red

❖ Isolation

After incubation at room temperature for 24 hrs. On Nutrient agar various types of colonies was observed. There are 48 organism we selected for further Study.

Fig No 1 : Colonies of organism on nutrients agar

➢ **Isolated organisms**

Sr.No	Organism	Sr. No	Organism
1	Aa 1	**25**	Bc 1
2	Aa 2	**26**	Bc 2
3	Aa 3	**27**	Bc 3
4	Aa 4	**28**	Bc 4
5	Aa 5	**29**	Bc 5
6	Aa 6	**30**	Ca 1
7	Ab 1	**31**	Ca 2
8	Ab 2	**32**	Ca 3
9	Ab 3	**33**	Ca 4
10	Ab 4	**34**	Ca 5
11	Ab 5	**35**	Ca 6
12	Ac 1	**36**	Cb 1
13	Ac 2	**37**	Cb 2
14	Ac 3	**38**	Cb 3
15	Ba 1	**39**	Da 1
16	Ba 2	**40**	Da 2
17	Ba 3	**41**	Da 3
18	Ba 4	**42**	Da 4
19	Ba 5	**43**	Db 1
20	Bb 1	**44**	Db 2
21	Bb 2	**45**	Db 3
22	Bb 3	**46**	Db 4
23	Bb 4	**47**	Dc 1
24	Bb 5	**48**	Dc 2

- **ACC Deaminase :-**

On DF minimal medium the spot inoculated isolates developed colony was consider positive for ACC deaminase.

Fig No 2: ACC Deaminase test organisms

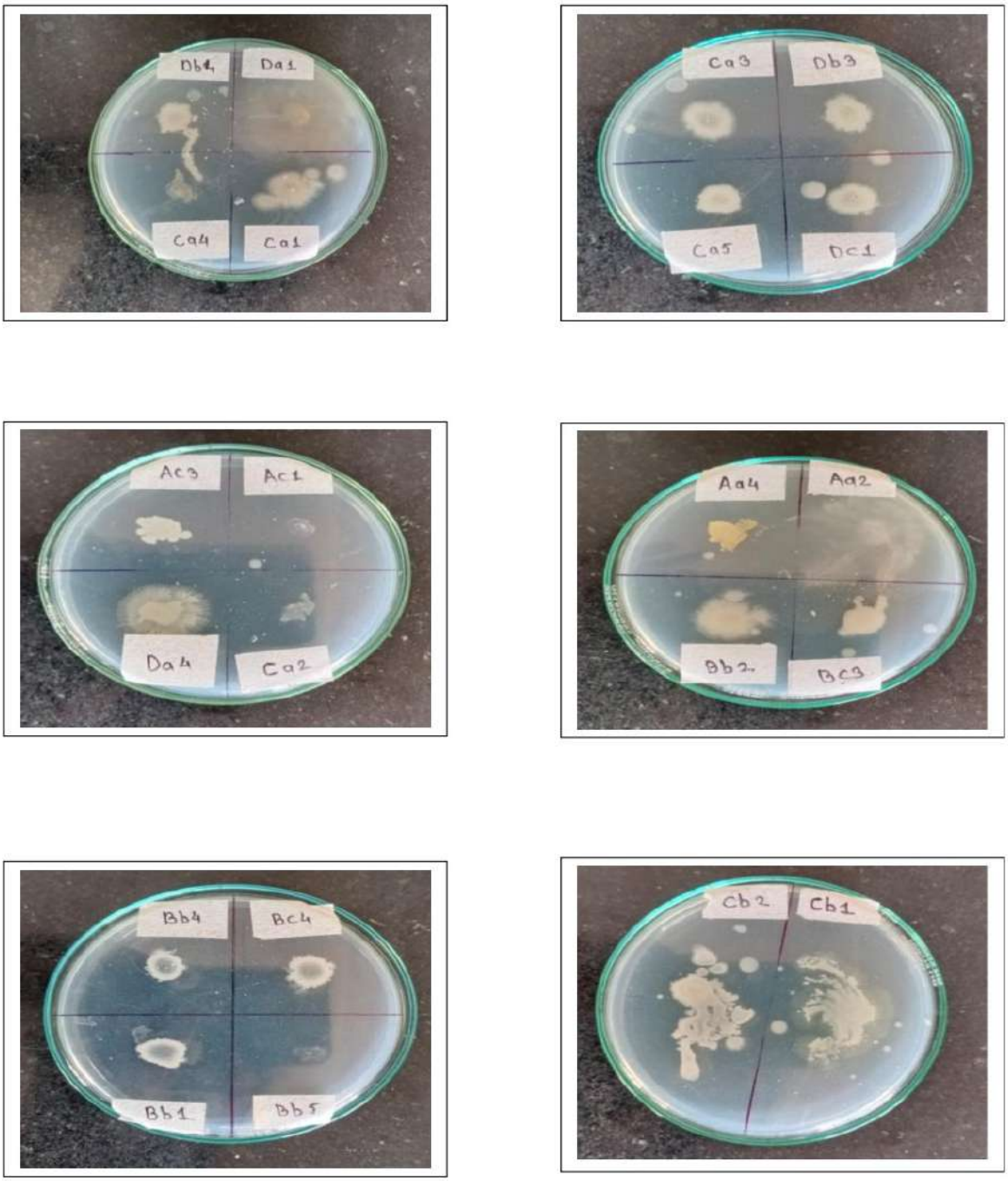


Table No. 1 :- Result of ACC Deaminase

Organism	Result
Cb1	Positive
Cb2	Positive
Aa4	Positive
Aa2	Positive
Bb2	Positive
Bb4	Positive
Bc4	Positive
Bb1	Positive
Bb5	Positive
Ac3	Positive
Ac1	Positive
Da4	Positive
Ca2	Positive
Db4	Positive
Da1	Positive
Ca4	Positive
Ca1	Positive
Ca3	Positive
Db3	Positive
Ca5	Positive
Dc1	Positive

❖ Screening of isolated organisms for their different abilities

➢ Phosphate Solubilising Bacteria Qualitative test :-

On Pikovskaya (Pk) agar medium the spot inoculated 4 isolates showed the Clear zone around colony after 48 hours. The spot inoculated some organisms shown the positive results

Fig No 2: phosphate solubilizing Qualitativetest

❖ Quantitative estimation of Phosphate :-

Fig No 2: Quantitative estimation of Phosphate

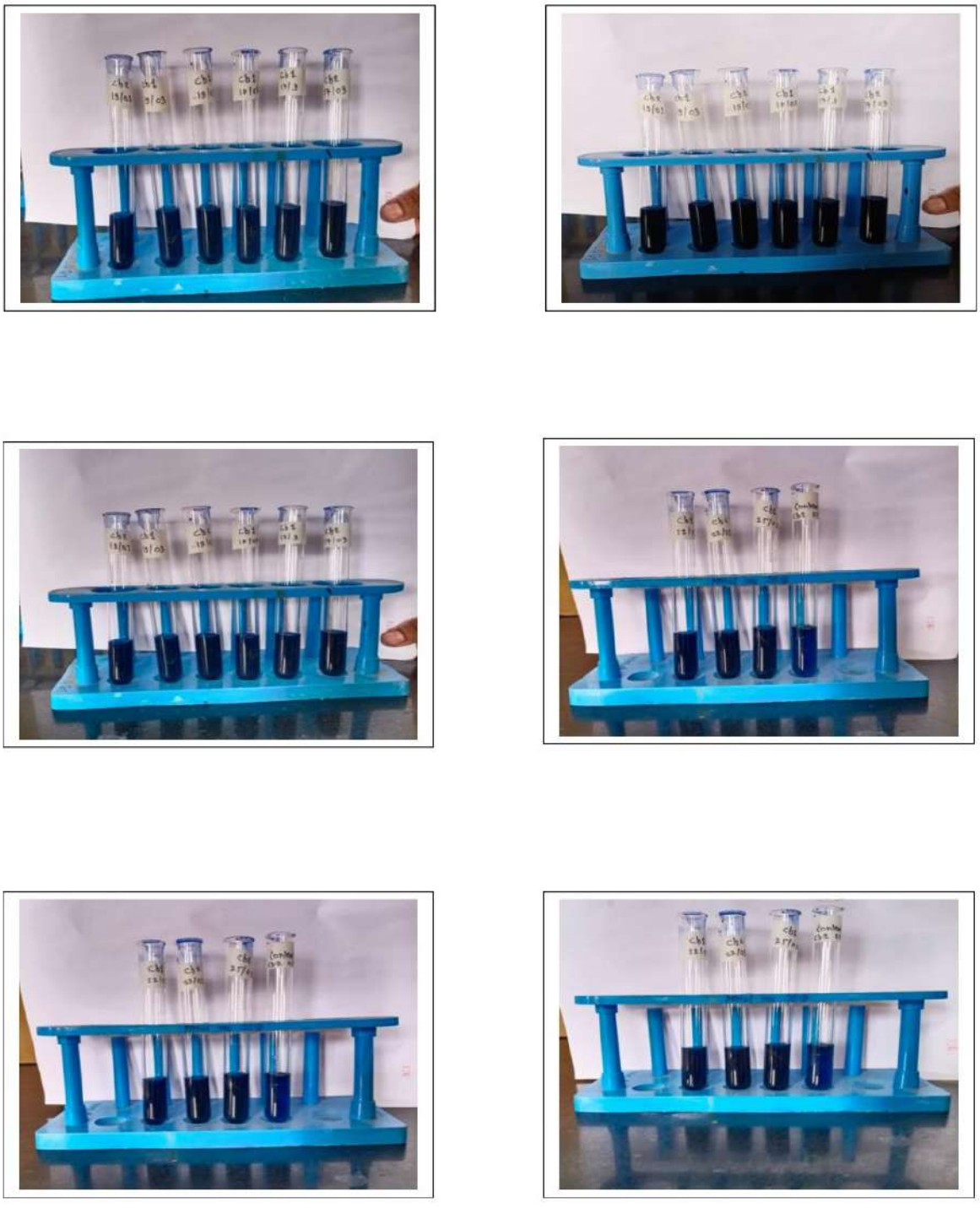
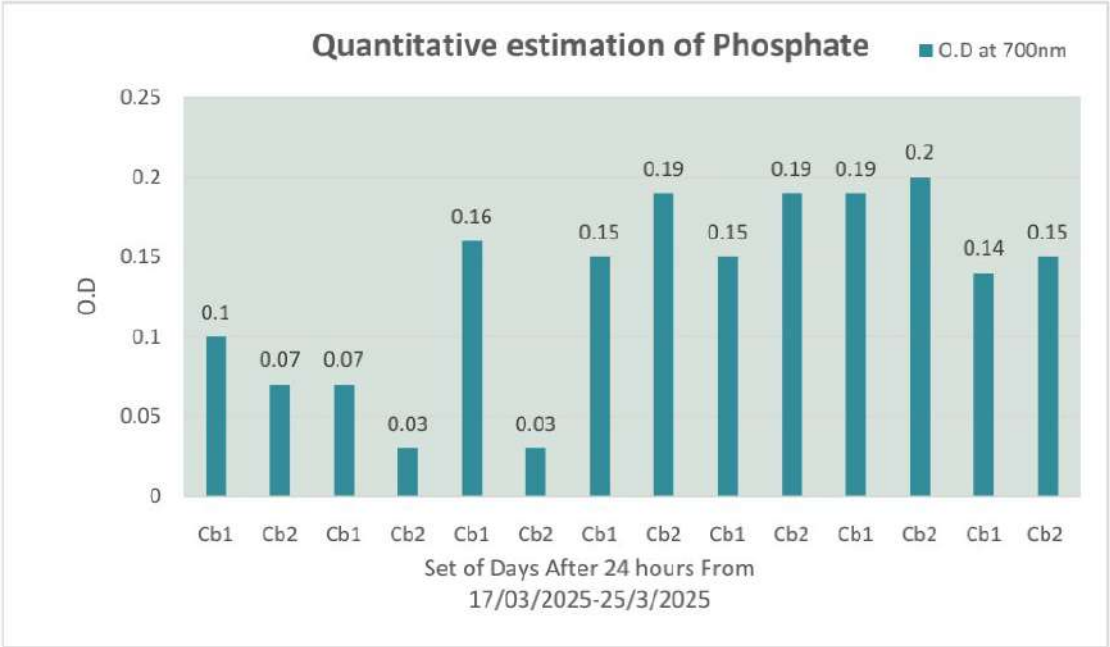


Table No. 2 :- Result of Quantitative estimation of Phosphate

Sr. No.	Organism	O.D at 700nm
1	Cb1	0.10
2	Cb2	0.07
3	Cb1	0.07
4	Cb2	0.03
5	Cb1	0.16
6	Cb2	0.03
7	Cb1	0.15
8	Cb2	0.19
9	Cb1	0.15
10	Cb2	0.19
11	Cb1	0.19
12	Cb2	0.20
13	Cb1	0.14
14	Cb2	0.15



Nitrogen fixation Ability-

The isolates were spot inoculated on Ashby's agar medium. After 48 hours, the clear zone around colonies were observed. The spot inoculated organisms shown the results were:

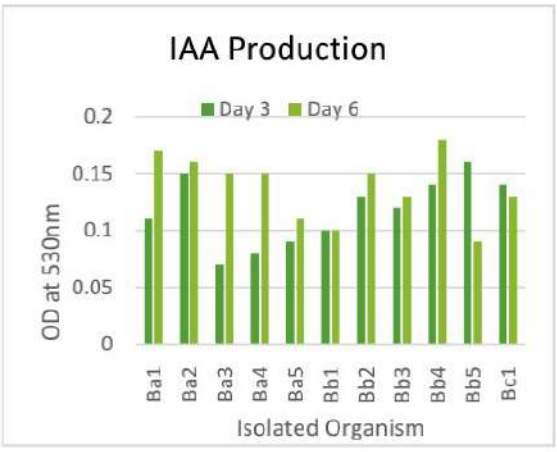
Fig No 3 : Organism showing nitrogen fixation ability

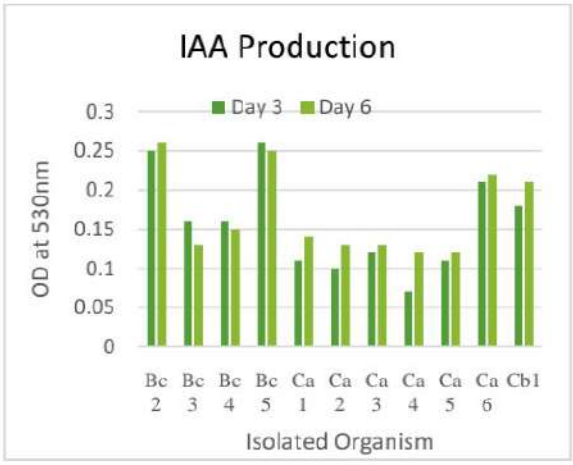
❖ IAA (Indole Acetic acid) :-

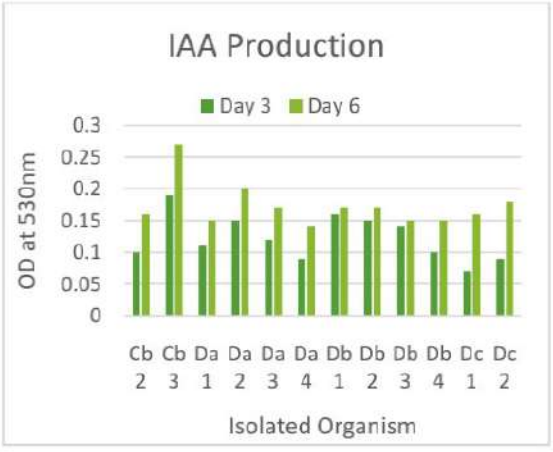
Result:- Qualitative and Quantitative Estimation of IAA

Tested IAA producing activity All of them are positive & show slightly pink colour.

Fig No 5: IAA produced by



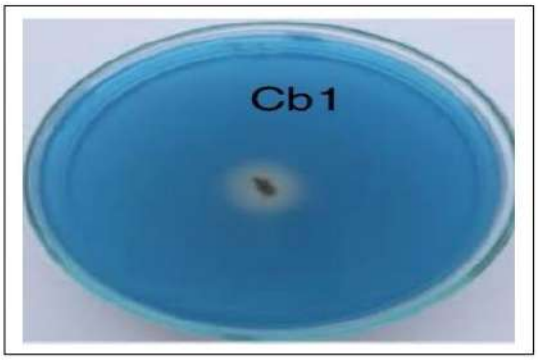


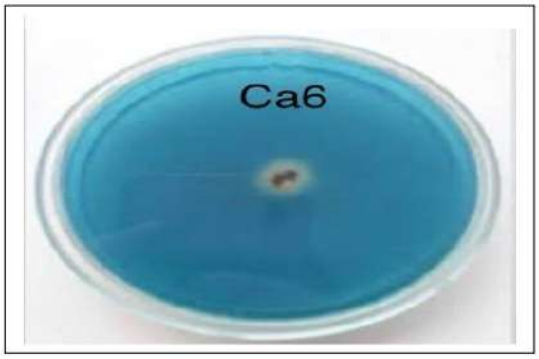


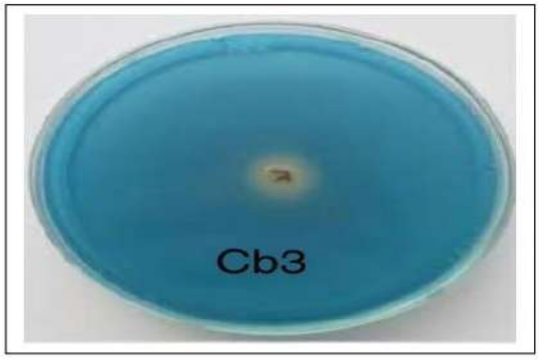
Siderophore :-

The isolates were spot inoculated on CAS medium. After 3-4 Days , Yellow colour zone around colonies were observed.

Fig No 4 :Organism showing siderophore production ability







❖ Exopolysaccharides :-

Result :- Exopolysaccharide Activity

Exopolysaccharides activity was studied alll of them are positive & they show white colour precipitated when chilled ethanol is added

Fig No 6 : Organism showing exopolysaccharides activity

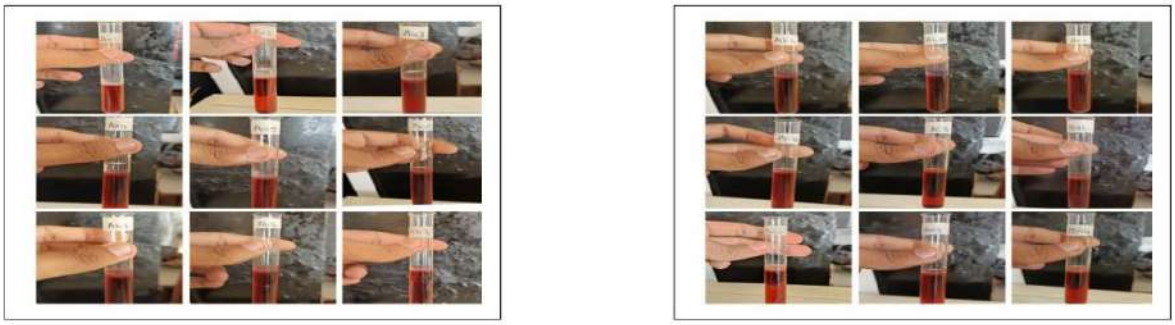


Table No 10:- Result of exopolysaccharide activity

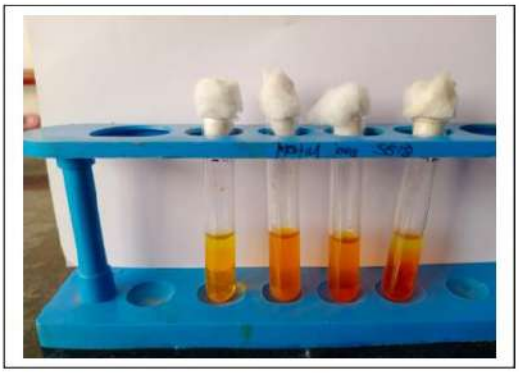
Organism	Gram Nature
Aa 1	Positive
Aa 2	Positive
Aa 3	Positive
Aa 4	Positive
Aa 5	Positive
Aa 6	Positive
Ab 1	Positive
Ab 2	Positive
Ab 3	Positive
Ab 4	Positive
Ab 5	Positive
Ac 1	Positive
Ac 2	Positive
Ac 3	Positive
Ba 1	Positive
Ba 2	Positive
Ba 3	Positive
Ba 4	Positive
Ba 5	Positive
Bb 1	Positive
Bb 2	Positive
Bb 3	Positive
Bb 4	Positive
Bb 5	Positive

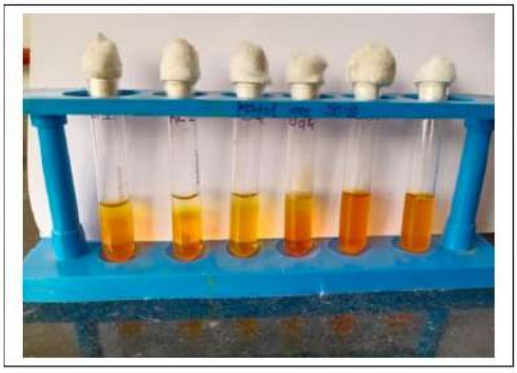
Organism	Gram Nature
Bc 1	Positive
Bc 2	Positive
Bc 3	Positive
Bc 4	Positive
Bc 5	Positive
Ca 1	Positive
Ca 2	Positive
Ca 3	Positive
Ca 4	Positive
Ca 5	Positive
Ca 6	Positive
Cb 1	Positive
Cb 2	Positive
Cb 3	Positive
Da 1	Positive
Da 2	Positive
Da 3	Positive
Da 4	Positive
Db 1	Positive
Db 2	Positive
Db 3	Positive
Db 4	Positive
Dc 1	Positive
Dc 2	Positive

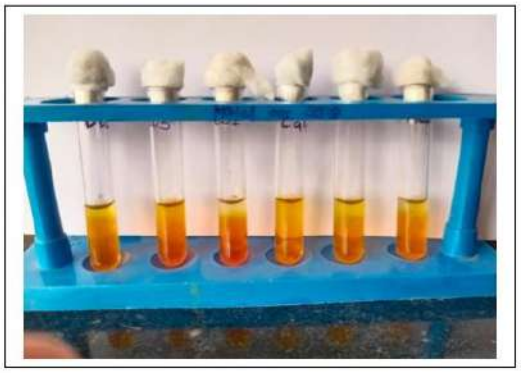
Ammonia production :-

After 24 hours, turbidity was observed in the peptone water. After addition of Nesslers reagent, brown to yellow colour was observed. Among all the isolate.

Fig No 2: Ammonia Production







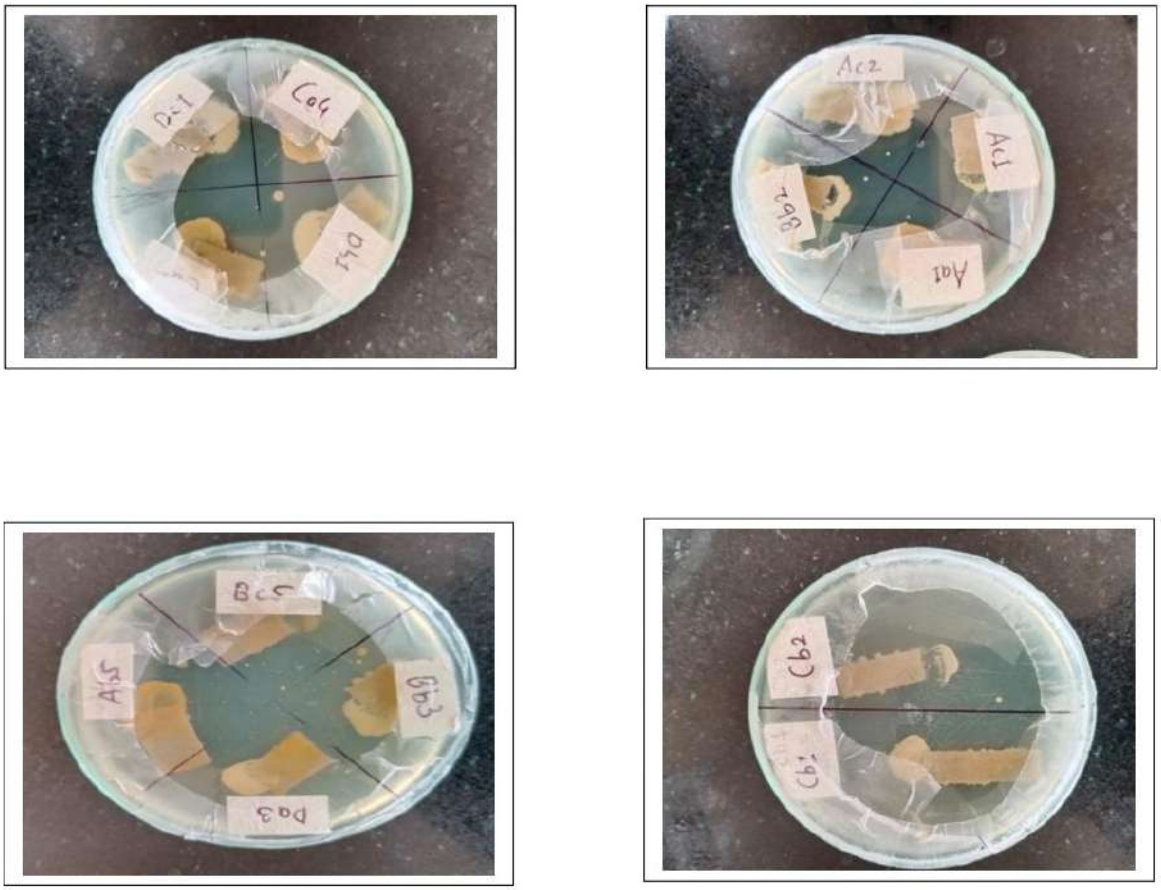
Ammonia productionResult :-

Organism	Result
Db3	Positive
Db1	Positive
Dc1	Positive
Ca2	Positive
Ca4	Positive
Cb2	Positive
Da4	Positive
Ba3	Negative
Ac2	Positive
Aa1	Positive
Ac1	Positive
Bb2	Positive
Bb1	Positive
Ca1	Positive
Cb1	Positive
Da1	Positive
Aa2	Positive
Ab1	Negative
Ab5	Negative
Da3	Positive
Bc5	Positive
Ba2	Negative

Hydrogen cyanide:-

Isolate were spot inoculated on nutrient agar plate covered with lead containing filter paper strip soaked with 1% picric acid and moistened with few drops of 10% $NaCo_3$

Fig No 2: Hydrogen cyanide

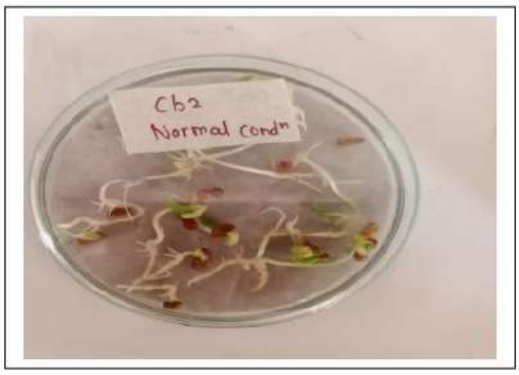


❖ Seed germination :-

Seedgermination of *V. Aconitifolia L* in petri plate was carried out for 7 days the growth pattern was observed in Plate the inoculum of cb1 and cb2 showed highest growth.

1 Day

3 Day



7 Day

❖ **Pot Trial :-**

Pot Trials of *V. Aconitifolia L* in petri plate was carried out for 14 days the growth pattern was observed in Pot the inoculum of cb1 and cb2 showed highest growth.

3 Day

6 Day

9 Day

12 Day

14 Day

Growth Parameter :-

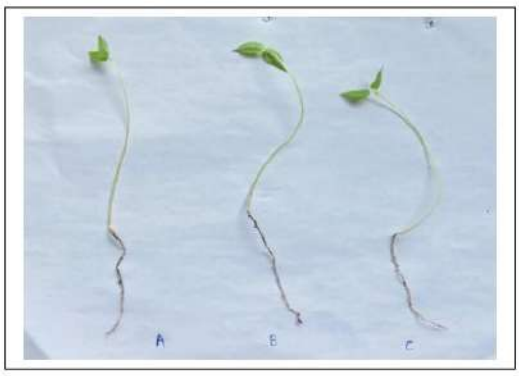
Cb2 Drought Stress

Cb1 Drought Stress

Control

Drought Stress

Cb1 Normal Condition



Cb2 Normal Condition

Cb2 Drought Stress	Seedling length (cm)	Root length (cm)	Fresh weight (gm)	Dry Weight (gm)
A	16.8	6	0.12	0.02
B	17.5	5.6	0.13	0.01
C	15.5	4.9	0.08	0.01

Cb1 Drought Stress	Seedling length (cm)	Root length (cm)	Fresh weight (gm)	Dry Weight (gm)
A	16	5	0.08	0.01
B	13.5	4.2	0.11	0.02
C	13.6	5	0.10	0.01

Drought Stress	Seedling length (cm)	Root length (cm)	Fresh weight (gm)	Dry Weight (gm)
A	12.4	2.9	0.07	0.1
B	14.4	1.2	0.10	0.01
C	17.9	4	0.13	0.03

Control	Seedling length (cm)	Root length (cm)	Fresh weight (gm)	Dry Weight (gm)
A	13	6.3	0.10	0.01
B	17.7	3.2	0.09	0.02
C	14.8	5.2	0.07	0.01

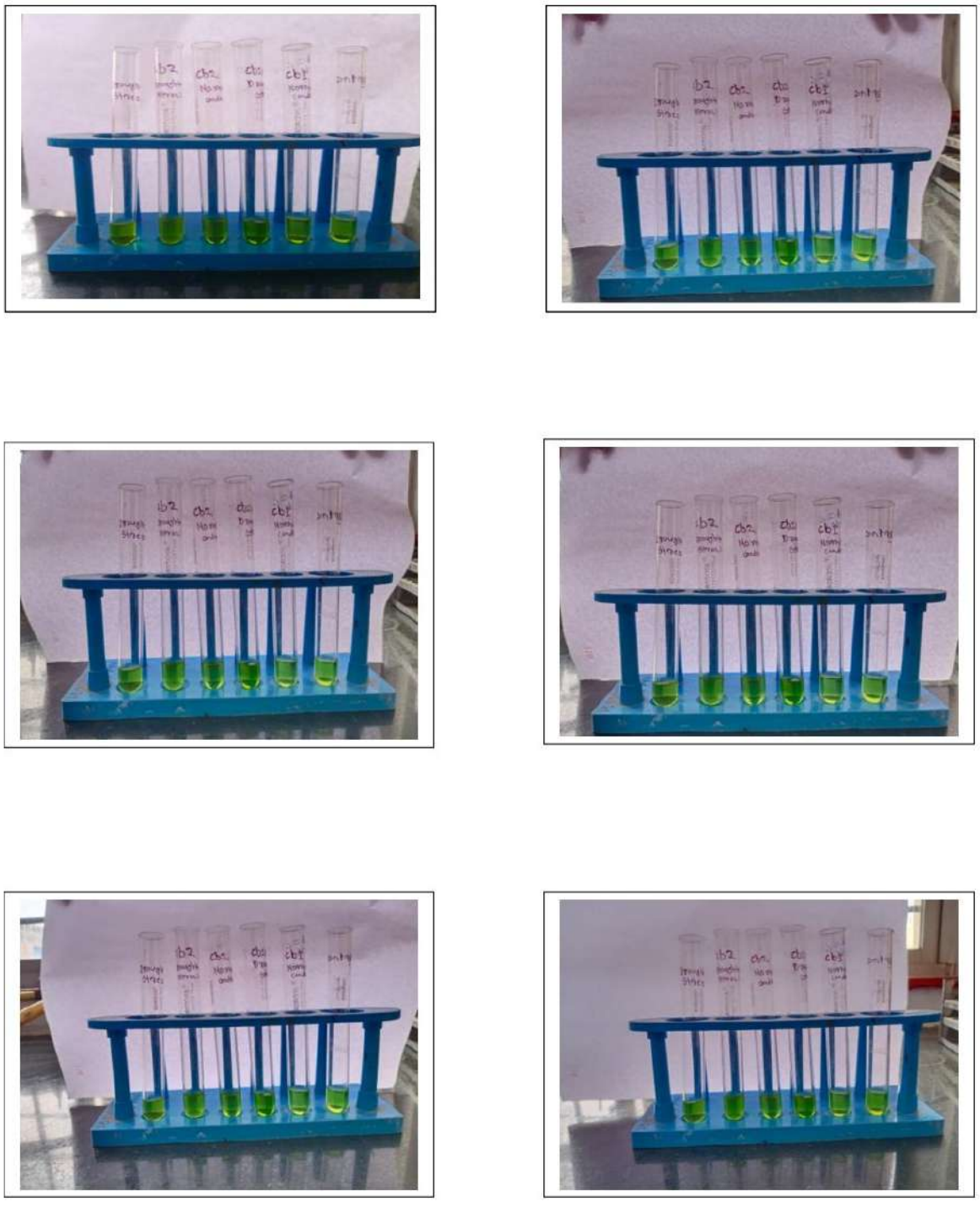
Cb1 Normal Condition	Seedling length (cm)	Root length (cm)	Fresh weight (gm)	Dry Weight (gm)
A	16.5	4.5	0.02	0.01
B	14	2.9	0.05	0.01
C	16.2	3	0.03	0.01

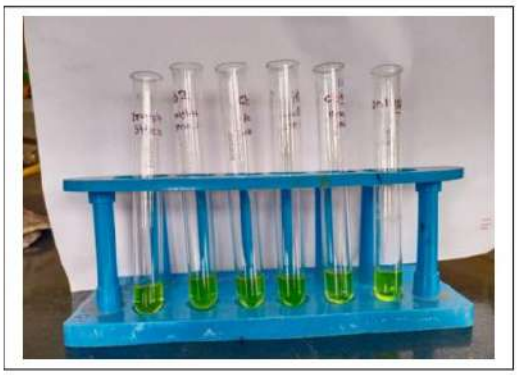
Cb2 Normal Condition	Seedling length (cm)	Root length (cm)	Fresh weight (gm)	Dry Weight (gm)
A	14.9	6.2	0.12	0.01
B	16	3.7	0.13	0.01
C	14.5	2.9	0.10	0.01

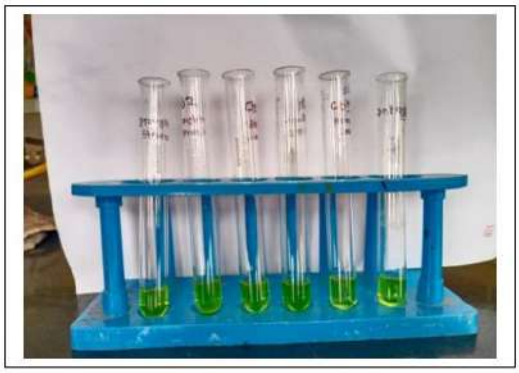
ChlorophyllActivity :-

Quantitative estimation of Chlorophyll

Fig No 2: Chlorophyll Activity

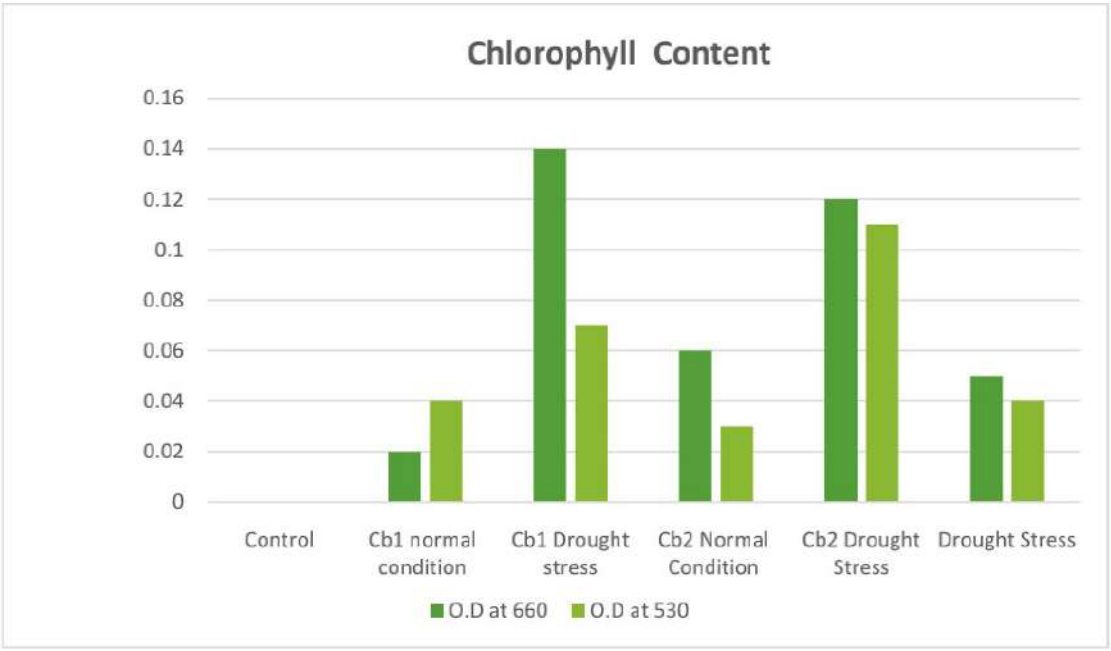




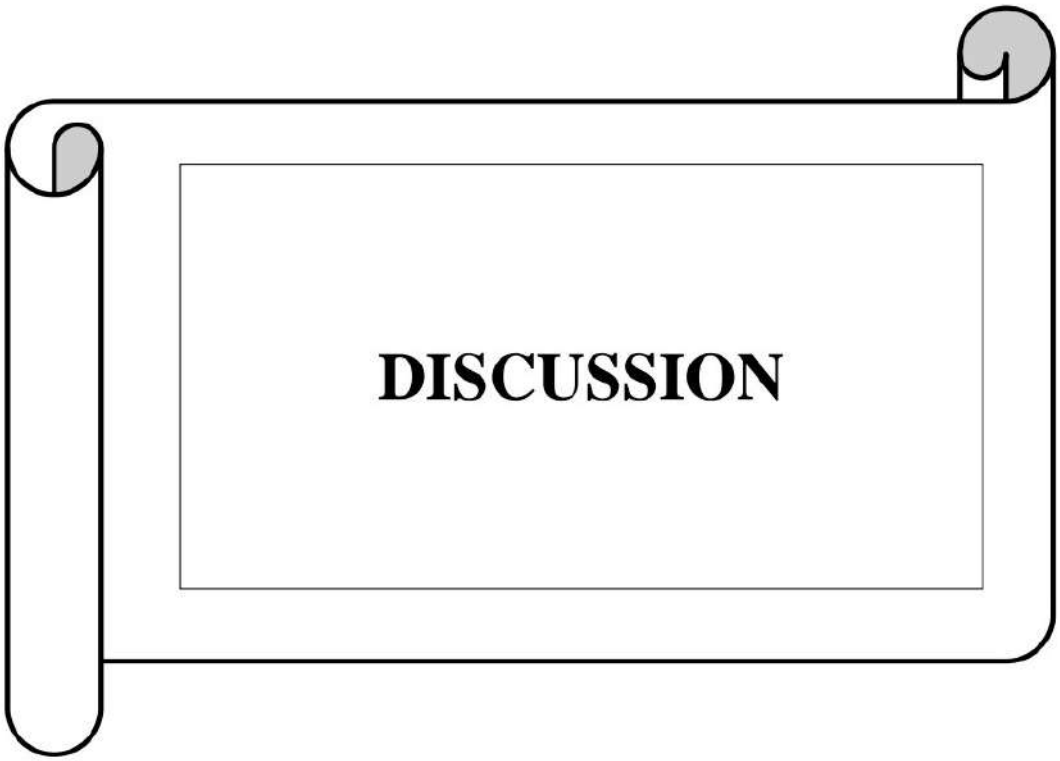


ChlorophyllActivity :-

	O.D at 660	O.D at 530
Control	0.00	0.00
Cb1 normal condition	0.02	0.04
Cb1 Drought stress	0.14	0.07
Cb2 Normal Condition	0.06	0.03
Cb2 Drought Stress	0.12	0.11
Drought Stress	0.05	0.04



➢ **Rhizosphericcompetence :-**



Discussion

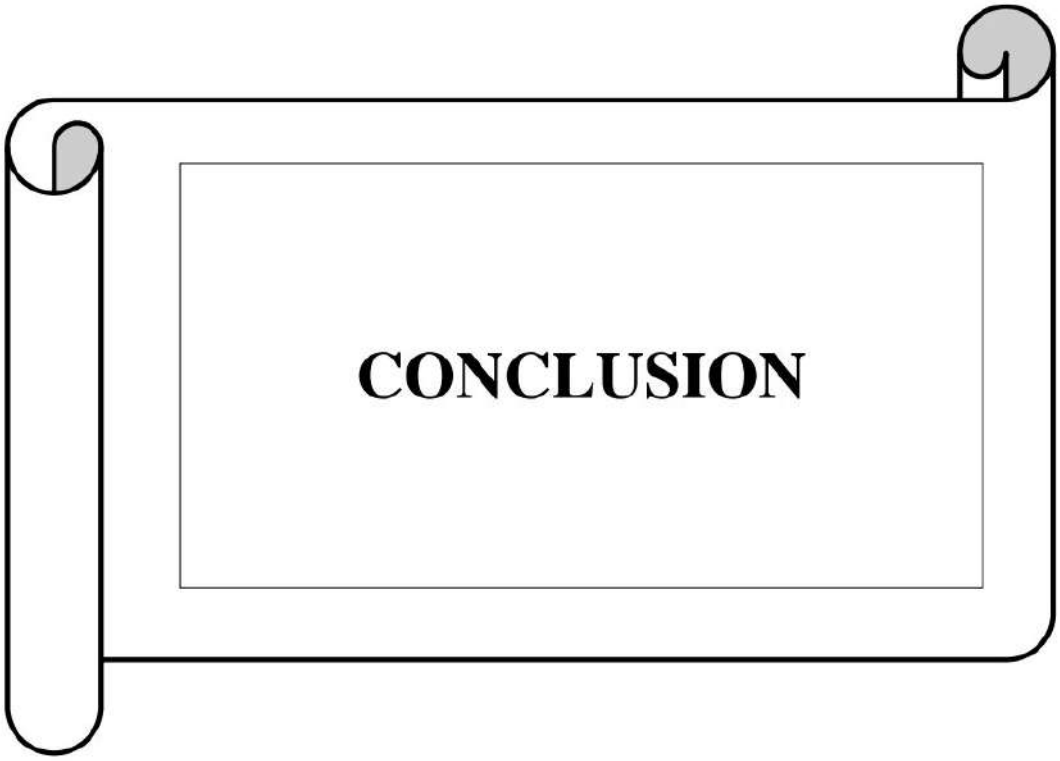
Plant growth-promoting bacteria (PGPB) contribute significantly to enhancing plant development and drought resilience by employing a wide range of mechanisms. In this study, selected bacterial isolates were examined for their abilities to fix atmospheric nitrogen, solubilize phosphate, produce siderophores, indole acetic acid (IAA), ammonia, hydrogen cyanide (HCN), exopolysaccharides (EPS), and to express ACC deaminase activity. In nitrogen-deficient soils, nitrogen fixation by PGPB plays a crucial role, as observed through the formation of foamy zones indicating nitrogenase activity. This facilitates the conversion of atmospheric nitrogen into plant-available forms, promoting greener foliage, stronger stems, and improved yield. Phosphate solubilization was demonstrated by the formation of clear zones on Pikovskaya's agar, particularly by isolates like Cb1 and Cb2. This process increases phosphorus availability, which is vital for energy transfer, root growth, and flowering, especially in nutrient-scarce conditions.

IAA production, confirmed by a pink coloration upon reaction with Salkowski's reagent, plays a vital role in stimulating root elongation, lateral root formation, and increasing the root surface area, which improves water and nutrient uptake under drought conditions. Siderophore production, observed as yellow halos on CAS agar, enhances iron uptake by chelating iron from the environment, supporting chlorophyll synthesis, reducing chlorosis, and contributing to improved enzyme activity and oxidative stress resistance. EPS production, detected by the formation of white precipitate in ethanol-treated supernatants, helps bind soil particles, improve soil structure, increase water retention, and protect roots by forming biofilms, which enhances plant survival during water scarcity.

Ammonia production was also detected, and it contributes directly to soil nitrogen content, enhancing fertility and promoting plant growth. Hydrogen cyanide (HCN) production by certain isolates plays a biocontrol role by inhibiting the growth of plant pathogens in the rhizosphere, thereby safeguarding root health. ACC deaminase activity, although not quantified here, is a critical trait that helps plants cope with drought-induced ethylene stress by breaking down the ethylene precursor ACC (1-aminocyclopropane-1-carboxylate), thereby facilitating continued root elongation and shoot growth even under water deficit. The practical implications of these traits were evident in seed germination tests and pot trials. Seeds treated with PGPB exhibited higher germination rates, faster seedling emergence, and more vigorous growth compared to untreated controls, indicating the

beneficial effects of bacterial inoculation at early growth stages.

Pot trials further confirmed that PGPB-inoculated plants maintained higher biomass, better shoot and root length, and greater survival under drought stress. Moreover, these plants showed higher chlorophyll content, indicating improved photosynthetic efficiency and better tolerance to oxidative damage. Elevated proline accumulation, a common stress marker, was also observed, which helps maintain osmotic balance, protect proteins and membranes, and scavenge free radicals during drought. Rhizosphere competency of the isolates was evident by their successful colonization of the root zone, even under stressful conditions. Traits such as EPS production and biofilm formation likely aided in their persistence, enabling them to maintain active interactions with plant roots. Together, these diverse PGPB traits not only enhance plant growth and yield under drought stress but also reduce dependency on chemical fertilizers, thus contributing to sustainable and eco-friendly agricultural practices.



CONCLUSION

Conclusion:

Drought stress remains one of the most significant abiotic challenges affecting agriculture globally. It limits crop growth, reduces yield, and impacts overall plant health by restricting water and nutrient availability. Additionally, drought conditions disrupt soil microbial biodiversity, further impairing soil health and plant performance. To combat this, sustainable and eco-friendly approaches are needed—one of which is the use of Plant Growth-Promoting Bacteria (PGPB), which offer biological solutions to enhance crop resilience under stress conditions.

In our project, we focused on isolating and characterizing drought-tolerant PGPB from various soil samples collected from drought-prone areas such asSangli, Bambawade, and surrounding regions. The main aim was to identify organisms capable of enhancing plant growth under water-limited conditions through multiple plant-beneficial mechanisms.

We successfully isolated several bacterial strains and evaluated them for key plant growth-promoting traits. These included phosphate solubilization, nitrogen fixation, siderophore production, indole-3-acetic acid (IAA) production, ammonia production, hydrogen cyanide (HCN) production, and exopolysaccharide (EPS) secretion. These traits are known to play crucial roles in nutrient availability, root development, and stress tolerance in plants.

After detailed screening, two bacterial strains were identified as the most potent based on their performance in laboratory tests. These strains showed strong abilities in multiple growth-promoting activities and were selected for further application. A bio inoculum was then prepared using these two efficient strains.

To assess the effect of the bio inoculum, we conducted seed germination tests and pot trials under drought conditions. The results demonstrated improved germination rates, healthier seedling growth, and better plant development in treated plants compared to controls. This confirmed that the selected PGPB strains could enhance drought tolerance and support plant growth even under limited water availability.

The success of this study highlights the significant potential of indigenous PGPB as a sustainable tool for agriculture, especially in regions frequently affected by drought. These bacteria not only help plants cope with abiotic stress but also contribute to overall soil health and nutrient cycling, reducing the dependency on chemical fertilizers.

The findings of this project support the development of low-cost, environment-friendly biofertilizers based on native drought-tolerant microorganisms. The positive results obtained from seed and pot trials encourage further exploration and field-level application of these bioinoculants.

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