SCHOOL OF GRADUATE STUDIES

SCREENING PLANT GROWTH PROMOTING ATTRIBUTES OF RHIZOSPHERIC PSEUDOMONAS SPECIES FOR ABIOTIC STRESS TOLERANCE ON SORGHUM

MSC THESIS

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NOVEMBER, 2023

WOLKITE, ETHIOPIA

Wolkite University

School of Graduate studies

Screening Plant Growth Promoting Attributes of Rhizospheric *Pseudomonas* Species for Abiotic Stress Tolerance on Sorghum

A Thesis Submitted to School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in General Biotechnology

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November, 2023

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School/ Department: Biotechnology

ACKNOWLEDGMENTS

First of all, I would like to thank Jesus Christ for His mercy, love, and perseverance, and his Mother, Saint Merry, for her help and unconditional love.

I would like to thank Dr. Tilahun Rabuma and Mr. Daniel Yimer for their support and guidance from proposal preparing to thesis writing. Their advice, mentoring, and keen judgment were crucial to the progress of my study and my overall development as a researcher. I am also grateful to my friends and classmates who helped me academically and emotionally. Their friendship made the difficulties of research easier to overcome. I would like to wholeheartedly thank the National Agricultural Biotechnology Research Centre, Holeta, Ethiopia, for their unwavering support in providing laboratory facilities and resources during my thesis research work. I would like to express my gratitude to Ambo University for financially sponsoring me to study for my M.Sc. study. I would also like to thank the Department of Biotechnology, Wolkite University, and all administrative staff for their constant support and the friendly environment they created.

Last but not least, I want to express deep appreciation to my family for their consistent support, inspiration, and tolerance as well as my son (Kine’ab) for his patience during my academic path. My biggest inspiration has been your confidence in me.

ABBREVIATIONS AND ACRONYMS

ACCD	1-aminocyclopropane-1-carboxylate deaminase
DNA	deoxyribonucleotide
IAA	Indole acetic acid
Mb	mega base pair
PEG	polyethylene glycol
P	phosphate
PCR	polymerase chain reaction
PGPR	plant growth promoting rhizobacteria
Spp.	Species
TSB	tryptone soya broth

TABLE OF CONTENTS

Contents Page No.

LIST OF FIGURES

Contents
Page

LIST OF TABLES

Contents

Page

LIST OF EQUATIONS

Contents Page

ABSTRACT

Sorghum is an economically important crop that is used for food, feed, and biomass production worldwide. Despite its economic importance, sorghum productivity is affected by biotic and abiotic stresses. By encouraging plant water-use efficiency, osmotic stress tolerance, and root development, PGPR (Plant Growth-Promoting Rhizobacteria) application assists in preventing drought and improves plant resilience and survival in environments where water is scarce. Therefore, the application of (PGPR) in sorghum demonstrates promising potential for sustainable and resilient agricultural practices. The present study aimed to screen the growth-promoting attributes of Rhizospheric Pseudomonas *spp. for abiotic stress tolerance in sorghum using a combination of techniques and approaches to gain a comprehensive understanding of the interactions between the* Pseudomonas *rhizospheric bacteria and the sorghum. Based on this there is a need to improve agricultural sustainability and chemical effects on the environment using PGPR. The sample was isolated from different areas of Ethiopia, and 210 isolated bacteria were screened using the serial dilution method. The response of PGPR was tested under temperature, pH, salt, and drought stress. The genes implicated in PGPR were amplified using PCR (polymerase chain reaction), and the bacteria were identified using* 16S RNA. *Based on biochemical tests 68 showed nitrogen fixation and 50 showed phosphate solubilization. Based on molecular examination, of the 68 isolates, 16 were positive amplifications for the* nifH *target amplicon and 10 for the* acdS *target amplicon, and of the 50 isolates, 21 were positive amplifications for the* pqq *target amplicon. Using the R software analysis, the* Pseudomonas *MS-22 isolate demonstrates its potential as a growth-promoting rhizobacteria; further research is required to identify optimal strains and application methods for sustained benefits. The implications of these findings extend to the realm of sustainable agriculture, where harnessing the potential of* Pseudomonas *MS-22 could pave the way for eco-friendly and efficient agricultural practices.*

Key Words: Abiotic stress, nitrogen fixation, Plant Growth-Promoting Rhizobacteria (PGPR), *pseudomonas* spp., sustainable agriculture

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a short-cycle C4 plant (Fan *et al.*, 2021) and hence can be harvested twice a year, producing a large amount of carbohydrates and fibers (López-Sandin *et al.*, 2021) with high photosynthesis capacity (Diene *et al.*, 2010). Sorghum ranks fifth among the most important cereal crops in the world after maize, rice, wheat, and barley (FAOSTAT, 2020). In 2023 projections indicate 62.18 million metric tons of production globally, surpassing last year's production of 57.34 million tons (Abello et al., 2023). Sorghum is the main staple crop for more than 500 million people in more than 30 countries (Abreha *et al.*, 2022). It is the main food crop for millions of people, especially in sub-Saharan Africa and South Asia (Khalifa and Eltahir, 2023). It is a significant food for the majority of the population in Ethiopia (Menamo *et al.*, 2021) and Ethiopia is the major source of genetic diversity (Cuevas and Prom, 2013) and adaptability to tropical and temperate conditions for sorghum (Gano *et al.*, 2021; Joshi *et al.*, 2021).

The whole part of this crop has been used for many purposes, i.e. the grain as food or feed, the leaves for forage, the stalk for fuel, and the fiber for animal feed (Almodares and Hadi, 2009). In addition, all components are economically valuable; for instance, bagasse has great biological value, such as evaluating the effects of different treatments on the solubilization of hemicellulose (glucoronoarabinonoxylan), xylose, and glucose synthesis (Martins *et al.*, 2021). It is also used for bioethanol production (Abdelhafez and Amer, 2015).

Despite its economic importance, environmental stress is much more closely related to the production of crops, affecting the quality and quantity of sorghum worldwide (Sarshad *et al.*, 2021). Therefore, discrepancies in its production can potentially jeopardize the food security of millions of poor people (Khalifa and Eltahir, 2023). Environmental stress is classified into two; abiotic and biotic stress. Thus, abiotic stresses are temperature, drought, salinity, and heavy metals (Ajeesh *et al.*, 2021). These stresses have affected plant morphology, physiology, biochemistry, and gene regulation (Kumar and Verma, 2018). About 80% of sorghum production in the world takes place under dryland conditions (Assefa *et al*., 2010), and in sub-Saharan Africa, sorghum is mainly grown in drought-prone areas, which account for almost

60% of the total area (Hadebe *et al*., 2017). In Ethiopia, 66% of the areas where sorghum is mainly grown are prone to frequent droughts (Geremew *et al*., 2004). Therefore, drought stress impairs the growth and development of sorghum, which ultimately leads to a significant reduction in grain yield (Bobade *et al*., 2019; Queiroz *et al*., 2019; Abreha *et al*., 2022). Assefa and his friends reported that drought stress is the type of abotic stress, reduces sorghum yield by more than 36 % and 55 % at the vegetative and reproductive stages (Assefa *et al*., 2010). Moreover, high temperatures (30-40 ℃) also inhibits photosynthesis, limiting the growth and development of sorghum seedlings (Yamasaki *et al*., 2002).

Different morphological and physiological traits are related to the ability of sorghum to maintain cellular metabolism and water uptake by reducing evapotranspiration and oxidative stress under conditions of drought, pH, salt and heat stress (Ndlovu *et al*.,2021). Moreover, several methods, including the use of film farming, nanoparticles, drought-resistant varieties of plants, super-absorbent hydrogels, and biochar, can be employed to improve abiotic stresses. However, most of these methods have few advantages and are costly and labor-intensive. Therefore, the effects of abiotic and biotic stress can be overcome by applying PGPR to plants (Babalola and Glick, 2021; Chaudhary and Sharma, 2021; Singh and Bhatia, 2021; Soumare *et al*., 2021).

PGPR help the growth and development of plants in stressful conditions (Kumar and Verma, 2018). Therefore, applying PGPR provides a preferential strategy that encompasses many direct and indirect benefits (Singh and Bhatia, 2021). They have numerous effects, including elongation of primary roots, differentiation of tissues, formation of pigments, stimulation of nitrogen fixation, and resistance to various stresses. The study by Santana *et al.* (2020) investigates how to increase Sorghum resistance to abiotic stresses by using microbial inoculants, specifically certain bacteria.

In Ethiopia's agricultural system, the roles of plant growth promoters in the rhizosphere and their biofertilizer capacities remain unknown. Thus, PGPR have a character in agriculture for the enhancement of products and yields while conserving soil fertility (Kumar *et al.* 2015). Hence, the current study aims to screen plant growth-promoting traits of rhizospheric *pseudomonas* spp for abiotic stress like heat stress, drought stress, salt stress and pH stress tolerance on *Sorghum*. The present study focused on screening different plant growth-promoting traits that *Pseudomonas* spp. displayed in the rhizosphere, with a focus on how well these traits worked to improve Sorghum resistance to abiotic stress.

The research explores the mechanisms of Pseudomonas spp. that promote plant growth, aiding in resilient and sustainable agriculture methods. It suggests using microbial fertilizers, specifically biofertilizers, instead of chemical fertilizers. The study also suggests using PGPR connected to sorghum roots to address drought stress, enhance plant productivity, and advance sustainable agriculture in Ethiopia. The research aims to close a knowledge gap in Ethiopian agriculture. In light of these considerations, the current study has the following objectives:

1.1 Objectives

1.1.1 General objective

- To evaluate plant growth-promoting traits of rhizospheric *pseudomonas* spp for abiotic stress tolerance on *Sorghum bicolor* (L.) Moench.

1.1.1 Specific objective

- To isolate plant growth-promoting traits that are associated with *Pseudomonas* spp. using biochemical tests and coding genes of the *nifH* gene, *pqq* gene, and *acdS* gene.
- To characterize the *pseudomonas* spp. using 16s rRNA gene
- To identify the optimal conditions of the isolated species on greenhouse evaluation.

2. LITERATURE REVIEW

2.1. Origin, domestication and distribution of sorghum

The sections *Chaeotosorghum*, *Heterosorghum*, *Parasorghum*, *Stiposorghum*, and *Sorghum* comprise the genus *Sorghum Moench*, according to De Wet (1978). *Rhizomatous Sorghum halepense* (L.) Pers. (2n = 40), *annual Sorghum bicolor* (L.) Moench (2n = 20), and *Sorghum propinquum* (Kunth) Hitchcock (2n = 20) are found in Section Sorghum. Originating from wild *Sorghum bicolor subsp. arundinaceum* from Sudan and Ethiopia, domesticated sorghums emerged (De Wet, 1978). Cultivated sorghum has developed from its original form into five major categories (bicolor, guinea, caudatum, kafir, and durra) and ten intermediate races (Harlan and de Wet, 1972).

Evolutionaries, botanists, and archaeologists disagree about the domestication and origins of sorghum. Sorghum is a multipurpose, drought-tolerant, and climate-resilient cereal that comes from African savannas. It feeds people in areas of drought who have fewer resources (Venkateswaran *et al.*, 2018). The northeastern region of Africa, which includes Ethiopia, Sudan, and East Africa, is known as the primary sorghum origin. It has the most diversity of both wild and farmed sorghums (Harlan, 2023).

Because of its resilience to a variety of situations, including genetic and environmental factors, sorghum yields remain stable (Roozeboom and Prasad, 2019). It grows in warm climates and is especially well-suited to areas with high temperatures and limited availability of water (Tesfaye, 2017). A hardy and adaptable grain crop, sorghum grows well in a variety of climates. Sorghum is adaptable in both semi-arid and dry conditions and is well known for its resistance to drought (Batista *et al.*, 2019). Although the crop can be grown in a variety of soil types, such as sandy and loamy soils, it usually thrives in soils that are well-drained and rich.

Sorghum is more tolerant to abiotic stresses than other crops (Reddy, 2019). However, Asadi and Eshghizadeh (2021) investigated that sorghum genotype responses to water shortage stress and that drought stress decreased the dry weight of the sorghum genotypes by reducing the traits related to plant performance and increasing the tolerance mechanisms. Heat and drought stress have been reported to affect the production and productivity of sorghum by reducing germination efficiency, reproductive, and yield of sorghum (Ndlovu *et al*., 2021). Sorghum can withstand drought, although, at key growth stages, it benefits from sufficient precipitation (Phiri *et al*., 2023; Sood *et al.*, 2023).

2.2. Taxonomic Classification of Sorghum

The current classification puts sorghum in the family *Poaceae, tribe Andropogoneae*, *subtribe Sorghinae*, section *Magnoliophyta*, class *Liliopsida*, order *Cyperales,* and genus *Sorghum* (Hariprasanna and Patil, 2015). The family *Poaceae* is a large family that comprises cereal crops, while the order *Poales* includes a variety of grasses. The genus Sorghum bicolor, as it is officially named, is a flowering plant that produces encased seeds. Sorghum is a member of the *Andropogoneae* tribe and the *Panicoideae* subfamily of the grass family. Sorghum is distinguished more precisely by the species Sorghum bicolor. This taxonomic classification, which is cited by Watson and Dallwitz in the botanical database, offers a methodical explanation of sorghum's position within the plant kingdom, emphasizing its connections to other plant species and advancing the area of botanical knowledge (Watson and Dallwitz, 1992).

2.3. Economic Importance of Sorghum

With an annual production of about 66 million metric tons in 2007, sorghum (*Sorghum bicolor* L. *Moench*) is the fifth most significant crop quantitatively, after maize, rice, wheat, and barley (FAOSTAT 2009). Sorghum *bicolor* L. Moench is a crop that is widely utilized as feed, food, fiber, and bioenergy (Rai *et al.*, 2016; Bakari *et al.*, 2023). The current classification puts sorghum in the family *Poaceae*, *tribe Andropogoneae*, *subtribe Sorghinae*, section *Magnoliophyta*, class *Liliopsida*, order *Cyperales*, and genus *Sorghum*. Moreover, a significant staple crop with great genetic diversity in Africa, sorghum boasts an extensive germplasm collection with around a quarter of a million accessions worldwide (Wang *et al*.,2016). For millions of people in Africa and Asia, sorghum is an essential part of their nutrition. Also, it is used for traditional farming as both food and fodder and is becoming more and more significant in the high-input commercial and biofuel sectors. Therefore, low-yielding sorghum fields make for more than 80% of the world's total sorghum production. Even though it is cultivated in more than 100 countries, more than 60% of the world's production comes from eight countries with one million hectares or more. The output of sorghum peaked globally in the middle of the 1980s and then began to drop, except for a few nations where cultivation is rising, such as Australia, Brazil, Ethiopia, and Sudan. Due to shifting tastes, sorghum is becoming less common as food, but it is becoming more common in industrial and animal feed. Also, sorghum will become more important in a changing environment, and it will need special care to provide food security and sustainability (Rakshit and Wang, 2016).

Furthermore, sorghum is a major contributor to the livestock sector and provides a staple diet for millions of people in Asia and Africa, making it a significant economic factor on a worldwide scale (Smith and Frederiksen, 2000; Singh *et al.*, 2010). Because of its high biomass and fermentable sugars, it is also becoming more and more popular in industrial applications, especially in the manufacture of biofuel (Yan *et al.*, 2010). Furthermore, sorghum has an impact on feed requirements and pricing in developed nations through its involvement in international trade, which is mostly related to the demand for animal products (FAO, 2021). Its ability to withstand harsh weather, notably drought, adds to its economic worth, particularly in areas where water scarcity is a concern (Ejeta, 2007). The economic relevance of sorghum is further influenced by trade policy, changing consumer tastes, and market dynamics (Taylor and Emmambux, 2010). Sorghum is an all-around adaptable and commercially valuable crop with a wide range of uses in many industries.

2.4. Production and Production Constraints Of Sorghum

In sub-Saharan Africa, sorghum (*Sorghum bicolor* [L.] *Moench*) is an important socio-economic crop that helps with food security and industrial needs in the face of population growth and climate change (Ahmad Yahaya *et al.*, 2022). Sorghum is fermented for malting and used to make beer in the area. In the industrial sector, sorghum is mostly utilised by businesses that make drinks, cereals for breakfast, and candies. A tiny portion of the grain is also utilised as animal feed. The stalks are fed to livestock and used to construct fences and shelters. The nation acknowledges the potential of sorghum for use as raw materials for the biofuel and other future uses (Mekonnen *et al.*, 2022).

The issues causing low sorghum production and product development include a lack of high-yielding sorghum varieties, decreasing soil fertility, drought stress, infestation of Striga, restricted access to production inputs, credit facilities, and financing (Sirajo *et al*., 2013). While farmers use diverse varieties, those introduced face challenges like lodging and poor grain quality. Thus, to over come this farmers must use the inoculated seed’s for high productivity. Farmers employ management strategies, but breeders must address these issues for wider acceptance. Constraints include poor soils, erratic rainfall, and pest-infested grain storage. Enhancing farmers' seed quality would benefit them, emphasizing the value of diversity (Kudadjie *et al.*, 2004). All this problem’s can be over comed by using PGPR for crop productions.

2.5. Improvement of Abiotic Stress Tolerance in Sorghum

To develop sorghum varieties that are resistant to soil acidity, breeders must consider several factors, including soil depth, rainfall, temperature, effective cation exchange capacity, natural content of essential elements, level of toxic ions, p-fixation capacity, and amount and quality of organic matter. Therefore, Reddy (2019) suggests that to generate plant types that are resistant to salinity, the molecular, physiological, biochemical, and metabolic components that contribute to salt tolerance must be combined.

Depending on the initiation, length, and intensity of the stress, abiotic factors can limit crop plant development and yield to varying degrees. According to estimates, the negative effects of environmental pressures cause crops to only yield approximately 25% of their potential (Zaidi *et al.*, 2019). Sorghum has developed complex mechanisms for responding to stress, and master regulators of stress-responsive genes, transcription factors (TFs), are encoded by genes that make good candidates for crop development. Recent research has demonstrated related strategies to improve stress tolerance in crop species, such as TF gene regulation and overexpression (Baillo *et al.*, 2019).

Despite being the world's fifth-most significant cereal, sorghum's full yield potential is not fully realized, especially in areas with low rainfall and challenging agronomic conditions. Thus, the crop's adaptability to abiotic stresses, particularly drought, makes it a crucial candidate for study. (Dalal *et al*., 2012) identify drought-related traits like stay-green post-flowering and the need for mapping genes associated with drought tolerance by using quantitative trait loci (QTL) and marker-assisted breeding approaches. Additionally, they discuss the challenge of aluminum toxicity in acidic soils, highlighting the identification of a

Multidrug and Toxic Compound Extrusion (MATE) gene in sorghum related to aluminum tolerance.

Moreover, to increase sorghum's low nutritional stress tolerance, family members of a few nutrient transporters, including the phosphate transporter (PHT), sulfate transporter (SULTR), and nitrate transporter (NRT), were found and described by (Maharajan *et al.*, 2021).

2.6. Plant Growth-Promoting Rhizobacteria

Rhizosphere

The word "rhizosphere," which is now often used to characterize this zone, was first used by Hiltner (1904) to refer to the soil microorganisms inside and around roots. The term "endophytes" now refers to bacteria living inside the root as opposed to those living on the root surface, or "rhizoplane" (Zhang *et al.*, 2019). Several microbial species as well as other creatures may be found in soil, and these organisms work together to build a very complex ecosystem (Lin *et al.*, 2019). Microorganisms are necessary for the breakdown of organic materials, the recycling of nutrients, and healthy plant growth (Ahmad *et al.*, 2007). The main areas where roots can grow are freely draining holes because roots are frequently larger than the water-filled pores at field capacity (pores with a diameter of 60 mm). The ability of roots to change their anatomy to fit into a pore that is typically smaller than their diameter is hindered if the soil will not deform (Gregory, 2006).

A variety of metabolic, physiological, and physical activities that result from root development, water and nutrient intake, respiration, and rhizo deposition distinguish the rhizosphere from the bulk soil. Additionally, these activities have a significant impact on plant physiology and microbial ecology (Hinsinger *et al.*, 2005). Plant growth-promoting rhizobacteria (PGPR), which are free-living soil bacteria that are beneficial to plant growth, can stimulate plant growth by colonizing the plant root. Rhizobacteria that promote nodule growth and plant health are both terms for PGPR. These are connected to the rhizosphere, an essential soil biological habitat for interactions between plants and microbes. The degree of closeness between plant roots and microbes in the soil grows nearer to the root surface, with the gradient of intimacy between the two increasing away from the plant root (Backer *et al.*, 2018).

2.7. Categories of Plant Growth-Promoting Bacteria

According to the report by Gray and Smith (2005), the PGPR can be separated into two categories *i.e.*, a) Symbiotic bacteria – they are a type of bacteria that live inside plants and exchange metabolites with plants directly, and b) Free-living rhizobacteria – the bacteria which live outside plant cells and can provide their metabolites. *Cyanobacteria* belonging to the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium*, and *Mesorhizobium* are examples of symbiotic nitrogen-fixing bacteria. It has been demonstrated that free-living or associative nitrogen-fixing bacteria, such as those from the species *Azospirillum*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*, may adhere to roots and effectively colonize root surfaces (Hayat 2010).

Free-living bacteria that attach very indiscriminately to plant tissues (mostly roots) and have strong ACC deaminase activity can shield plants from various abiotic and biotic challenges by reducing ethylene levels uniformly throughout the plant. On the other hand, symbiotic rhizobia with low enzyme activity, which typically binds tightly only to the roots of particular plants, promotes nodulation by locally reducing ethylene levels. It is unknown whether the significant differences in enzyme activity seen between free-living bacteria and rhizobia are due to variations in the amount of an enzyme synthesized by one type of bacteria as opposed to the other or variations in the specific catalytic activity of the different types of bacteria's enzymes (Glick, 2014a).

By supplying bio-available phosphorus for plant uptake, fixing nitrogen for plant use, securing trace elements like iron for plants by siderophores, producing plant hormones like auxins, cytokinins, and gibberellins, and reducing plant ethylene levels, free-living as well as symbiotic PGPR can directly enhance plant growth (Khan, 2005). *Pseudomonas fluorescens biotype G, Enterobacter cloacae ss disolvens*, and *Serratia marcescens ss marcescens* were used as three potential PGPR strains to examine the impact of either individual or consortium PGP bacterial inoculation on the growth, yield, and grain nutrient uptake of teff varieties (Tsegaye *et al.*, 2022). They discovered that the PGPR consortium could be used as inoculants to increase teff production and productivity because it performed better in promoting plant growth, yield, and grain nutrient uptake of teff varieties than individual PGP bacterial applications.

2.8. The Economic Importance of Plant Growth-Promoting Rhizobacteria

Through processes such as the synthesis of phytohormones, ACC deaminase, exopolysaccharides, siderophores, organic acids, nitrogen fixation, and more, PGPR efficiently overcomes biotic and abiotic challenges, reduces dependence on chemical fertilizers, and increases crop yield (Khoshru *et al.*, 2020). PGPR are important agricultural microorganisms that have significant economic benefits. PGPR improves plant nutrient availability through processes like nitrogen fixation, phosphate solubilization, and the synthesis of growth-promoting chemicals like phytohormones. Farmers can save money as a result of this decreased reliance on chemical fertilizers (Bhattacharyya and Jha, 2012). Furthermore, by improving the structure, nutritional content, and water-holding ability of the soil, PGPR promotes plant growth and may lessen the requirement for soil amendments (Khoshru *et al.*, 2020). According to (Nadeem *et al.*, 2009), the stress tolerance that PGPR confers against variables like salinity, drought, and diseases helps to stabilize crop production and reduces the financial risks that come with crop failures. Because PGPR is ecologically benign, it promotes long-term agricultural sustainability and reduces pollution in the environment in line with sustainable farming practices (Rai *et al.*, 2020). Furthermore, some PGPR strains' biological pest control abilities provide a low-cost, all-natural alternative to chemical pesticides, potentially saving costs associated with pest management (Compant *et al.*, 2019). The adoption of PGPR corresponds with market preferences as consumer demand for sustainable agriculture rises, providing farmers with new avenues for economic growth (Bhattacharyya and Jha, 2012). Higher crop yields, less reliance on chemical inputs, improved soil health, increased stress resistance, and alignment with sustainable farming practices all demonstrate the economic significance of PGPR in agriculture.

2.9. The Role of PGPR for Abiotic Stress Tolerance Improvement in Sorghum

One significant abiotic stressor reducing agricultural output is drought. The variety of microbiomes in the rhizosphere and roots is reduced by abiotic stresses (Qi *et al.*, 2022). Carlson *et al.* (2020) used rhizobacterial isolates to study how to induce systemic tolerance in sorghum against abiotic stress. Significant metabolic reprogramming was found for treating plants by untargeted metabolomics. *Bacillus* and *Pseudomonas* were the two isolates that showed 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. They increased antioxidant capacity, hormone upregulation (gibberellic acid, indole acetic acid, cytokinin), early activation of systemic tolerance signaling, osmolyte production (proline, glutamic acid, choline), epicuticular wax production (docosanoic acid), and ACC deaminase activity all contributed to the enhanced drought tolerance in primed sorghum. These factors ultimately resulted in lower ethylene levels.

Furthermore, (Govindasamy *et al.*, 2020) identify rhizobacterial endophytes from sorghum plants, such as *Ochrobactrum* sp. EB-165 and other species. Due to their stress tolerance, these bacteria help plants develop during droughts, especially in their roots. These isolates improve root length, dry weight, and surface area when seeded into a sterilized soil-rite mix. Additionally, the endophytes enhance physiological reactions including proline accumulation in leaves and osmotic adjustment. Upregulation of genes responsive to drought, such as sbP5CS2 and sbP5CS1, is seen, suggesting induced stress tolerance. The results point to the potential of these rhizobacterial interactions for stress resilience and sorghum growth enhancement.

2.10. Machinery of Plant Growth-Promoting Bacteria in Sorghum

There are two levels of complexity that PGPR and hosts can relate to the rhizospheric relationship. In many rhizospheric relationships, the PGPR attaches to the surface of the plant (Andrews and Harris, 2000). Endophytic relationship PGPR exists within apo plastic spaces inside the host plant (Vessey, 2003). Bruno *et al.*(2020) explore the effects of elevated atmospheric temperature on *Sorghum bicolor*, revealing that chromium-reducing, thermotolerant bacteria of plant growth-promoting bacteria (PGPB) can enhance plant growth, antioxidant defenses, and reduce chromium accumulation. In addition to this, there are two types of plant growth-promoting rhizobacteria (PGPR) found in the root zone *i.e.*, a) extracellular plant-growth-promoting rhizobacteria (ePGPR) and b) intracellular plant growth-promoting rhizobacteria (iPGPR) (Gray and Smith 2005). The sorghum-associated bacteria exhibited growth-promoting characteristics such as the generation of auxin, nitrogen fixation, and phosphate solubilization (da Silva *et al.*, 2018).

The extracellular PGPRs could exist inside the rhizosphere, on the rhizoplane, or inside the territories between the cells of the root cortex while intracellular plant growth-promoting rhizobacteria (iPGPR) exist in some cases inside the specific nodular arrangement of root cells

(Bhattacharyya and Jha, 2012). The bacterial genera such as *Agrobacterium, Arthrobacter, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Azotobacter, Azospirillum, Bacillus, Burkholderia, Micrococcus, Pseudomonas, and Serratia* are a good representative for ePGPR (Ahemad, 2014), while *Allorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium* are representative examples for iPGPRs (Bhattacharyya and Jha, 2012).

Three different types of machinery make up the rhizosphere; the first, the rhizosphere is the soil zone regulated by roots through the release of substrates and that affects microbial activity. The second, rhizoplane is the root surface that strongly binds soil particles, and the final, root is colonized by microorganisms (Barea *et al*., 2005). Ashry *et al*., (2022) isolated bacteria from the soil using serial dilution to examine drought-stress-resistant bacteria and investigated the drought-stress potential of the cultures using Polyethylene glycol (PEG) (6000). Based on these approaches, the rhizospheric bacteria were isolated from the root surface. A naturally occurring polymer that is nonionic, water-soluble, and has a molecular weight of 6000 is called polyethylene glycol (PEG) (Muscolo *et al*., 2014).

PEG 6000 is proven to imitate drought stress and causes osmotic stress, which lowers the plant's water potential (Ahmad *et al.*, 2020). PEG can control water's gradual decrease in a way that is homogeneously linear to the water potential of the soil (Daszkowska-Golec and Szarejko, 2013). Moreover, Ju *et al.*, (2021) utilized the process of serial dilution to identify bacteria to reduce salt stress on seedlings using plant growth-promoting bacteria from the soil. Moreover, PGPR is defined by three intrinsic appearances:- (i) They must colonize the root, (ii) they must survive and multiply in microhabitats associated with the root surface at least for the time needed to express their plant promotion/protection activities, and (iii) they must promote plant growth (Barea *et al*., 2005).

2.10. The Role of Plant Growth Promoting Rhizobacteria in Sorghum

Studies on the impact of PGPR on crops were not well coordinated in the middle of the 20th century, although up to 50–70% yield increases were reported. Additionally, the PGPR's mechanism was mostly unknown at this time (Bashan *et al.*, 2014). Increases in germination rates, root growth, yield (including grain), leaf area, chlorophyll content, magnesium content, nitrogen content, protein content, hydraulic activity, drought tolerance, shoot and root weights, and delayed leaf senescence are just a few of the benefits of PGPR addition to plant growth.

According to Creus *et al*, (2004), bacterial inoculation into the plant's rhizosphere increased crop output when drought stress was present. They found that pre-inoculating wheat with *Azospirillum brasilense* Sp245 increased crop yield and mineral quality while also improving water potential, relative and absolute water content, cell wall flexibility, and apoplastic water fraction, indicating an increase in drought resistance. The plant's acquired disease resistance, frequently referred to as "biocontrol," is another important advantage of PGPR use. The difficulty of PGPR in colonizing plant roots has frequently been cited as a reason for both their failure as biological control agents and their lack of effectiveness in the field (Lucy, 2004).

Moreover, PGPR can solubilize inorganic and organic compounds in abiotic and biotic stresses to promote the growth of sorghum (Mishra *et al.*, 2008). Three factors make up the ameliorative actions of PGPR in sorghum: their capacity to defend themselves against hyperosmotic circumstances and abnormal NaCl concentrations; their ability to assist plants in tolerating high salinity; and their capacity to enhance soil quality (Kumar *et al.*, 2020). The PGPR is influenced by several biotic factors (plant genotypes, plant developmental stages, plant defense mechanisms, and other members of the microbial community) and abiotic factors (soil composition, soil management, and climatic conditions) (Vacheron, Desbrosses, M. L. Bouffaud, *et al.*, 2013). It is believed that sorghum can benefit from a sufficient amount of ethylene produced from the existing supply of ACC to activate plant defensive responses to stress stimuli, such as temperature extremes, droughts or flooding, insect pest damage, phytopathogens, and disturbance (Abeles *et al*., 2012). Fortt *et al.*, (2022) claim that they use the ESL007 PGPR strain displaying ACC deaminase activity to discover the PGPR-mediated processes involved in the regulation of salt stress in plants and evaluate their impact on lettuce plants under salt stress.

However, when present at high concentrations in plant tissues, an elevated ethylene accumulation, also known as stress ethylene or the larger peak of ethylene in the biphasic model, may have damaging effects on plant growth (such as chlorosis, abscission, and senescence) and even result in plant death (Glick, 2003; Glick, 2014a). The reduction of the increased ethylene levels and subsequent indirect support of plant growth is greatly aided by the synthesis of ACC deaminase (Hatran *et al*., 2021). Through the deamination of 1-aminocyclopropane-1-carboxylic acid, PGPR is more frequently able to reduce plant ethylene levels. (ACC). The ACC deaminase gene (*acdS*), which converts ACC into ammonium and -ketobutyrate, is present in several PGPR genomes (Penrose and Glick, 2003; Contesto *et al.*, 2008). It has been discovered that many *Rhizobium* spp. carry the *acdS* gene and may manufacture ACC deaminase in a free-living environment. For instance, 27 *Rhizobium* isolates from Saskatchewan (Canada) in a collection of rhizobial isolates had an *acdS* gene and could make ACC deaminase, demonstrating that *acdS* genes are found in all *Rhizobium* isolates (Duan *et al.*, 2009). Out of phytohormones, auxins (e.g., Indole Acetic Acid (IAA)) are the main plant growth regulators. although it is generated by various plant-associated bacteria, including PGPR. (Spaepen, 2011). Through the synthesis of bacterial phytohormones and related metabolites as well as through major morphological changes in the roots, the PGPR has the potential to boost plant growth and assist in the control of plant diseases and abiotic stresses in the soil. These modifications enhance the relationship between plants and water, boost nutritional status, and activate plants' defense mechanisms to combat unfavorable environmental factors (Goswami, 2020). Many PGPRs are capable of producing phytohormones and secondary metabolites, including auxins, 2,4-diacetyl phloroglucinol (DAPG), and nitric oxide (NO), that block the plant auxin pathway (Vacheron *et al.*, 2013). Exogenous IAA regulates a wide range of processes involved in plant growth and development: low concentrations of IAA can promote primary root elongation, whereas high concentrations of IAA promote the growth of lateral roots, shorten primary root length, and promote the creation of root hairs (Patten and Glick, 2002; Perrig *et al.*, 2007). Cell division, elongation, and differentiation are a few of the growth and development processes that the signal molecule IAA controls in plants (Asgher *et al.*, 2015).

Different biochemical, physiological, and molecular processes are involved in plants' systems for drought tolerance. Thus, in plant tissues, hormonal interactions control these activities (Khan *et al.*, 2018). According to Scippa *et al.*, (2004), a histone H1 variation that is responsible for the production of drought stress-responsive genes for stomata closure is induced by drought stress. Additionally, the plant hormone abscisic acid (ABA) enhances drought tolerance by correctly regulating stress-responsive genes. Numerous ABA signaling genes, including OsNAP, OsNAC5, and DSM2, have been shown to increase grain output in drought-prone environments. Additionally, it is possible to harness these non-stomatal changes that ABA causes in plants to raise crop yields both qualitatively and quantitatively According to recent research, plants that overexpressed the ABA-induced GhCBF3 gene were able to tolerate drought by maintaining correct levels of water, chlorophyll, and proline (Ma *et al.*, 2016).

2.11. Mechanism of PGPR Involved In Promoting Sorghum Growth and Development

Direct and indirect processes are the two main ways that PGPR might promote plant growth and development. Thus, when PGPR uses one or more of the many different pathways to stop or lessen some of the negative impacts of plant diseases, this is known as indirect growth promotion (Glick, 2017). These include improving the host plant's resistance to harmful organisms or producing chemicals that inhibit pathogens (Cartieaux *et al*., 2003). (Manasa *et al.*, 2021) they identify the most potent isolates (*Bacillus mojavensis* and *Bacillus cereus*), assessing their compatibility as a consortium, that used the direct mechanism of plant growth promotion. Thus, microbial consortia enhance plant growth, yield, and nutrient content in sorghum.

Direct growth promotion (figure 1) can occur in a variety of ways, including by supplying the host plant with advantageous chemicals created by the bacteria and/or by aiding the uptake of nutrients from the soil environment (Santoyo *et al.*, 2021). The knowledge of the role of phytohormone synthesis as a direct mechanism of plant growth promotion by PGPR has improved through the use of molecular techniques using microbial and plant mutants altered in their ability to synthesize or respond to certain phytohormones (Nussaume and Robaglia, 2003). In traditional human health systems, sorghum is an essential grain crop that suffers considerable problems from biotic stressors (Bizoza *et al.*, 2020). To overcome this PGPR used an indirect mechanism.

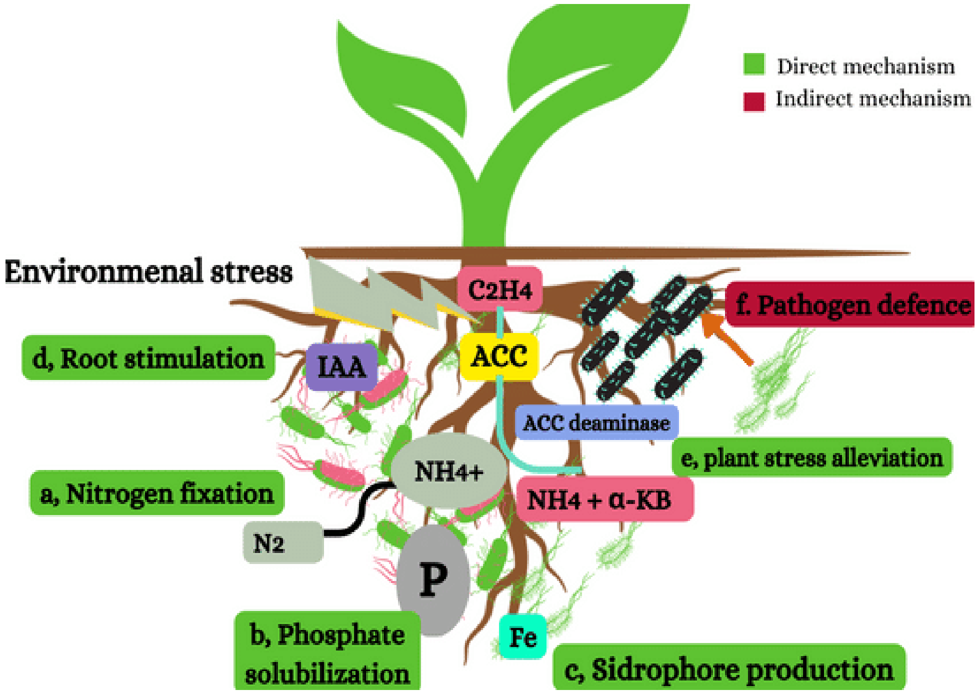

Figure 1. Mechanism of Plant growth promoting rhizobacteria

Indirect and direct mechanisms (figure 1) are located by microbes to promote plant growth and development during stress conditions (Gray *et al*., 2018). These two mechanisms are used by microbes to promote growth and development. These include nutrient fixation, neutralizing biotic and abiotic stress, and producing volatile organic compounds (VOCs) and enzymes to prevent disease (Kumar and Verma, 2018). Phytohormones are produced by plant growth promoter rhizobacteria in direct mechanism (PGPR) (Mishra *et al*., 2008). Gibberellic acid (GA), cytokinin (CK), auxin, abscisic acid (ABA), jasmonic acid (JA), and ethylene are plant growth regulators that enable plants to alter their responses and adapt to drought stress (Basu *et al*., 2016). However, the mode of action of different types of PGPR varies according to the type of host plant (Garcia *et al.* 2015).

Plant growth-promoting rhizobacteria are also considered bio-stimulants because microbes interact with plants at the roots (Backer *et al*., 2018). They improve nitrogen fixation, phosphate, and potassium solubilization, or produce phytohormones, or biocontrol agents when they produce antimicrobial compounds with activity against phytopathogens (Hakim *et al*., 2021; Strafella *et al*., 2021).

The majority of research on the methods PGPR uses to promote plant growth has concentrated on bacterial features without considering the physiological reactions of the host plant (Bloemberg and Lugtenberg, 2001). At various stages of a plant's growth, these two mechanisms may operate either simultaneously or sequentially (Chaparro *et al.*, 2013). The PGPR's numerous modes of action and advantages for plants include everything from simple occupying of void biological spaces to ecological connections like symbiosis, antibiosis, competition, and predation, among others (Kloepper *et al*., 2004).

They are divided into three major groups according to their activities, which correspond to three growth promotion methods (Figure. 2):

i. Biofertilizers improve nutrient availability and plant nutrient uptake.

ii. Phytostimulants, also known as biostimulants, create helpful compounds like PGRs, which are not nutrients, pesticides, or soil-improving agents.

iii. Biocontrol agents: they prevent the spread of pathogens.

either through the synthesis of antimicrobial metabolites or rivalry for resources and space (Subramaniam *et al*., 2020; Soumare *et al*., 2021).

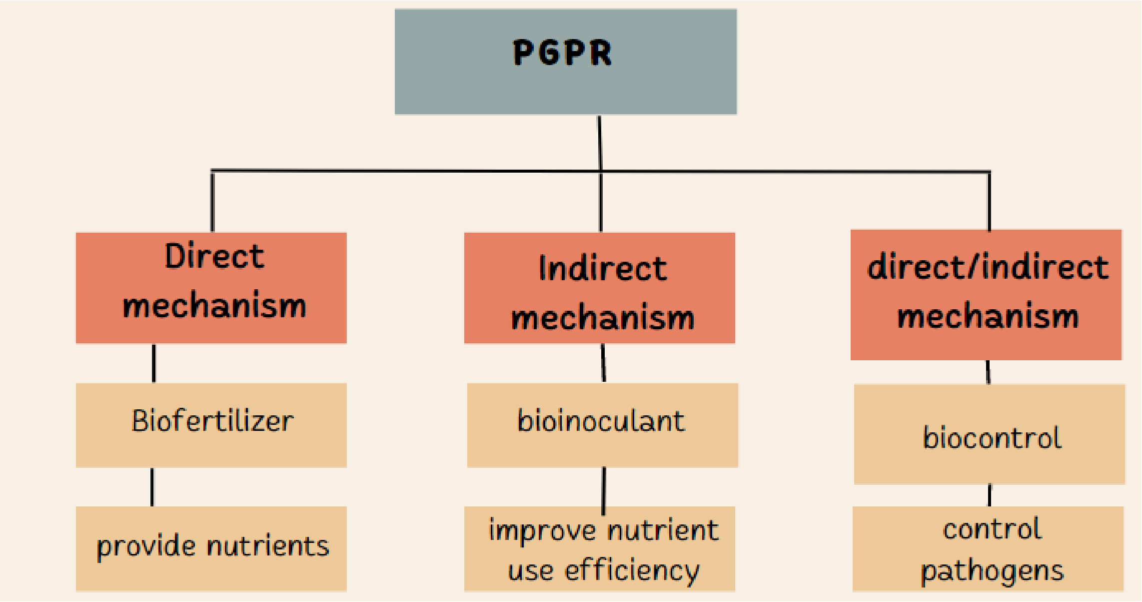

Figure 2. Role of Plant growth promoting rhizobacteria (PGPR).

In addition to encouraging the uptake of nitrogen and phosphorus, PGPR can also efficiently encourage the uptake of other nutrients and plant growth (Ahmad *et al*., 2008). Without fertilization, significant increases in the uptake of potassium, calcium, and magnesium were observed in a calcareous soil when wheat was inoculated with *Pseudomonas* sp. or *Bacillus* sp. (Mehmet *et al*., 2011). Some PGPRs can exhibit two or three processes that aid in encouraging plant development. PGPRs have an impact on all facets of plant life through their multifunctional activities, including seed germination, nutrition, growth, and reaction to biotic and/or abiotic stimuli (Weyens *et al.*, 2009; Subramaniam *et al.*, 2020).

2.11.1. Direct mechanisms

Plant growth and development are aided by mechanisms such as nutrient absorption or increased nutrient availability through nitrogen fixation, mineralization of organic compounds, solubilization of mineral nutrients, and phytohormone synthesis (Bhardwaj *et al.*, 2014).

Direct methods include those that change the balance of plant growth regulators, whether microorganisms release growth regulators that integrate into the plant or function as a sink for plant-released hormones, and plant metabolism that improves adaptive capacity (Govindasamy, 2008; Glick, 2014).

A. *Nutrient fixation*

Plants take up nitrogen from the soil in the form of nitrate (NO^{-3}) and ammonium (NH4+), both of which are necessary for growth. In aerobic soils where nitrification occurs and is taken by plants, nitrate is frequently the most abundant form of accessible nitrogen (Xu, 2012). Recently, (Minorsky, 2008) described a PGPR strain, *Pseudomonas fluorescens* B16, colonizing tomatoes' roots vigorously and increasing plant height, blossom quantity, and total fruit weight.

Salinity reduces soil water potential, making it difficult for plants to absorb water and nutrients, resulting in osmotic stress. Cations such as Na^+ (sodium), Ca^{2+} (calcium), K^+ (potassium), anions Cl (chloride), and NO^3 (nitrate) induce salinity in soil under a variety of situations (Kumar and Verma, 2018). In another study, inoculating salt-resistant and salt-sensitive chili peppers with the growth-promoting bacterium *Pseudomonas stutzeri* reduced the deleterious effects of soil salinity (Bacilio, 2016).

B. *Nitrogen fixation*

Nitrogen (N) is a key plant nutrient that has become a limiting issue in agricultural environments owing to high rainfall and mineral leaching losses (Bhattacharyya and Jha, 2012). Bacterial strains with the ability to fix nitrogen are divided into two groups. The first group contains root/legume-associated symbiotic bacteria that can infect roots and create nodules, such as Rhizobium strains. The so-called free-living nitrogen fixers are another type of bacterium that isn't plant-specific (Oberson, 2013).

C. *Phosphate solubilization*

Phosphorus (P) is a major nutrient used by plants for growth(Adhikari *et al.*, 2021). It requires plant-based metabolic processes like photosynthesis, energy transfer, signal transduction, and macromolecular biosynthesis (Glick and Babalola, 2017). Rhizobacteria that encourage plant growth can either stimulate ion transport mechanisms in the root or directly improve nutrient delivery in the rhizosphere. Two basic categories of bacterial activity can be thought of in terms of enhanced nutrition supply. First off, one important impact of PGPR on plant nutrition is phosphate solubilization. Although frequent fertilizer treatments leave soils with vast amounts of phosphorus, only a small part of this is useful to plants(Vacheron, Desbrosses, M. L. Bouffaud, *et al.*, 2013).

Soil microbes can uptake P and mobilize it through plant roots (Richardson and Simpson, 2011). *Mesorhizobiumciceri* and *Mesorhizobiummediterraneum*, both isolated from chickpea nodules, are effective phosphate solubilizers (Parmar and Sindhu, 2013). Soil microorganisms can transform an unavailable form of P to orthophosphate (Pi), a simple form of P that is used directly by plant roots. These microorganisms are referred to as phosphate-solubilizing microorganisms (PSMs) (Kalayu, 2019; Babalola, 2021).

D. *Phytohormone production*

Phytohormones control all cell processes in photosynthetic organisms and help to organize a variety of signal transduction pathways during the plant's response to abiotic stresses (Pieterse *et al.*, 2009). They are organic substances generated in the plant's interior to control plant development and yield. Despite that, they cause plants to develop resistance to abiotic and biotic stressors. Abiotic stressors activate signal transduction pathways that are linked to baseline processes and are transduced by plant hormones (Khan *et al.,* 2020).

Plant growth, development, reproduction, and survival are all regulated by phytohormones, which are tiny chemicals. They operate as signal molecules and are found in small amounts. Abscisic acid (ABA), auxins, cytokinins, ethylene (ET), and gibberellins are classic phytohormones, although tiny signaling molecules like brassinosteroids, jasmonates (JAs), and salicylic acid (SA) are also classified as phytohormones (Pieterse *et al.*, 2009).

E. *Siderophore production*

Microorganisms make siderophores, which are tiny organic compounds that increase iron absorption capability when iron is scarce. Siderophores have gotten a lot of interest in the last ten years because of their unusual ability to collect iron metal ions (Saha *et al.*, 2016). As PGPR, *Pseudomonas* sp. makes use of siderophores generated by other microorganisms in the rhizosphere to meet its ion requirements (Gray *et al.*, 2018).

2.11.2. Indirect mechanisms

The method of biological control through which rhizobacteria indirectly promote plant development is by lowering the effect of illnesses, such as antibiosis, systemic resistance, and competition for resources and habitats (Miransari, 2014). Growth-promoting rhizospheres have indirect effects on plant health by suppressing phytopathogens and other deleterious microorganisms through parasitism, competing for nutrients and niches within the rhizosphere, producing antagonistic substances (such as hydrogen cyanide, siderophores, antibiotics, and antimicrobial metabolites) and lytic enzymes (such as chitinases, glucanases, and proteases), and inducing systemic resistance in plants against a wide range (Berg *et al.*, 2017; Meena *et al.*, 2020). Antifungal compounds produced by rhizobacteria include HCN, phenazines, pyrrolnitrin, 2,4-diacetyl phloroglucinol, pyoluteorin, viscosinamide, and tensin (Bhattacharyya and Jha, 2012).

Any type of stress stimulates the production of reactive oxygen species (ROS) such as H_2O_2, O_2, and OH radicals. The overproduction of reactive oxygen species (ROS) generates oxidative stress, which harms plants by oxidizing photosynthetic pigments, membrane lipids, proteins, and nucleic acids (Gray *et al.*, 2018). The use of PGPR in plant abiotic stress management has been extensively researched using bacterial strains such as *Pseudomonas putida* and *Pseudomonas fluorescens*, which can scavenge cadmium ions from soil and thereby offset the damaging effect of cadmium pollution on barley plants (Jila Baharlouei Yancheshmeh, 2011). Stimulating rhizobacteria in plant roots produces a signal, that travels throughout the plant and boosts the defensive power of distant tissues against pathogen invasion (Thakker, 2013).

A. *Antibiotics and Enzymes*

As bio-control agents develop resistance to particular antibiotics due to an overreliance on bacteria that produce antibiotics, several researchers have used bio-control strains that produce hydrogen cyanide and one or more antibiotics to stop this from occurring. While hydrogen cyanide might not have much bio-control action on its own, it seems to work well with antibiotics that are encoded in bacteria, making this strategy effective (Kim *et al.*, 2008). Chitinases, cellulases, β-1, 3 glucanases, proteases, and lipases are just a few of the enzymes that some biocontrol bacteria produce. These enzymes can partially break down the cell walls of many pathogenic fungi (El-tarabily *et al.*, 2000).

B. *Induced Systemic Resistance*

Induced systemic resistance (ISR), which is phenotypically similar to systemic acquired resistance (SAR), is a phenomenon that can be brought on in plants by PGPR. SAR happens when plants activate their defensive mechanisms in response to infection by a pathogenic agent (Pieterse *et al.*, 2009).

ISR-positive plants are thought to be "primed" to respond to pathogen attacks by triggering defense systems more quickly and forcefully. ISR doesn't focus on any particular disease. Instead, it might work well to manage disorders brought on by several pathogens. Jasmonate and ethylene signaling in the plant is a component of ISR, and these hormones promote the host plant's immune responses to a variety of pathogens (Verhagen *et al.*, 2004).

3. MATERIALS AND METHODS

3.1. Sample Collection

3.1.1. Plant material collection

Recently published genotypes of sorghum that offer high yield production are Teshale, Argity, Gambella 1107, Degalit, Meko, Melkam, and Abshir. Those genotypes were therefore collected from the Melkassa Agricultural Centre and taken to the Microbial Biotechnology Laboratory, National Agricultural Biotechnology Research Center (NABRC), Holeta. About 20 kg of soil samples were collected from three different locations, namely Kemise Special Zone, Artumafursi Woreda (10.31'19.8", 039.55'24.00"), Jiletumuga Woreda (10.17'57.4", 039.58'23.8") and Semenshewa Zone, Ifat, and Gidim Woreda (10.27'33.3", 039.56'12.4") at a depth of 15 to 20 cm from each site and carefully transported to the Microbial Biotechnology Laboratory, (NABRC).

3.1.2. Soil sample collection and seed germinations

The entire experiment was conducted at the National Agricultural Biotechnology Research Center in Holeta, Ethiopia, in the Oromia region, which is 29 km from Addis Ababa. The collected soil samples was well-sieved and air-dried for 4 hours and then the soil was put in the 50g sterilized plastic pot (figure 3A). The collected seeds of Sorghum were surface sterilized with 95% ethanol and 3% sodium hypochlorite solution for 10 seconds and 1 minutes, respectively. Seeds were rinsed five times with sterilized distilled water and allowed to germinate for three days at 25°C on water agar (1%). Five pre-germinated seeds were planted in each surface sterilized plastic pot (figure 3B). All pots were placed in the greenhouse and watered every three days. After 30 days from germination as shown in figure 3C, sorghum seedlings were selected from each pot and carefully uprooted with their rhizosphere soil (figure 3D).

Figure 3.The process of harvesting rhizospheric soil from the pot. A, putting the soil into pot B, and Sowing the seeds in the prepared pot C, After thirty days of germination, the sorghum seed looks like this D, the rhizospheric soil extraction from the root surface of the plant.

3.2. Isolation and Determination of Strains

The rhizospheric soil sample was transeferred by using sample bag (figure 4A) and one gram of the soil sample was weighed (figure 4B) and mixed with 9 ml of distilled water in a sterilized falcon tube. Then the serial dilution method was applied to obtain the appropriate concentration of bacteria. One ml of the supernatant solution was diluted from 10^{-1} to 10^{-6}. The bacterial colonies were isolated from the soil and purified using the spreading method on King-B agar and incubated at 28 °Cfor 24 hours (Gibbs and Hayes, 1988). After 24 hours, the single colony was spread on TSA (Tryptic Soy Agar) and incubated at 28℃ incubator for 24 hours. After that, a single colony was transferred into an Eppendorf tube that contained TSB (Tryptic Soy

Broth) medium and incubated at 28℃ for 24 hours. The culture was stored in 20% glycerol and kept at -80℃ for further processes.

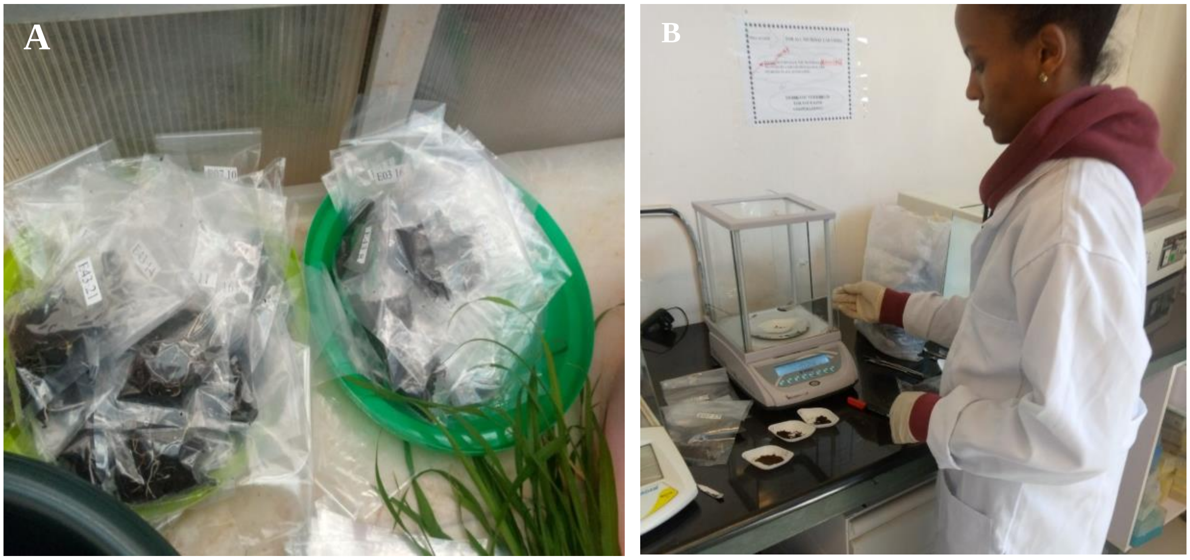

Figure 4. Transferring the rhizospheric soil by sample-containing bag into laboratory B, measuring 1g of rhizospheric soil in the laboratory.

3.3. Bacterial Biochemical Characterization

3.3.1. Phosphate solubilization test

A total of $10\mu l$ of the bacterial culture was inoculated on a Pikovskaya agar plate in three different locations per plate and incubated for 5 days at 28℃ for a phosphate-solubilizing test as a method used in Pikovskaya and Ri (1948). Then, the formation of the clear zone was measured in millimeters (mm) using a ruler, and isolate variation was made using the phosphate solubilization index formula (Hariprasad and Niranjana, 2009).

Equation 1 phosphate solubilization index

Solubilization index = [the ratio of the total diameter (colony + halo zone) - colony diameter]

3.3.2. Nitrogen fixation ability test

The nitrogen-fixing ability of isolated bacteria was tested by using BG-11 semi-solid medium without (NH_4NO_3) as a method used in Hardy *et al.*(1968). All isolate was inoculated in a 25 mL flask containing 10 mL and then incubated at 28℃ for 3 days. The color change from green to blue and the formation of pellicles were detected and recorded in the test tube. The green color changes into a blue color and the formation of pellicles indicates that the bacteria can fix the nitrogen. The media without culture was used as a control.

3.3.3. Monitoring Indole Acetic Acid (IAA) Production

Indole Acetic Acid (IAA) production test was performed using Patten and Glick protocols (Patten and Glick, 1996). The isolate was grown in Luria broth supplemented with L-tryptophan (0.5g ml-1) for 72 h. Then, the culture was centrifuged at 10,000 rpm for 10 min. To quantify the amount of IAA produced by *Pseudomonas* spp., 1ml of the supernatant was allowed to react with 1.5 ml of Salkowsky reagent (1.5 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) at 28℃ for 30 min as the method described in Ehmann (1977). Then, the absorbance was measured using a spectrophotometer at 530 nm.

3.4. Evaluation of Plant Growth Promoting Attributes and Extreme Growth Properties *In Vitro*

3.4.1. High-temperature tolerance test

The samples of soil that isolated using serial dilution and grow on the king's B agar were refreshed in a TSB medium and incubated at 28℃ for 24 hours. Approximately 1 ml of TSB was added to an autoclaved 96-deep-well microtiter plate, and 1 µl of fresh culture of the strains was inoculated. The 96-well deep well plates were adjusted to assess heat tolerance at different temperatures and also incubated at 35℃, 40℃, 45℃, and 50℃ (Patel *et al*., 2017). Subsequently, the OD630nm of the culture was measured after 0 and 24 hours using a Multiskan FC microplate reader (Thermo Fisher Scientific Instrument Co. Ltd. Shangai, China) (Mahdi *et al.*, 2021).

3.4.2. Salt tolerance test

The isolated culture was refreshed in a TSB medium and incubated at 28℃ for 24 hours. TSB was prepared in four different salt concentrations: 0.5M, 1M, 1.5M, and 2M (Thant *et al.*, 2018). TSB with each salt concentration was provided in four different bottles. Approximately, 1 ml of TSB was added to an autoclaved 96 deep-well microtiter plate, and 1 µl of fresh culture of the strains was inoculated and incubated at 28℃ for 24 hours. Subsequently, OD630nm was measured after 0 hours and after 24 hours using a Multiskan FC microplate reader (Thermo Fisher Scientific Instruments Co. Ltd. Shangai, China). Three replicates of each isolate were measured at the same concentrations while a sterile medium was used as a blank (Mahdi *et al.*, 2021).

3.4.3. Drought tolerance test

The isolated culture was refreshed in a TSB medium and incubated at 28℃ for 24 hours. TSB was supplemented with four different concentrations of polyethylene glycol (PEG 6000): 10%, 15%, 25%, and 32.6%. About 1 ml of TSB was added to an autoclaved 96-deep-well microtiter plate, and 1 µl of fresh culture of the strains was inoculated and incubated at 28℃ for 24 hours. Then, the OD630nm of the culture was measured at 0 and 24 hours using a Multiskan FC microplate reader (Thermo Fisher Scientific Instruments Co. Ltd., Shangai, China), and a sterile medium was used as a blank. Three replicates of each isolate at the same concentrations were measured (Ashry *et al*., 2022).

3.4.4. PH tolerance test

The isolated culture was refreshed in a TSB medium and incubated at 28℃ for 24 hours. About 1 μl of the fresh culture of the strains was inoculated on 1 ml of TSB previously dispensed in an autoclaved 96-deep-well microtiter plate. The 96-well deep well plates were adjusted for different pH (2, 3, 4, and 5 and incubated at 28℃. Then, after 0 and 24 hours of incubation periods, the OD of the culture was measured at 630nm using a multiskan FC microplate reader (Thermo Fisher Scientific Instruments Co. Ltd. Shangai, China) (Mahdi *et al.*, 2021).

3.5. Molecular Screening of Plant Growth-Promoting Traits Using Specific Primers

Genomic DNA was extracted using the EZ-10 spin column DNA cleanup miniprep kit protocol (Bio-Basic) with some modifications. The cultures were refreshed by TSB broth medium and incubated at 28℃ for 24 hours. DNA quality and concentration were measured by a Nanodrop spectrophotometry (NanoDrop ND-1000). Furthermore, the quality of the extracted genomic DNA was checked using 1% gel-electrophoresis at 100 volts for a 45-minute.

3.5.1. DNA amplification of *Pseudomonas* strains using 16s rRNA primer

The bacterial strains having potential in plant growth promotion and preserving multiple beneficial traits were identified by partial sequencing of the 16s rRNA gene. The amplification of the PCR product were checked by 0.8% agarose gel electrophoresis and ultraviolet spectrophotometry, respectively. High-quality DNA samples were amplified using primers listed in (table 1) targeting the full-length regions of the bacterial 16S rRNA gene as the method used in Edward, (1984). PCR reactions were containing genomic DNA, forward and reverse primers, deoxynucleotide triphosphate, $MgCl_2$, 10× buffer, and Taq DNA polymerase.

The PCR reaction mixtures containing 7.5 μl DNAase free water, 1 μ l of forward and reverse primers at 20 μm final concentration, 12.5 μl PCR master mix, and 3 μl of DNA sample. Thus, the total PCR reaction mixture volume is 25 μl were prepared. The amplification process was performed based on the optimized primer's temperature using gradient PCR (Bio-Rad Thermo cycler T100).

The following PCR reaction conditions were used for PCR amplifications: 1 cycle of 94°C for 5 min;35 cycles of 94°C for 30 s, 56 °C for 30 s, 72 °C for 1 min, and 1 cycle of 72°C for 10 min. The amplified product was run on 0.8% agarose gel for 45 minutes at 100 volts, and the PCR amplicon band capturing was performed using a gel documentation system (Omni Doc, Cleaver Scientific Ltd., Taiwan). Then, 5 μl of each PCR product sample was run for 50 minutes at 100 volts on 1% agarose gel and quality was checked on UV spectrophotometry (Nano Drop ND-8000 8 sample spectrophotometer).

3.5.2. PCR amplification for the *nifH* gene fragments

A total of 100ng of DNA was used as a template in a PCR amplification reaction. Selected forward and reverse primers of *nifH*F and *nifH*R (Table 1) were used to amplify a 317-bp region of the *nifH* coding sequences. The PCR amplification reaction was performed for 35 cycles at 94℃ (5 min and 30 sec), 1 min for the annealing step at 55℃and 72℃ (2 min) elongation reactions, and 10 min final extension at 72℃ as the method described in Poly, (2001). The amplified product was run for 50 minutes at 100 volts on 0.8% agarose gel and the amplified band product was captured using a gel doc system (Omni Doc, Cleaver Scientific Ltd., Taiwan).

3.5.3. PCR amplification for the *pqq* gene fragments

Primer pairs listed in Table 1 were used to amplify the region on the *pqq* gene that was used for phosphate solubilization. Polymerase chain reaction (PCR) was performed on a SimpliAmp thermal cycler (Applied Biosystems). The following conditions were used for PCR: 1 cycle of 94℃for 5 min; 35 cycles of 94℃ for 30 s, 56℃for 30 s, 72 ℃for 1 min, and 1 cycle of 72°C for 10 min. After PCR, the amplified product was run on 0.8% agarose gel, and photo documentation was performed using a gel doc system.

3.5.4. PCR amplification for the *acds* gene fragments

The PCR reaction conditions for amplification of *acds* gene were set as 1 cycle at 94°C for 5 min, 35 cycles for 1 min at 94 °C, 55 °C for 1 min, and 72 °C for 1 min, and the final extension step of 72 °C for 10 min (Kamala-Kannan *et al.*, 2010). Then finally, the PCR amplified product was run on 0.8% agarose gel for 50 minutes at 100 voltage, and photo documentation was performed using a gel doc system.

Table 1. List of primers used to amplify the pgpr traits such as nitrogen fixation, phosphate solubilization, and 1-aminocyclopropane-1-carboxylic acid deaminase associated with pseudomonas spp.

Name of gene-specific Primers	**Primers**	**Sequence**	**Length (bp)**	**Tm**	**GC%**	**Amplicon Size**
16s rRNA	F	AGAGTTTGATCCTGGCTCAG	20	51.8 °C	50.00%	1,300–1,500 bp
	R	AAGGAGGTGATCCAGCCGCA	20	55.9 °C	60.00%	
nifH	F	TGCGAYCCSAARGCBGACTC	20	53.8-60 °C	55 - 70.00%	360bp
	R	ATSGCCATCATYTCRCCGGA	20	51.8- 55.1°C	50-60 %	
pqq	F	GGCTGCTGGCCGAACTGA CTT	21	58.3°C	62%	670 bp
	R	GGCCGCAAGAAGCATTATTAG	21	52.4°C	48%	
acdS	F	GCAACAAGACGCGCAAGYTN GARTAYN	27	56.7-64.3 °C	41-59%	754 bp
	R	GTGCATCGACCCCTCRW ANACNGGRT	26	61.5-67.2 °C	48-60%	

3.6. Evaluation of Selected Isolates for Abiotic Stress Tolerance on Sorghum

3.6.1. Germination assay

To evaluate the ability of the selected bacterium to stimulate early plant development, Gambella-1107 sorghum seeds were used for *in vitro* seed bacterialization. After sorting, the seeds were surface sterilized with 3% sodium hypochlorite for one minute, soaked in 70% ethanol for one minute, rinsed five times with sterile distilled water, and air-dried in a laminar flow hood. The bacterial pellets (OD600nm = 0.2) were separated from an overnight bacterial culture by centrifugation for 5 minutes at 5000 rpm. The plates were wrapped with parafilm to stop evaporation and contamination. The seed of the sorghum germination assay was examined for a total of five days. Germination rates were checked 24 and 48 hours after incubation on the plates, which were tagged and kept at 25℃ in the dark.

To calculate the plates' morphological features, namely total length and fresh and dry weight, they were kept at room temperature in a day/night cycle (~12/12 h) for an extra 72 hours on the third day. The following formulae were used to determine the vigor index and germination percentage (Bybordi, 2010; Hossain, 2016; Mahdi *et al.*, 2021). Three milliliters of sterile distilled water were used aseptically to moisten each filter paper. The plates were wrapped with parafilm to prevent evaporation and contamination. Germination rates were checked 24 and 48 hours after incubation on the plates, which were labeled and stored at 25℃ in the dark. To calculate the morphological characteristics of the plates, i.e. total length and fresh and dry weight, they were kept at room temperature for a further 72 hours on the third day in a day-night. The following formulae were used to determine the vigor index and germination percentage: (Bybordi, 2010; Hossain, 2016; Mahdi *et al.*, 2021).

Equation 2 Germination percentage

Germination percentage (%) = (n/N) × 100………………………. (1)

n: number of germinated seeds and N: total number of seeds.

Equation 3 vigor index

Vigor index = Germination percentage (%) × Total seedling length (cm)……………… (2)

3.6.2. Greenhouse pot experiment

In vivo, pot experiments were carried out to test the effect of Pseudomonas sp. on the development of the sorghum plant in the greenhouse of the National Agricultural Biotechnology Research Center in Ethiopia. Before filling the pots, the soil was autoclaved twice at 121 °C for 20 minutes. After sterilization, the soil was mixed with tricalcium phosphate powder (2 g/kg) and mixed thoroughly. Six replications of pots for each experiment were performed. Each pot contained 1 kilogram of soil for phosphate dissolution and 1 kg of sand for nitrogen fixation. All pots (twenty-four pots) were placed under natural light (sunlight) at an average temperature of 25 °C during the day and 8 °C at night. The sorghum seeds were disinfected by immersion in 70% ethanol for 1 minute and 3% (v/v) sodium hypochlorite for 2 minutes. Then, the seeds were extensively washed with sterilized distilled water five times for five minutes. Seeds were put into 9-cm sterile Petri plates on filter paper at a ratio of 20 seeds per plate for germination. After germination, thinning to one seedling per pot was performed and plant growth promoter was conducted under greenhouse conditions.

After the emergence of seedlings and sowing of germinated seeds, 5 mL of bacterial inoculum (10^8 CFUmL^{-1}) was poured close to the stems of plants (per plant for each inoculation step) depending on the duration of the experiments at 0, 14, and 28 post days of sowing (Kandeler *et al.*, 2019). The pots that used as a Control will not be poured by the culture of the bacteria but replaced by 200 ml of distilled water. The plants were watered with 200ml of distilled water every single day. The experiment was laid out in complete randomized design (CRD) with four treatments A0 (Control contains Tri Calcium Phosphate + water and soil), A1 (TCP + Bacteria culture and soil), A2 (sand + Water), A3 (sand + Bacteria Culture) (Nagrale *et al.*, 2023). There were six replications for each treatment with five plants per pot. On the 30 days of post-sowing, growth parameters such as root length, root dry weight, shoot dry weight, shoot root ratio, percentage of root and shoot dry weight increase over control, and disease incidence were recorded. An experiment containing 5 plants in each pot, and a total of 10 plants were taken for mean comparison of the data, which was subjected to one-way ANOVA using R Software.

4. RESULTS

4.1. Morphological Characteristics of Isolates

Using serial dilution up to 10^{-6}, a complete set of 210 isolates was obtained from soils associated with seven different genotypes of sorghum. While figure 5B shows the isolation of a single colony at a dilution of 10^{-1}, figure 5A shows the rapid growth of *Pseudomonas* colonies at a dilution of 10^{-3}. After selecting the most notable isolates based on unique morphological characteristics, they were purified using multiple streaking techniques on the same culture medium. To accomplish further separation, this procedure entails streaking a sample onto a solid King's B medium, enabling isolated colonies to grow, and then moving a tiny amount of these colonies to a fresh TSA broth. The separated strains' purity is enhanced by this repetition.

After 3–4 days of incubation, morphological examination revealed that *Pseudomonas* sp. colonies had small diameters (1.5–2.5 mm) and an intact, round edge on King's B agar at 15 °C. *Pseudomonas* sp. colonies showed reduced size (1.5–2.5 mm diameter) with a complete, circular border and a creamy coloration on King's B agar under 28 °C conditions after 3 days, but no creamy color was seen on nutritional agar (figure. 5C and 5D).

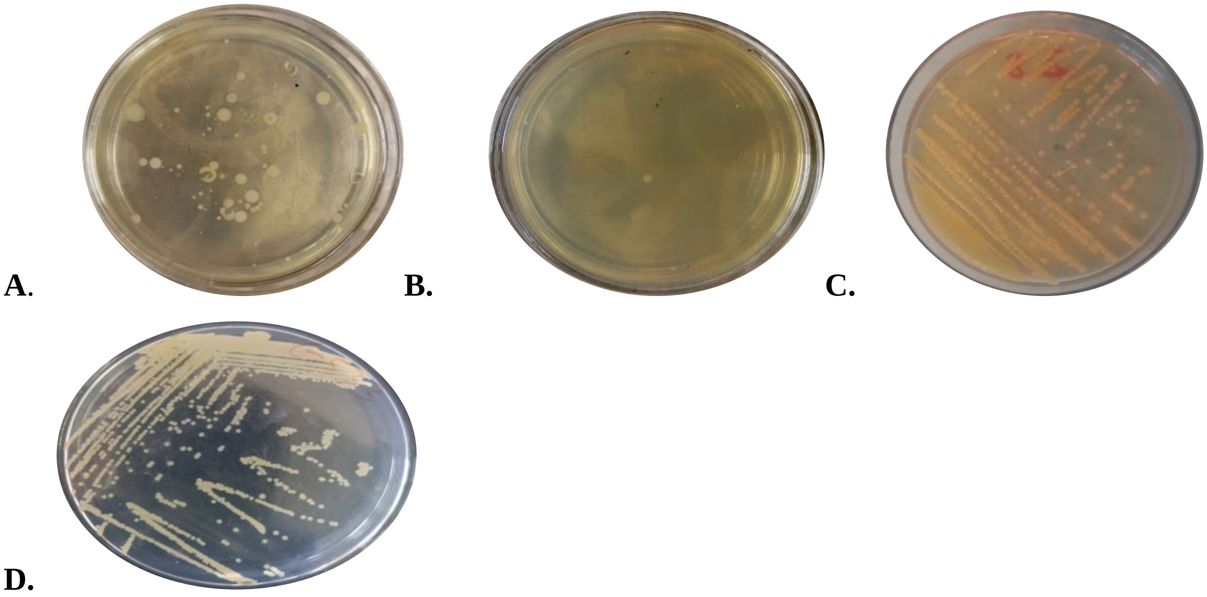

Figure 5. A and B Serial dilution at 10^{-5} and 10^{-1}, C growth of pseudomonas spp. on nutrient agar D, growth of Pseudomonas spp. on King's B agar.

Apart from their morphological features, the observed changes in colonies indicate that *Pseudomonas* sp. may have adapted to varying environmental conditions, such as temperature and nutrition. The different behaviors exhibited by the colonies could point to a flexible and robust microbial community, which could have consequences for comprehending the ecological dynamics of bacteria linked to sorghum under different environmental conditions.

4.2. Abiotic Stress Response of PGPR

4.2.1. Temperature stress test

The heat tolerance of the bacterium isolates was revealed that all strains were grown between 35 and 50℃, although ts28, gs19, ns35, bs12, bs15, ns37, ds14, bs17, and ts110 strains could grow at 55 ℃ and produce an OD value of less than 0.6 nm. However, all strains are capable of growing above the range of 0.5–1.25 at 600nm at 35 °C. Bs22, bs31, ns38, and ms22 displayed rapid development at 50°C and provided greater than 0.8 or (10^8 cfu/ml) OD value at 600nm which was above typical at this temperature as shown in figure 6A. Although, they were able to grow at 40℃ and 45℃.

4.2.2. pH stress test analysis result

In the current study, PGPR's capacity to survive in both acidic and alkaline soils was verified to have a wide pH range tolerance. The results of this study show that isolated bacteria can grow in a wide variety of pH demonstrating their broad tolerance to acidic environments. The Gs19, bs310, as22, and ts29 isolates demonstrated the highest growth at a pH value of 5, followed by a pH value of 4 and a pH value of 3, whereas a pH value of 2 was observed the least amount of growth from the majority of the bacterial isolates. Whereas, as-36, ns-32, and bs-310 displayed more growth in the acidic environment (figure 6B). The isolates were able to grow at a variety of temperatures between 35 and 50 °C, however, their best growth curve was shown between 35 and 40℃ (figure 6A). It could tolerate a pH of up to 5 (figure 6B). Therefore, in this experiment; bacterial growth was closely correlated with both an increase in temperature and a decrease in pH. The growth of the bacteria decreased as the pH became more acidic (figure 6B).

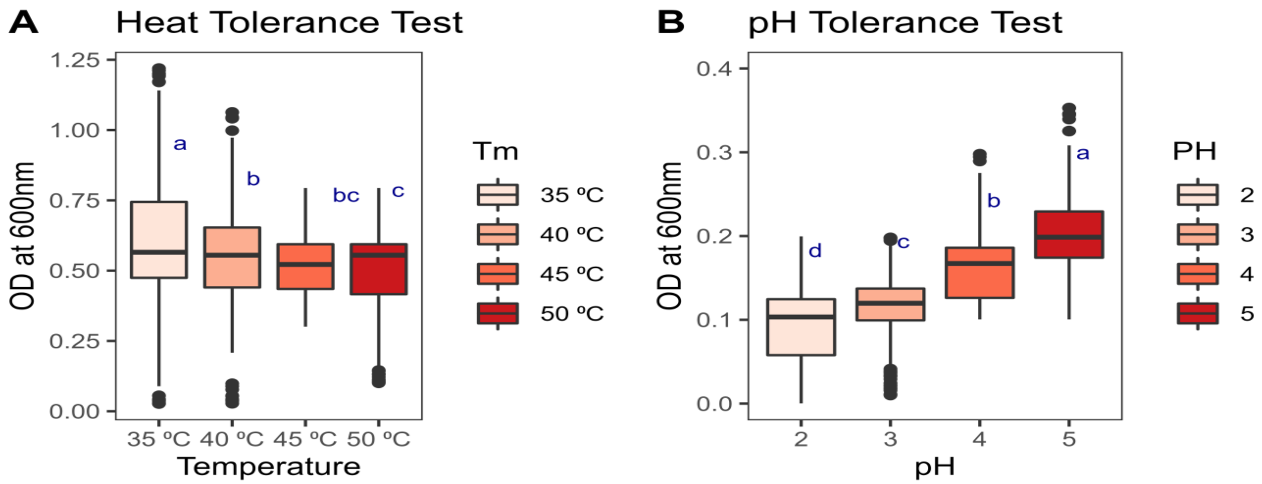

Figure 6. Boxplot and Tukey test of bacteria growth at different temperatures and pH levels: Letters show a comparison of bacterial growth means per OD medium at the 95% confidence level. Number of isolates = 3. A significant ($P < 0.05$). By increasing the temperature, the bacteria's growth decreased. Bacterial growth is reduced when the pH is reduced.

4.2.3. Salinity stress test

we examined the isolates' capacity to develop at various NaCl concentrations of 0M (broth only that used as a control), 0.5M, 1M, 1.5M, and 2M (figure 8B). When compared to 0 M (positive control without any salt concentration; positive standard), bs35, ms22, ds210, gs38, ts17, and so on isolates demonstrated the maximum NaCl tolerance at 0.5M. Additionally; gs11, gs15, ts26, ms22, and as24 isolates were shown to be least tolerant at 2M. However, the growth of bacterial isolates was significantly suppressed at increasing NaCl concentrations. The extreme properties of salt tolerance showed that the isolates were tolerant to 0.5M NaCl. In figure 7, the graphic illustrates bacterial growth across distinct wells within a laboratory deep well plate, each exposed to varying concentrations of NaCl. This visual representation gives perspectives into how the presence of different salt levels influences bacterial proliferation, providing valuable information in the context of the experimental setup using a 96-well configuration.

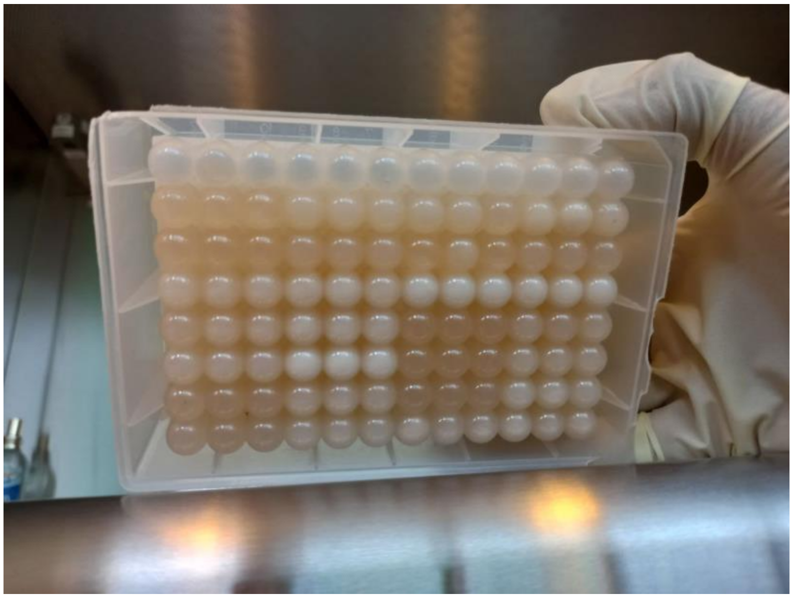

Figure 7 Growth of bacterial culture on the 96-well deep well plates after 24hrs incubation time.

4.2.4. Drought stress test

For drought tolerance performance evaluation of bacterial isolates different concentrations of PEG (6000): 10%, 20%, 25%, and 32.6% were used. The gs13, ds19, ds33, ns11 isolates can grow at 10% PEG (6000) and are categorized as completely tolerant to DS with an OD > 0.4, then at 20% PEG (6000), ts22, ts210, as21, ts12, ms11 and ds210 isolates are categorized as tolerant with an OD of 0.3, at 25% PEG (6000), as sensitive with an OD of 0.2, and at the final 32.6% PEG (6000), they are grouped as completely sensitive with an OD of 0.1 at 600nm (figure 8A). To sustain a high water activity level, isolates can tolerate polyethylene glycol concentrations of up to 10% (w/v) and stress levels of up to 32.6%. The productivity and output of crops used to feed people and animals will increase as a result of plant tolerance to abiotic stress. In figures 8A and 8B, when the concentration of PEG (6000) and NaCl increased, the Optical Density (OD) value decreased, demonstrating a direct correlation between the concentration of PEG (6000) and NaCl and the OD value.

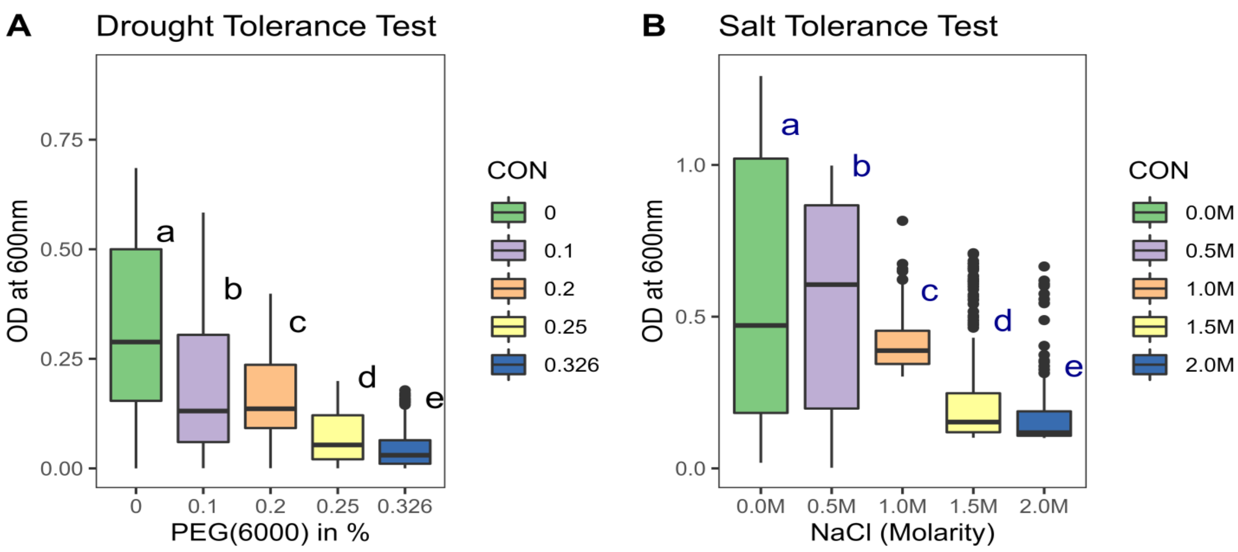

Figure 8. Boxplot and Tukey test of bacteria growth at different PEG (6000) concentrations and NaCl concentration: Letters show a comparison of bacterial growth means per OD medium at the 95% confidence level. Number of isolates = 3. A significant ($P < 0.05$). By increasing the PEG (6000) concentration, the bacteria's growth decreased. Bacterial growth is decreased when the NaCl concentration is increased.

4.3. Primary Screening of Selected PGPRs

4.3.1. Phosphate solubilization

The result for phosphate solubilization testing 210 bacterial isolates indicated that the bacteria can form clear zones On PVK agar plates (table 2). The bacterial isolates were then further chosen by using the molecular screening method to see if they could solubilize phosphate as shown on the plate agar method, which was based on their remarkable ability to solubilize inorganic P on plates as evidenced by the halos surrounding colonies (Figure 9A and 9B).

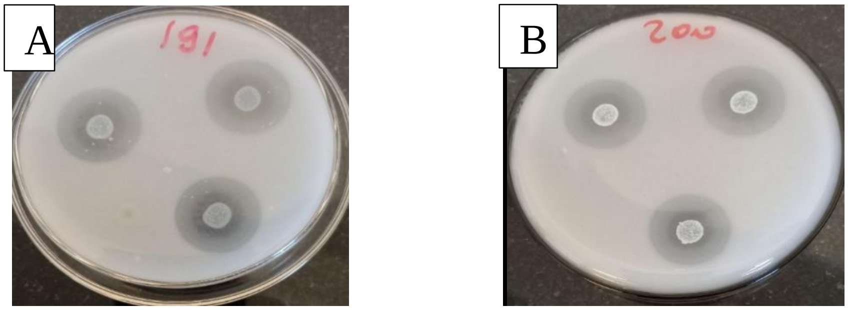

Figure 9. Phosphate solubilization test on PVK agar plate. A 191-coded sample (ns31) and B 200-coded sample (ns310) was that showed a high clear zone diameter on the PVK agar plate.

Based on the clear zone surrounding the bacterial colonies, although qualitative assessments were performed based on the ruler measurement in mm, as shown in Fig. 9, and only 50 of the 210 isolates were taken into account when estimating how much phosphate was solubilized quantitatively based on the ruler measurement. Figure 10 indicates that the graph of Phosphate solubilization of bacterial strains showed more than 5mm of clear zone on the PVK agar plate.

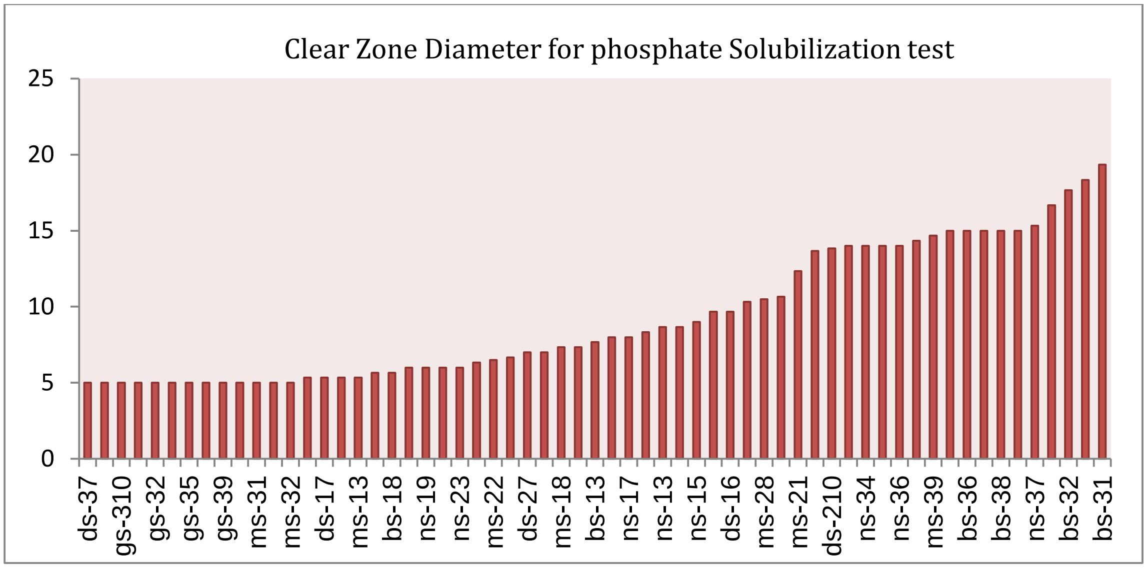

Figure 10. Clear halo zone diameter measurement result for Phosphate solubilization. The X-axis represents the isolated bacteria the clear zone index is > 5, Y-axis represents the clear zone index in mm.

Table 2. Lists the necessary PGPR characteristics (Nitrogen fixation and phosphate solubilization) out of 210 isolated bacteria.

No	Isolates	Clear Zone diameter(mm)	Nitrogen Fixation
	as11	0	-ve
	as110	0	-ve
3	as13	0	-ve
4	as19	3.7±2.08	-ve
5	bs 11	0	-ve
6	bs34	0	-ve
7	bs37	14.3±0.57	-ve
8	ds14	0	-ve
9	ds26	4.2±2.02	-ve
10	ds37	5.0±0	+ve
11	gs12	4.3±0.57	-ve
12	gs26	0	+ve
13	gs32	5.0±1	+ve
14	ms11	0	-ve
15	ms15	0	+ve
16	ms210	2.8±0.76	-ve
17	ms31	5.0±0	+ve
18	ms310	0	-ve
19	ms32	5.0±1	-ve
20	ms38	4.3±0.57	-ve
21	ns110	5.7±0.57	+ve
22	ns31	15.0±1	-ve
23	ns34	14.0±1	+ve
24	ns35	16.7±1.15	-ve
25	ns37	15.3±1.15	-ve
26	ns38	14.0±1	-ve
27	ns39	18.3±1.52	+ve
28	ts11	3.3±0.57	+ve

p-value <2e-16 ***. It is significant.

Values are mean ± standard deviation, +ve: indicates the positive result, and –ve: indicates the negative result of the nitrogen fixation. The ANOVA results indicate that there is a statistically significant difference in the mean values of the diameter among the isolates.

4.3.2. Nitrogen fixation

In this study, the isolated bacteria can fix nitrogen as seen in figure 11 and Table 2. Based on the findings, 68 of the 210 isolates indicated positive interactions, turning the green color of the broth medium blue as an indication of their ability to fix nitrogen**.** We found in our investigation that while certain bacterial strains are capable of fixing nitrogen on BG11 (which lacks sodium nitrate, the source of nitrogen) solid media (figure. 11B). The diversity of microbial contributions to soil nutrient availability, which affects plant growth, is demonstrated by the variety of bacterial behavior. As illustrated in figure 11A, the occurrence of pellicle formation is obvious, an occurrence associated with nitrogen fixation. The figure visually captures the development of a pellicle, providing a direct representation of the observed process during nitrogen fixation.

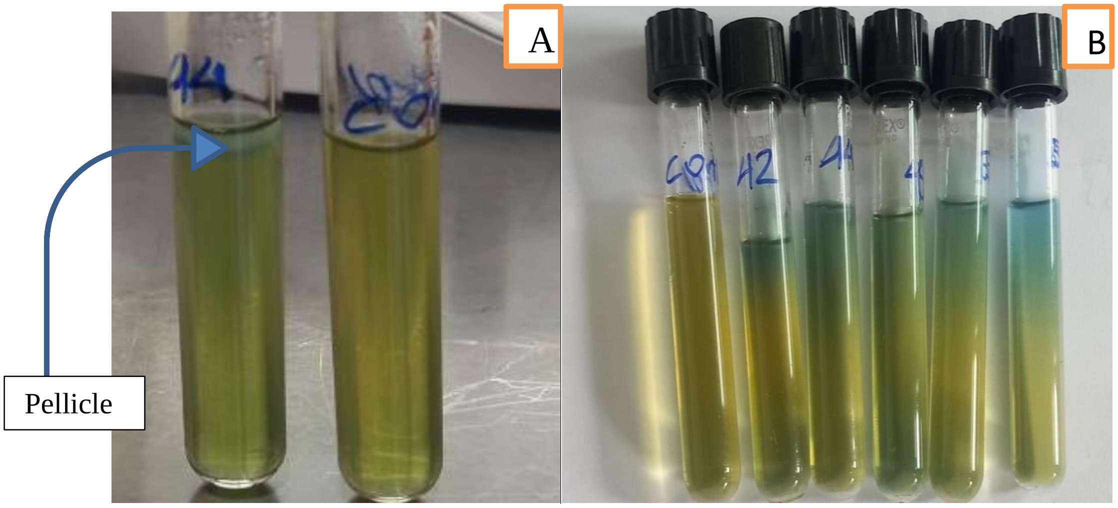

Figure 11. Nitrogen fixation ability of Pseudomonas spp. ds37, gs26, gs32, ms15, ms210, and ms31 are the bacteria samples that can fix nitrogen: A. Pellicle as an indicator of the fixation of nitrogen. B. An indicator nitrogen fixation process was carried out on bacteria with the decrease in pH (pH >7.6) produced by the creation of ammonia and nitrates.

4.4. Molecular Screening of Plant Growth-Promoting Traits Using Primers

4.4.1. Amplification of pyrroloquinoline quinone (*pqq*) gene

Among the 50 bacterial strains forming the clear zone on the PVK agar, only 21 bacterial strains exhibited the amplicon size of the gene as determined by molecular characterization using the pyrroloquinoline quinone (*pqq*) gene for phosphate resolution analysis. These strains of twenty-one bacterial species are as follows: bs31, bs33, bs36, ns33, ms21, ns34, ns17, ds16, ns19, ms22, gs31, ds19, ns26, bs14, bs15, ms13, bs18, ns110, ds32, gs39, and gs35 (figure 12). The *pqq* gene is of crucial importance for bacterial metabolism and regulates enzymes involved in redox reactions, dehydrogenase activity, and gluconic acid production. The amplicon size of the pyrroloquinoline quinone gene was 670 bp. A specially designed primer was used to confirm the exact region for *pqq* gene amplification. Furthermore, out of 50 bacterial isolates, only twenty one showed positive *pqq* gene amplification (670 bp band), while twenty nine showed no *pqq* gene amplification.

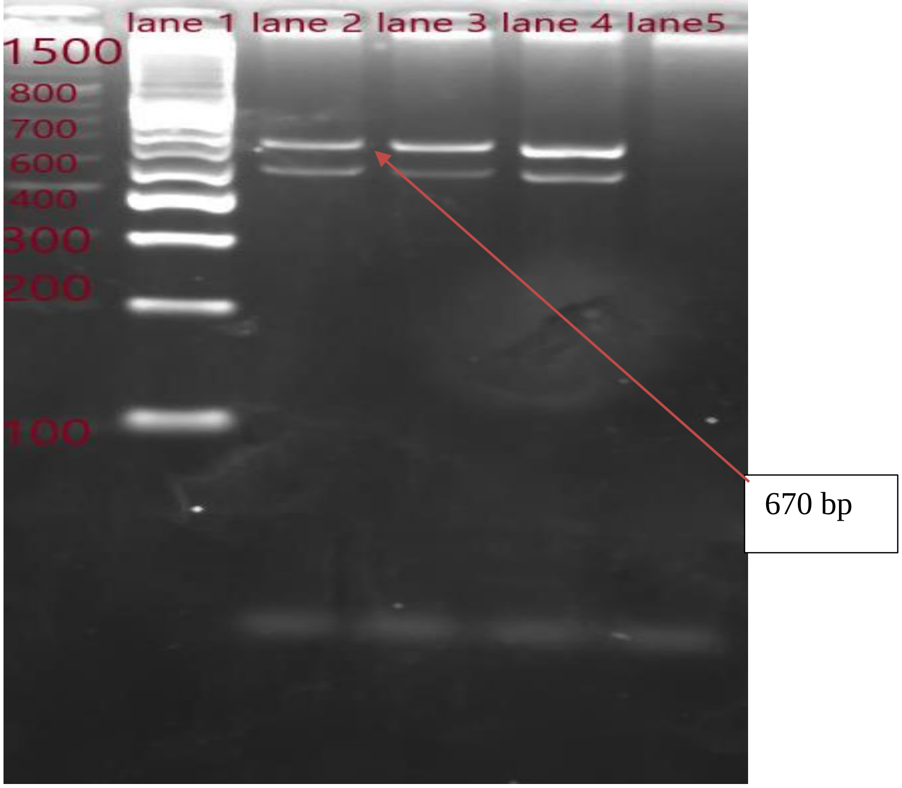

Figure 12. PCR product of *pqq* gene among Bacterial strains. Lane1, 100bp Ladder (100 bp – 1500bp); lane 2, ms22; lane 3, ns34; lane 4, bs14; lane 5 no amplicon present.

4.4.2. Amplification of nitrogen fixation specific *nifH* gene (Poly gene)

The gene *nifH*, which is associated with nitrogen fixation with 360bp amplicon size was detected in sixteen strains among the sixty-eight bacterial strains isolates, namely ms21, ns34, ns32, ms39, ns12, ns15, ds16, ds21, ms22, ds19, bs14, ns110, ds33, gs310, gs39 and gs32. A specially designed primer was used to confirm the exact region for amplification of the *NnifH* gene. Out of 68 bacterial isolates, only 16 isolates had an amplicon size of the *nifH* gene amplification (390bp), while 52 isolates showed no *nifH* gene amplification. Not amplified, and no band was discovered; this could be because the DNA sample used for PCR amplification was too concentrated.

4.4.3. PCR amplification of ACC deaminase production (*acds*) gene

The PCR amplification product for the *acds* gene was exhibited in only ten of sixty-eight bacterial strains. These bacterial strains that had an amplification product with an amplicon size of 754bp for the accds gene were ts13, as14, gs17, ms12, ms22, ds19, bs14, ns110, gs39, and gs32 (figure 13). Fifty-eight isolated bacteria thus showed no amplification of the *acds* gene. Not amplified, and no band was discovered; this could be because the DNA sample used for PCR amplification was too concentrated.

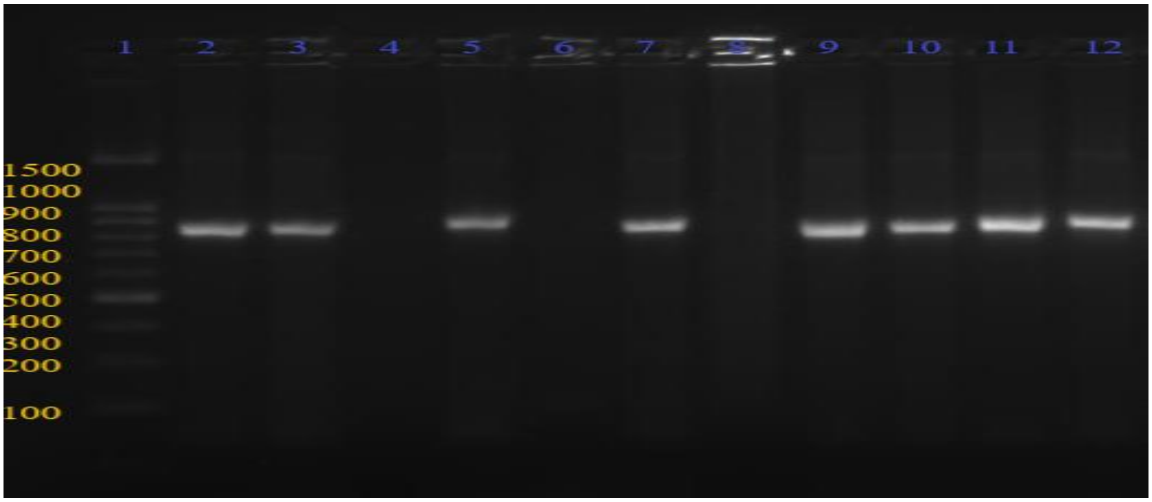

Figure 13. PCR product of acds gene among Bacterial strains. Lane1, 100bp Ladder (100 bp – 1500bp); lane 2, ts13; lane 3, as14; lane 4, no amplicon; lane 5, ms22; lane 6, no amplicon; lane 7, gs17; lane 8, no amplicon; lane 9, ms14; lane 10, ms12, lane 12, bs310 and lane 11, gs32.

4.5. Secondary Screening of *Pseudomonas* sp. Ms22 for Plant Growth Promoting Parameters

4.5.1. Phytohormone production

Eight isolated bacteria that were screened for accds production were also screened for IAA production to determine if the bacteria could produce IAA based on the standard curve of the IAA. Figure 14 illustrates the standard curve for measuring the amount of IAA production on the UV spectrophotometry at 530nm. The standard curve for Indole-3-Acetic Acid (IAA) is applied to quantify IAA levels, assess microbial IAA production, monitor IAA responses to treatments, ensure quality control in commercial products, compare plant varieties, optimize agricultural IAA applications, and investigate IAA biosynthesis pathways.

Of the eight isolated bacteria in IAA production, the ms22 isolates showed a positive interaction by developing a pink color, followed by ns34, ns110, and bs14 (figure 14). The *Pseudomonas* sp. Ms22 produced 14.8 mg/ml IAA in the presence of L-tryptophan (0.5 g/ml) at 30°C after 72 hours (figure 16). Compared to the other eight PGPRs, Ms-22 has produced a suitable quantity of IAA.

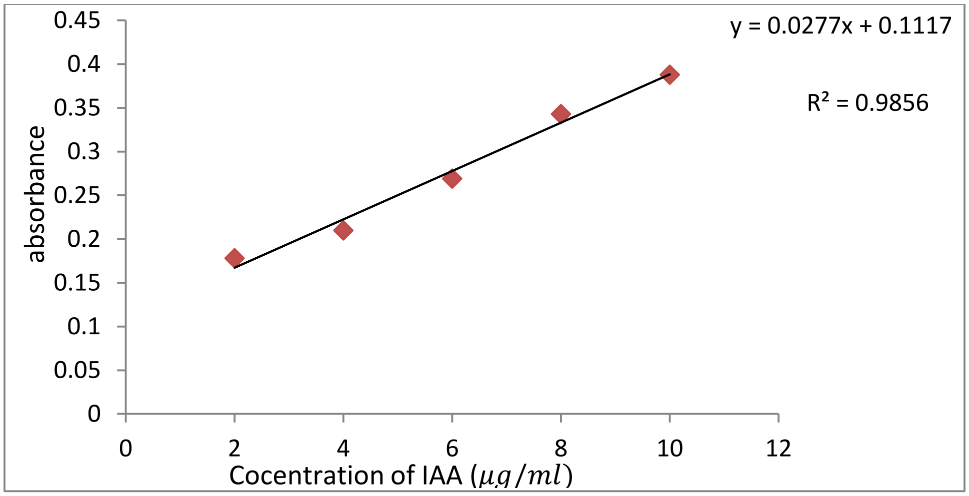

Figure 14. Standard curve for the IAA production at 530nm: A straight-line curve shows that the amount of red color by the salkawski reagent and IAA concentrations are directly correlated.

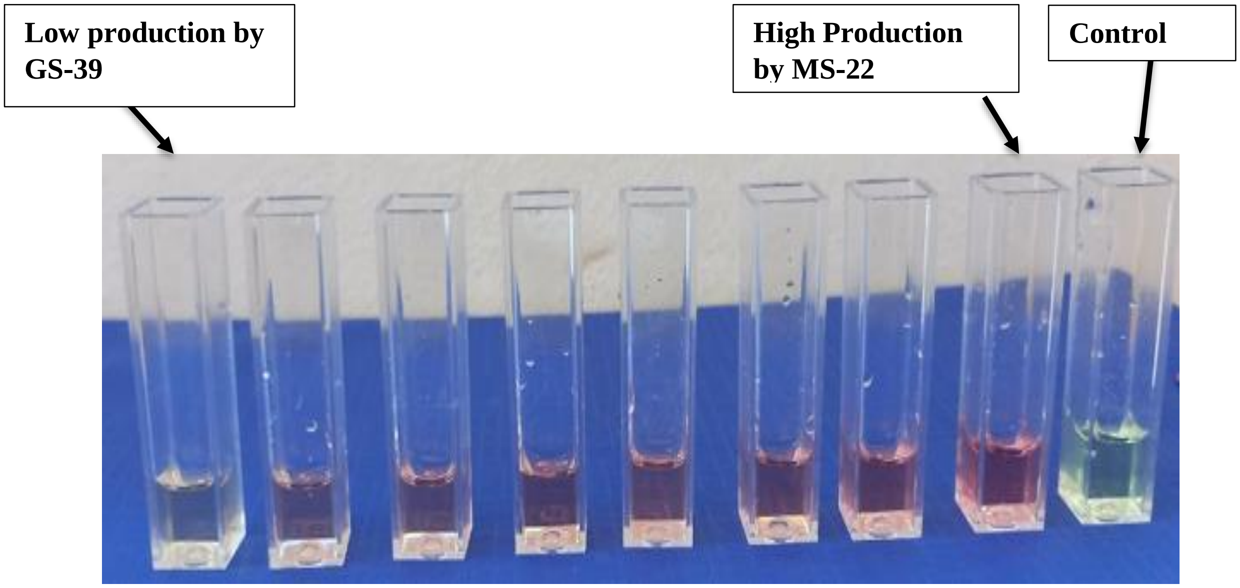

Figure 15. IAA production of selected bacterial strains showed a pink color.

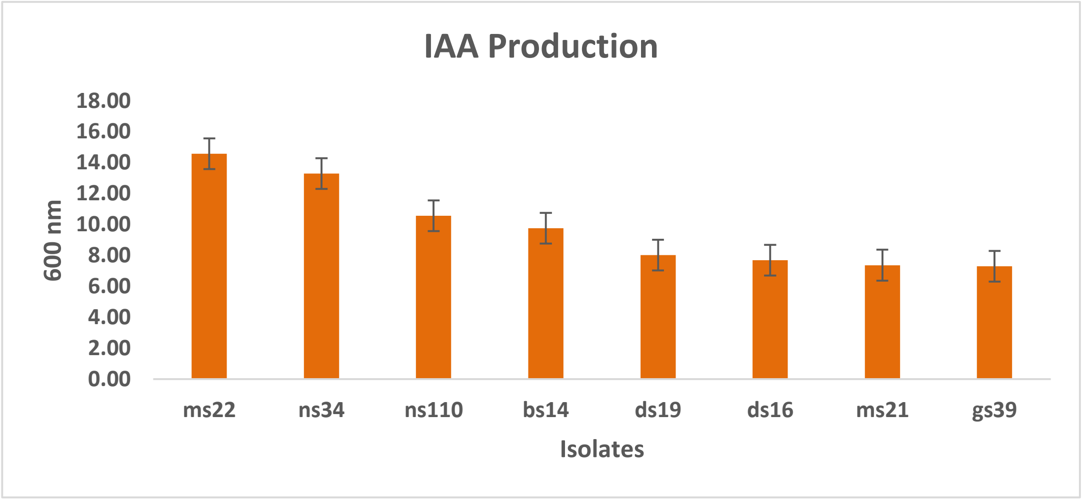

Figure 16. Error Bar diagram for IAA production assay using the UV spectrophotometry results at 530nm. Salkowski's reagent was used to compare the stranded curve of known IAA concentrations to determine the production of IAA.

4.5.2. Germination seedling assay

The genotype of the seed used for germination assay was Gambella-1107. It is a local variety and more stable for the environment as well (figure 17A). Ms-22 showed a high plant length when compared with the control that was immersed in phosphate buffer saline solution (figure 17B). The result shows the performance of the bacterial strain, especially 24 hours after incubation, declined rapidly, along with the sorghum seed germination rate in both inoculated and control. At 48 hours post-incubation, MS-22 significantly increased the germination rate of the seeds (figure 18). Using the bacterial strains ns110, ds16, and gs39, seed germination was reduced by 75 % compared to ms22, gs34, and ns34 and by 60 % compared to the control seeds (figure 19A).

Inoculation with the Ms-22 strain notably increased the overall length of seedlings. Surprisingly, the percentage of germination did not show a significant difference compared to the control, as depicted in figures 19A and B. Among the bacterial strains tested bs14, ms21, ds16, and gs34 showed the lowest seedling length and Ms-22 resulted in the highest seedling length, followed by ns34 and ds19 strains in order of increasing seedling length, as illustrated in figure 18E. Moreover, the dry weight of sorghum seedlings exhibited an increase under all bacterial culture treatments, suggesting a positive influence on growth in terms of both length and dry weight.

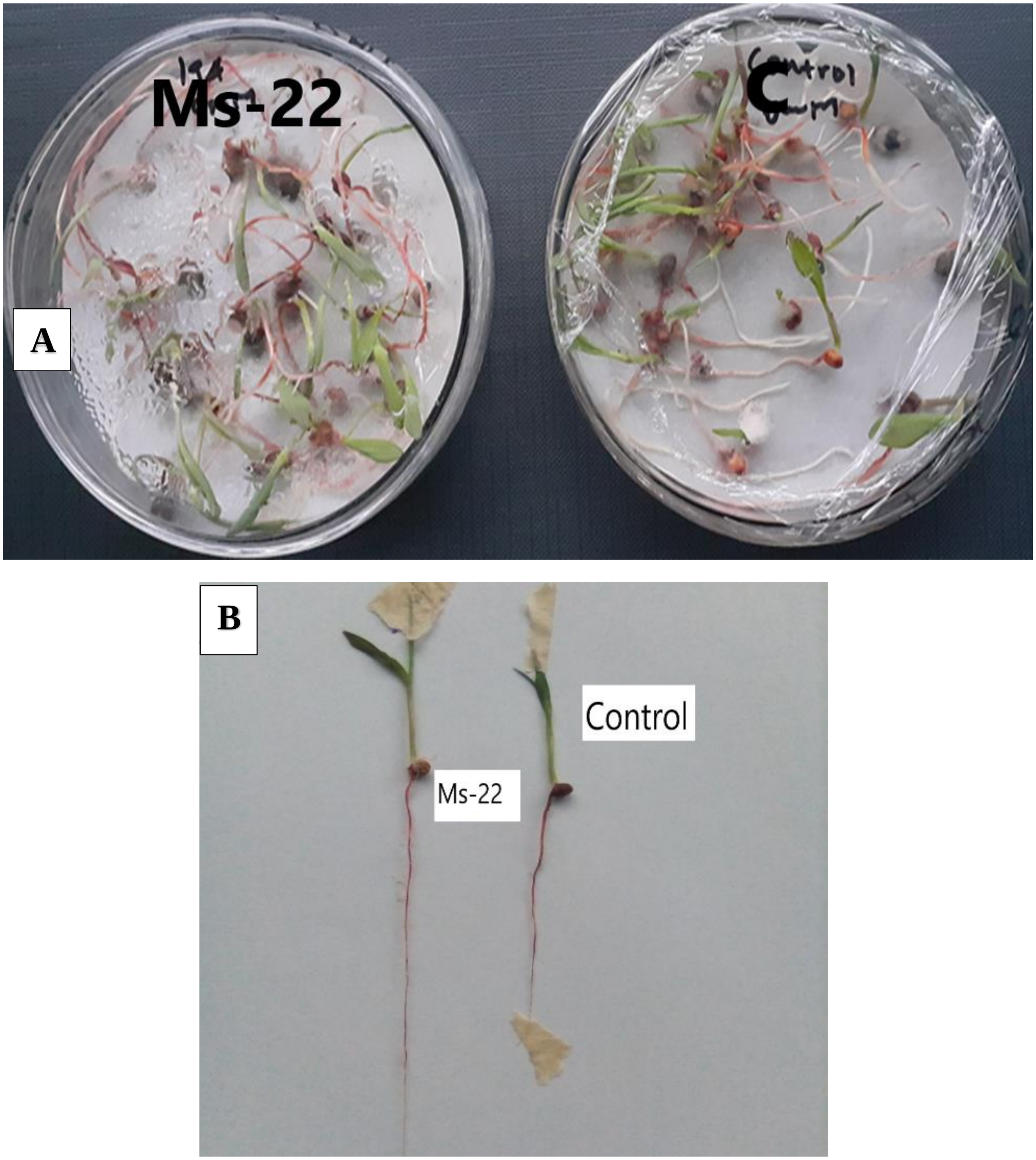

Figure 17. A. Germination on plate and B. germination of sorghum genotype (Gambella-1107) with inoculated (Ms-22) and C; Control (seed treated with PBS).

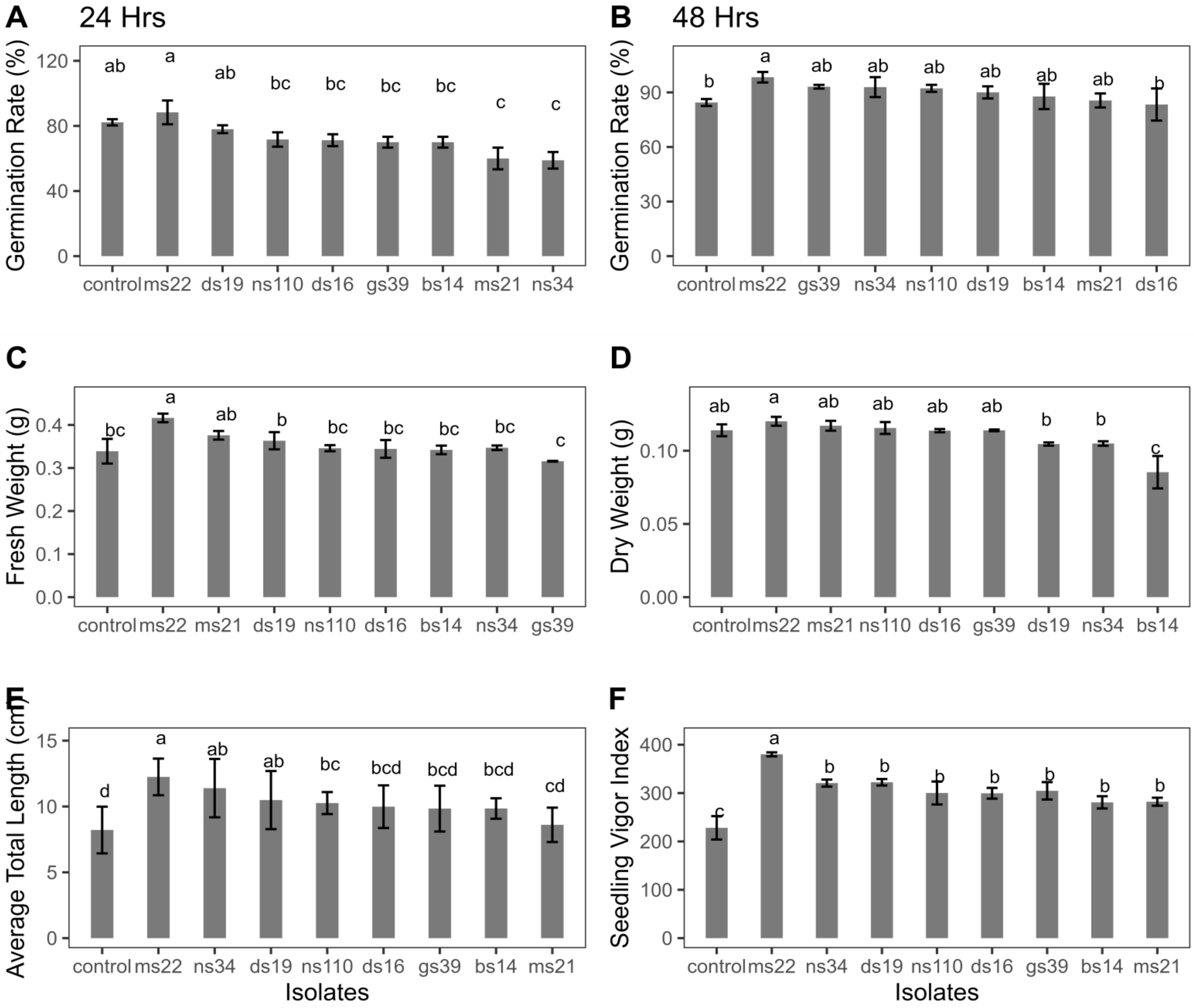

Figure 18. Effect of bacterial strains on sorghum seed germination after 48 h of incubation at 25 ˚C. C− (Control; Seeds inoculated with phosphate-buffered saline (PBS)), ms22, ns34, ds16, gs39, ds19, ns110, bs14 and ms21 (Seed treated with the bacteria strain): A. Germination rate after 24 h of incubation, B. germination rate after 48 h of incubation, C. average of dry weight of seedlings, D. average of fresh weight of seedlings, E average of total length of seedlings, and F. seedling vigor index. The values represent the means of three replicates (n = 3) ± SD. The different letters in superscript (a–d) indicate the statistically significant difference at 95% between treatments.

4.6. Greenhouse Pot Experiments

Compared to the control group, the Ms-22 isolate shows potential for promoting growth (figure 19). In this investigation Ms-22 showed high shoot length and root length compared with the un-inoculated seed as shown in Figures 19A and B. Although, Ms-22 displayed high shoot fresh weight and root fresh weight with a comparison with the control (figure 19C and 20D) and Ms-22 revealed high yield on the root dry weight and shoot dry weight with the comparison with the control (figure 19E and 20F). However, the effectiveness of this capability depends on the genotype of the source. Specifically, when compared to bacteria isolated from other sorghum genotypes, those obtained from the melkam genotype significantly enhanced all growth-related parameters. This suggests that the genotypes of the source sorghum influence the type and potential of Plant Growth-Promoting Rhizobacteria (PGPR).

Significant differences between the Ms-22 isolate and the control group were observed for each parameter studied, indicating distinct impacts on sorghum growth (figure 20). The entry mean squares for every agronomic parameter were significant ($p<0.01$), suggesting that the PGPR rhizosphere bacteria exhibited diverse growth-promoting abilities based on their origin.

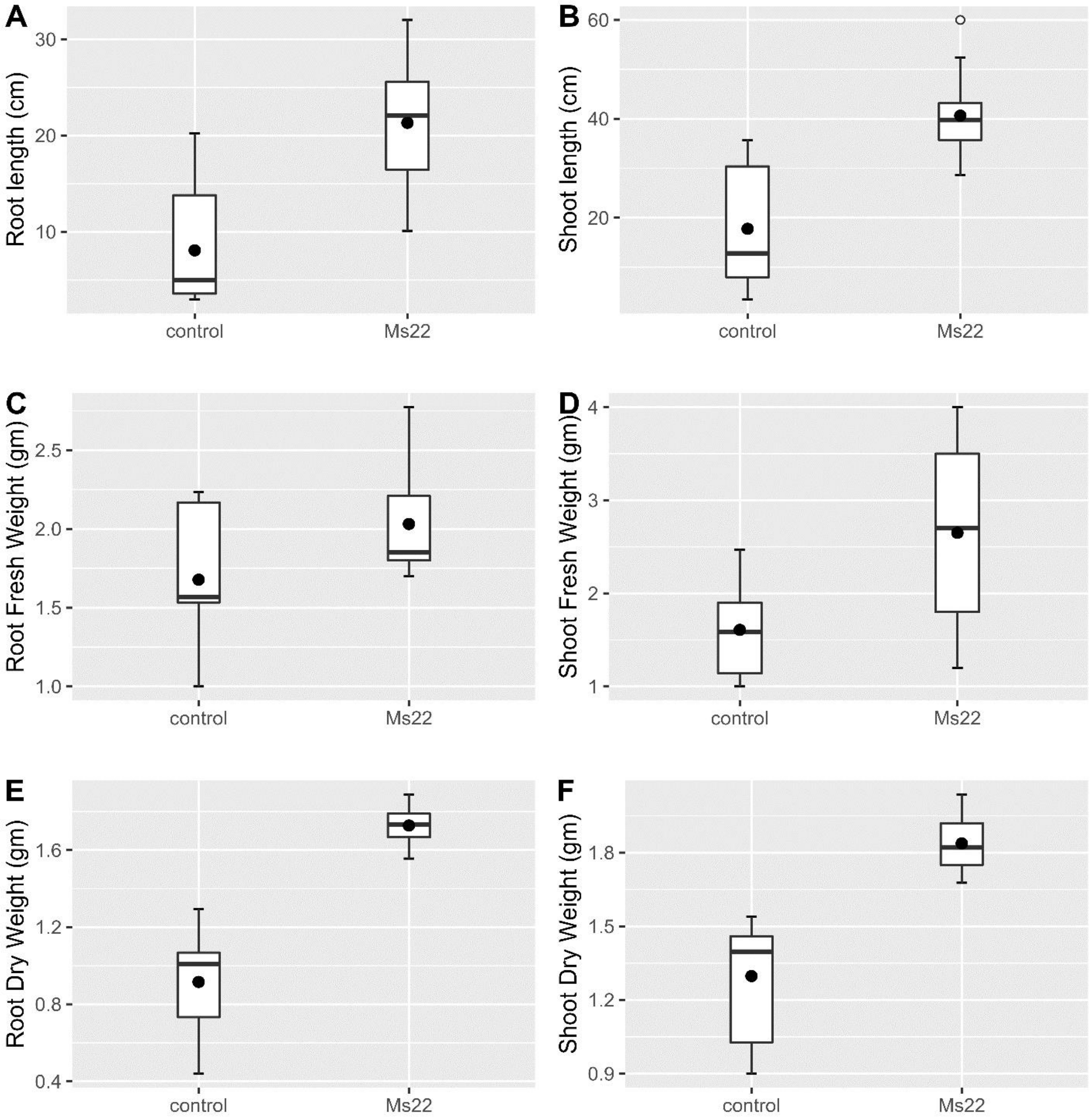

Figure 19. Effect of potent Ms-22 isolates on physio-morphology parameters: A. Root Length; B. Shoot Length; C. Root Fresh weight; D. Shoot Fresh weight; E. Root Dry weight; F. Shoot Dry Weight; Control, plants from un-inoculated seeds with 2 g TCP; Ms-22, seeds inoculated with bacteria with 2 g Tri Calcium Phosphate. Columns represent Mean values while boxes represent Standard deviation (n = 3).

Table 3 Table of ANOVA for showing the significance of the greenhouse result

Code	*Residual*	*DF*	*Mean±SD*	*F value*	*P value*	*(>F)*
RL	46	1	21.32±6.8	49.76	7.55e-09	***
Control			8.07±6.19			
SL	46	1	40.65±6.8	63.72	3.14e-10	***
Control			17.75±6.19			
RFW	34	1	2.03±6.8	6.819	0.0133	*
Control			1.67± 6.18			
RDW	34	1	1.72±6.8	141	1.2e-13	***
Control			0.91±6.18			
SFW	34	1	2.62±6.8	16.14	0.000309	***
Control			1.6±6.18			
SDW	34	1	1.83±6.8	79.64	1.98e-10	***
Control			1.29±6.18			
Total	228	6				

(RL: root length, SL: shoot length, RDW: root dry weight, SDW: shoot dry weight, RFW: root fresh weight, SFW: shoot fresh weight, Significance. codes: 0 ‘***’, 0.01 ‘*’. The asterisks (*) are commonly used to indicate the significance of code. The following is a standard protocol for p-value interpretation: Significant at the 0.05 level is **0.05: ***; at the 0.01 level, it is **0.01: **. **0.001: * (Valid at the 0.001 threshold), less than 0.001: (Extremely important))

In this study, we conducted a rigorous statistical analysis to evaluate the impact of different treatments on various plant growth parameters. The data presented reveals the results for six different treatment groups, each representing a distinct plant growth condition. The focus is on six parameters: RL (Root Length), SL (Shoot Length), RFW (Root Fresh Weight), RDW (Root Dry Weight), SFW (Shoot Fresh Weight), and SDW (Shoot Dry Weight). The key statistical measures include Residuals, Degrees of Freedom (DF), Mean ± Standard Deviation

(Mean±SD), F value, and P value (P > F), which collectively provide insights into the treatment effects.

For the RL parameter, the treatment significantly influenced plant growth (F value = 49.76, P value = 7.55e-09). The mean root length for the control group was 21.32 ± 6.8, while the treatment exhibited a distinct mean of 8.07 ± 6.19. The statistical significance, denoted by '***,' suggests a substantial impact of the treatment on root length (table 3).

Similar findings were observed for SL in table 3, with a highly significant F value of 63.72 (P value = 3.14e-10). The control group displayed a mean shoot length of 40.65 ± 6.8, contrasting sharply with the treatment mean of 17.75 ± 6.19. The '***' notation emphasizes the significance of the treatment effect.

In table 3 the analysis for RFW demonstrated a statistically significant effect (F value = 6.819, P value = 0.0133). The control exhibited a mean root fresh weight of 2.03 ± 6.8, while the treatment mean was 1.67 ± 6.18. The ' * ' notation indicates a significant but less pronounced impact compared to RL and SL.

The RDW parameter exhibited the most significant treatment effect (F value = 141, P value = 1.2e-13). The control group's mean root dry weight was 1.72 ± 6.8, contrasting dramatically with the treatment mean of 0.91 ± 6.18. The '***' notation emphasizes the substantial impact of the treatment on root dry weight (table 3).

A statistically significant effect was observed for SFW (F value = 16.14, P value = 0.000309). The control group's mean shoot fresh weight was 2.62 ± 6.8, while the treatment exhibited a mean of 1.6 ± 6.18. The '* ' notation suggests a significant but moderate impact of the treatment on shoot fresh weight (table 3).

SDW demonstrated a highly significant treatment effect (F value = 79.64, P value = 1.98e-10). The control group's mean shoot dry weight was 1.83 ± 6.8, whereas the treatment mean was 1.29 ± 6.18. The '***' notation highlights the substantial impact of the treatment on shoot dry weight (table 3).

The cumulative analysis across all parameters for the total of 228 observations and 6 degrees of freedom further underscores the robustness of the study. The presented statistical results provide compelling evidence of the treatment's influence on plant growth, with varying degrees of significance across different parameters. These findings contribute valuable insights into optimizing plant growth conditions and have implications for agricultural and horticultural practices.

Bacteria isolated from the melkam sorghum genotype, in combination with soil collected from Jiletumuga Woreda, significantly improved six metrics: shoot length, root length, shoot dry weight, root dry weight, and root fresh weight. The process of how the greenhouse performed under a controlled environment for extracting the plants for analysis is shown in figures 20A, B, C, D, E, and F. These enhancements could be attributed to factors such as plant genotype, soil type, or external elements influencing the potential of Plant Growth-Promoting Rhizobacteria (PGPR). The variations in rhizosphere bacteria that contribute to sorghum development and growth-related characteristics, encompass shoot length, fresh weight, and dry weight, as well as attributes linked to root length, fresh weight, and dry weight illustrated in figure 20.

Figure 20. A, Autoclaved soil sample preparation. B, Tri-calcium phosphate was added to the soil. C, Tri Calcium Phosphate was added to the soil. D, Plant root was isolated from the pot. E, plant root was washed to extract the soil. F, length of root and shoot were measured. G, uninoculated soil sample with Gambella-1107 seed genotype of sorghum, Ms-22 bacteria inoculated seed with Gambella-0911 seed genotype of sorghum.

4.7. DNA Amplification of *Pseudomonas* Strains Using 16s rRNA

In figure 21, a 1.8% (w/v) agarose gel is used to separate the 16S rRNA PCR product. Thus, Pseudomonas spp. was present around this region. The 16S rRNA was amplified to a size of around 1500 bp. The 16S rRNA region of the four groups of the bacterial isolate Ms-22 was amplified using both primer pairs during the amplification and sequencing process. It was found that each isolate had DNA fragments that had been amplified by PCR that weighed about 1500 bp. Additionally, several isolates had their DNA amplified.

The sample was poured into each well in a volume of 5 μl. The target sequences were 1500 bp. There was 1.8% gel present. Sigma Aldrich Chemical Pvt. Ltd. in India provided the forward primer and the reverse primer. Gel electrophoresis was used to separate amplified DNA fragments of various lengths. The speed of DNA migration through the gel pores is influenced by the size of DNA pieces. The buffer, which serves as a conductor and regulates pH, helps maintain the stability of biological molecules.

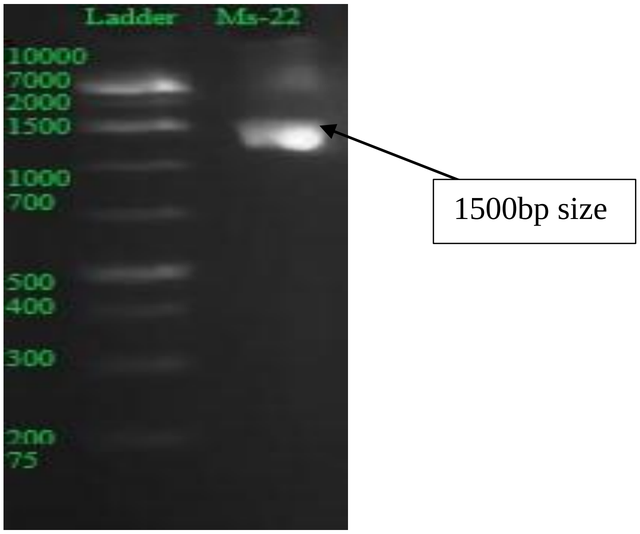

Figure 21. PCR product electrophoresis gel image Marker size: 10 kb. Lane 1: ladder; Lane 2: Ms-22.

5. DISCUSSION

5.1. Abiotic Stress Tolerance Test

The rhizosphere bacteria known as plant growth-promoting rhizobacteria (PGPR) can promote plant development by several different processes, including phosphate solubilization, siderophore production, biological nitrogen fixation, IAA generation, ACC production, and others. Several bacteria have been identified for isolation and screening from diverse rhizospheres and soils (Majeed *et al.*, 2015). Based on Mahdi *et al.*(2021), abiotic stress tolerance was investigated using PGPR characteristics. Gohil *et al.* (2022) isolated *Bacillus* spp. had examined the potential of the bacteria under different abiotic stresses from the rhizosphere. In the current study high growth of the bacteria was exhibited at the 35°C–45°C optimal temperature. *Pseudomonas* spp. can tolerate high temperatures because of the presence of heat shock proteins (Rangeshwaran *et al.*, 2013).

So far, a research study have identified the possible *Pseudomonas* species found in rhizospheric soil that tolerate high temperatures. For instance, Manasa *et al.*(2017) isolated *Pseudomonas* AKM-P6 strains from rhizospheric soil to promote plant development for sorghum seedlings' resistance to high temperatures (47–50°C). In their investigation, rhizobacterial isolates RR-1, GGP-1, and GNR-1 were all capable of surviving high temperatures (45°C) and demonstrated a variety of advantageous plant growth-promoting traits. In addition, Ali and the others inoculate wheat with the high-temperature (45 °C) *Pseudomonas putida* strain AKMP7 and results best productivity (Ali *et al.*, 2011). In the current finding supports the finding of Manasa *et al.*(2017) on the result of growth of bacteria on the high temperature. Selecting suitable isolates for high-temperature regions requires careful consideration of temperature.

The current study also focused on the growth of bacteria in different salinity levels. Thus, at high salinity concentrations, there is a decline in bacterial growth. This implies that the growth of bacterial growth is directly proportional to the concentration of NaCl (Santos *et al.*, 2018). Although, Singh and Jha (2017) collected PGPR from a Sorghum *bicolor* soil sample for increasing wheat plant development under salt stress and improving wheat's defense against the fungal disease "*Fusarium graminearum*". Our study's isolated bacteria demonstrating optimal growth at 2M (molarity); they were reported to be saprophytic. The present study finding supported the report of Amini Hajiabadi *et al.* (2021) that their isolated bacterial strain exhibited opitimal growth at 2M. It appears that the cells' synthesis of proline, glutamate, glycine, betaine, and trehalose is the cause of this high osmotic strength. Na^+ buildup reduces water conductivity, soil aeration, and porosity. Elevated Na+ ions also disrupt enzymatic functions and interfere with K^+ and Ca^{2+} (Paul and Lade, 2014). It is also important to be aware of a possible isolate's resilience to salt soils before choosing them for PGP attributes. While increased sodium chloride (NaCl) concentrations negatively affected bacterial development (Amini Hajiabadi *et al.*, 2021), our bacteria under study demonstrated tolerance to a broader range of NaCl concentrations.

Our findind on the bacterial isolate test on a range pH showed that the isolates were exhibited good growth from pH 2 to 5 but at pH 2, 3, and 4 at a significantly low rate compared to pH 5 (Figure 6A). However, most *Pseudomonas* species are often inhibited by extremely high acidity, making it challenging to identify the specific pH range they tolerate for agricultural productivity. However, the strain that Kumar *et al.* (2021) isolated *Pseudomonas lurida* strain EOO26 showed notable capacities for phosphate-solubilization and indole-3-acetic acid synthesis over a range of Cu concentrations (0–100 mg L−1) at different pH levels (5.0–8.0). This implies that strain EOO26 may be able to perform well under a variety of abiotic stress situations, such as those with varying pH levels.

A severe abiotic stressor, drought can result in significant losses in production in arid and semi-arid locations when rain is the only source of agriculture (Minakshi *et al*., 2013). For this problem, we examined the plant growth-promoting attributes of *Pseudomonas* spp. on different PEGs (6000) to examine their drought tolerance. Different levels of PEG (6000), 10%, 20%, 25%, and 32.6% are used in this study based on Bruno *et al.* (2020) and Uzma *et al.* (2022), which work with some modifications. Ansari *et al*. (2021) identified a new strain of *Pseudomonas*, called *Pseudomonas azotoformans* FAP5, that showed resilience up to 15% PEG (─0.49 MPa) and tolerant to water stress conditions caused by PEG in LB medium. (Ali *et al*., 2014) isolate nine fluorescent *Pseudomonas* sp. from rhizosphere and non-rhizosphere soils and these isolates are tolerant to a substrate metric potential of −0.30 MPa (15 % PEG 6000). In this study, *Pseudomonas* species displayed higher resistance, overcoming up to 10% - 20% PEG (6000) level. This suggests that isolates have a good chance of surviving dry conditions in crops. Based on Ashry *et al.*(2022) classification of drought-tolerant isolates, we also classified isolates based on their OD.

5.2. Primary Screening of Selected PGPR

A phosphorus deficiency will usually cause premature growth of the stem and roots, poor flowering, and no seed or fruit production (Mahdi *et al.*, 2021). The phosphate solubilizing ability of the potential isolate was examined on PVK agar plate. However, Gupta *et al.* (2022) examined the ability of bacterila growth which is in agreement with our finding that the bacteria isolates have shown solubilizing the phosphate and grown well on PVK agar plate

A varous published research documents the solubilization of phosphate and nitrogen fixation by *Azotobacter*, *Pseudomonas*, and *Bacillus* (Bhattacharyya and Jha, 2012; Zeng, Wu, and Wen, 2016; Li *et al.*, 2017; Gohil *et al.*, 2022; Tsegaye *et al.*, 2022). Uzma *et al*. (2022) found the maximum clear zone diameter (3.33mm) by using *Pseudomonas aeruginosa* strain MK513748. Related to this, our finding is more interesting because BS-31 bacterial isolates yielded the maximum clear zone (19.3±0.57mm). Like this 50 isolates showed the best growth which means greater than 5mm. It will be determined that the presence of phosphate solubilizing bacteria (PSB) in soils is an indication that the bacteria can be used as biofertilizers to increase crop productivity (Majeed *et al.*, 2015). Gupta *et al.* (2022) discuss that the isolates showed an increase in P solubilization capacity as the time interval increased. The highest P solubilization at the time interval increased because the isolate produced organic acid, which causes insoluble phosphate solubilization in the medium. Thus, in our study, we incubated the PVK agar plate for 5 days. Thus, also Gupta *et al.* (2022) used 7-day incubation.

Nitrogen fixation is the process through which neutral N2 gas transforms in the soil into a form that can be metabolized (Hakim *et al.*, 2021). The nitrogen-fixing bacteria can change the color of the media from green to blue when they fix the nitrogen from the environment. A visible indication of active nitrogen fixation is the change in color of the pH indicator bromothymol blue from green to blue, which is caused by the by-product of alkaline ammonia released by some bacteria during nitrogen fixation. The alkalinity of ammonia is the cause of the color change (Tang *et al.*, 2020). In our investigation, the color of the media was changed from green to blue as the same result (Tang *et al.*, 2020). PGPR even in saltwater conditions, halotolerant rhizobacteria that fix nitrogen can continue to promote growth (Ding *et al.*, 2005). It has long been recognized that *K. pneumonia* and *P. stutzeri* are capable of fixing nitrogen (da Silva *et al.*, 2018).

Increased root elongation, lateral root production, and root hair formation result from Indole acetic acid (IAA) modification (Amora-Lazcano *et al.*, 2021). *Pseudomonas* spp. used in this study were able to produce IAA. In IAA production the amount of tryptophan provided directly relates to the amount of IAA produced. Although tryptophan serves as an IAA precursor, bacteria are also capable of producing IAA (Cavalcante da Silva *et al.*, 2020). To produce indole acetic acid, a plant hormone, Reetha *et al.* (2014) isolate two rhizobacteria, *Pseudomonas fluorescens* and *Bacillus subtilis,* and apply them to onion plants, indicating possible uses for growth-promoting agents. In our investigation, Ms-22 showed 14.8 mg/mL IAA production after 3 - 4 days of incubation time on a shaker at 120 rpm, consistent with previous research correlating IAA production with L-tryptophan concentration (Bharucha *et al*., 2013).

5.3. Molecular Screening of Plant Growth-Promoting Traits Using Specific Primers

Molecular studies on PGP microbes provide insights into their genetic composition, functional abilities, and interactions with plants. Further investigation into these details is crucial for future agricultural research. Meena (2018) underscores the importance of addressing the challenges facing the agriculture industry and suggests that a detailed understanding of complex biochemical and molecular systems is essential for more productive collaborations. Our study explored the nitrogen-fixing capacity of *Pseudomonas* spp., determining the amplicon on 390 - 400 *bp* size of the *nifH* gene based on (Solanki *et al.*, 2018) and (Raes *et al.*, 2020).

This gene, responsible for nitrogen fixation in *Pseudomonas* spp., was further investigated in our study. The exploration of these genes holds significance in understanding ecological functions, nutrient cycling, and plant-microbe interactions, aligning with previous research emphasizing the critical role of *nifH* genes in agricultural and environmental consequences ((Hussain *et al.*, 2009); (Tripathi *et al.*, 2020) and (Singh *et al.*,2022). Several *nif* genes, particularly *nifH*, encode the nitrogenase enzyme responsible for nitrogen fixation (Masood, Qiang, and Fang, 2020). The microbial *nifH* gene has been employed as a reference gene in metagenomic investigations to identify the diazotrophic communities present in sorghum and to validate their involvement in delivering bioavailable N to sorghum, as well as in studies of rhizospheric and endophytic microbial diversity (Rodrigues Coelho *et al.*, 2008; Coelho *et al.*, 2009). Because of its remarkable conservation, the amino acid sequence of nifH is commonly used to investigate the evolution of bacteria that fix nitrogen (Hakim *et al.*, 2021). Using PCR amplification, Padda *et al*. (2019) discovered that 14 isolates had the *nifH* gene present. However, only 16 of the 68 isolates in our investigation tested positive for the *nifH* gene.

Pyrroloquinoline quinone (*pqq*) contributes significantly to advantageous characteristics including antifungal activity and induced systemic resistance (ISR) in addition to its function in P-solubilization. This may be because it serves as a cofactor for multiple enzymes, including glucose dehydrogenase (GDH) (Han *et al.*, 2008). Enzymes expressed by the pqq gene create pyrroloquinoline quinone (*pqq*), which helps with phosphate solubilization in some *Pseudomonas* species (Anyia *et al.*, 2009; Yadav *et al.*, 2021). According to Kim *et al.*, (2003) the pqq gene amplified to a size of 670 bp in our investigation. The targeted area was used to amplify 21 bacterial isolates. However, in another study, the development of the antibiotic pyoluteorin was increased by inactivating the *pqq* genes in *Pseudomonas fluorescens* CHAO. These genes are involved in the biosynthesis of pyrroloquinoline quinone, a cofactor of several hydrogenases (Hölscher and Görisch, 2006). Another application of *pqq* is, According to Choi *et al.* (2008), *P. fluorescens* B16 uses pyrroloquinoline quinone (*pqq*) as a major growth-promoting agent in plants. Mutants of B16 did not manufacture *pqq*; wild-type B16 did. Tomato height, flower number, fruit number, and total fruit weight all increased as a result. *pqq* also functioned as an antioxidant in cucumber leaf discs and raised the fresh weight of cucumber seedlings. Thus, using this gene provide many application for agricultural sectors.

By deaminating 1-aminocyclopropane-1-carboxylic acid (ACC), PGPR can more widely reduce plant ethylene levels. A gene called *acdS,* which codes for an ACC deaminase that breaks down ACC into ammonium and α-ketobutyrate, is present in many PGPR genomes (Blaha *et al.*, 2006; Bouffaud, *et al.*, 2013). Using defective primers, the ACC deaminase (*acdS*) gene was amplified using PCR. In this experiment with ten isolated bacteria, an expected product of around 755 bp was seen, supporting the findings of the ACC deaminase tests. Primers were annealed at locations 146 and 900 of *Pseudomonas putida* WU4's acdS reference nucleotide sequence, which corresponds to an anticipated amplification product of about 754 bp (Niu *et al.*, 2018). Additionally, it has been demonstrated that the rhizobia that produces ACC deaminase are effective nitrogen fixers. According to Nascimento *et al.* (2012), the nif promoter regulates the structural gene of ACC deaminase (*acdS*) in *Mesorhizobium* sp. This promoter also normally controls the nif gene responsible for nitrogen fixation. Based on this, we use isolates that can fix nitrogen. Out of 68 nitrogen-fixing bacteria on the medium, only 10 isolates are positive for the *acds* target gene.

5.4. Secondary Screening of *Pseudomonas* Sp. Ms22

Bacteria play an essential role in promoting sorghum growth, as evident in our study. *Pseudomonas* strains inoculated into various plants have shown significant enhancements in growth parameters, as observed in previous studies (Santillana Villanueva, 2006; Alarcon, 2010; Zambrano-Mendoza *et al.*, 2021). Our investigation, involving the bacterial strain "Ms-22," demonstrated a notable capacity to promote sorghum development. The findings are consistent with research by Gujral *et al.*(2013), repeating the strain's potential to enhance sorghum growth. In addition, our study assessed the impact of Ms-22 on seed germination, root growth, and shoot growth. The results revealed a significant variation in plant biomass, with PGPR-treated plants exhibiting 20% higher biomass than the control group. This observation is crucial, as higher biomass is often associated with higher crop yields, offering potential benefits for greenhouse operations (Condon *et al.*, 2002).

However, it is significant that the use of the Ms-22 strain had a substantial impact on the vigor index, with a p-value of 0.05. A p-value of 0.05 is commonly considered the threshold for statistical significance. This suggests that the utilization of the Ms-22 strain significantly affected the vigor index. In summary, while Ms-22 positively affected seedling length and dry weight in sorghum, it notably impacted the vigor index. The percentage of germination, however, did not differ significantly from the control.

In our study, PGPR-treated plants exhibited higher concentrations of Tri calcium phosphate (TCP). Comparing Ms-22 to non-inoculated controls, there was a significant promotion of growth. Most agricultural soils have a large P reserve in them. However, soil typically contains very little soluble P (0.4–1.2 g/kg) (Awasthi *et al.*, 2011; Fernández *et al.*, 2014). Mei *et al.*(2021) examined bacterial strains under greenhouse conditions with 0.5 g/mL Tri calcium phosphate. By comparing this in our experiment, we employed 2 g/mL to assess Ms-22's capacity in a controlled greenhouse environment. The results demonstrated significant plant height and biomass output, consistent with Fernández *et al.* (2007). Based on these findings, we suggest the use of PGPR bacteria to enhance global agriculture productivity and sustainability. The implications extend beyond better plant growth, encompassing economic and environmental benefits. The results of this study underscore the positive impact of PGPR on plants, supporting its integration into sustainable agriculture practices.

6. CONCLUSIONS

The current study's findings have shown that Ms-22 included PGP triats, which are utilised in the agriculture industry. The results showed that naturally occurring PGPR extracted from soil samples has the potential to be used in applications that promote growth in the nation's agricultural sector. The outcomes also imply that the PGPR strains are suitable candidates for selection as growth promoters and biofertilizers.Through the use of bacteria that promote plant development in the rhizosphere, the study is expected to enhance sorghum production as well as efficiency in Ethiopian agriculture. Out of the 210 isolates tested, 21 isolates can have the *pqq*, 10 isolates can have *acdS*, and 16 isolates have *nifH* genes. Thus, this strain possesses important PGP traits, including IAA production, phosphate solubilization, and nitrogen fixation, which are essential for plant growth promotion.

In a germination experiment, the most promising isolate "Ms-22" was assessed, and its growth-promoting capability was shown to be significant. All isolates greatly stimulate sorghum development, as shown by the fact that the mean square of all isolates for all examined attributes was significant. The findings hold promise for developing a PGPR strain that might actively promote sorghum growth, which would be advantageous for sustainable agriculture. The experiment was carried out in west showa Ethiopia, however, it is feasible to carry out comparable studies over a wider range of agro-ecologies in Ethiopia to identify more possible PGPR strains. To improve crop production efficiency and grain output of sorghum and other cereal crops, further research is required to evaluate various types of efficient and suitable PGPR strains and sources of crop and environment.

In conclusion, our data analysis highlights the positive impact of PGPR attributes on plant growth, endorsing its inclusion in sustainable agriculture. The potential to improve crop yield, reduce costs, and contribute to environmental responsibility makes PGPR a valuable asset. These results introduce confidence in incorporating PGPR attributes into sustainable agricultural practices for enhanced crop output, cost reduction, and environmental responsibility, emphasizing the need for more comprehensive research to ensure optimal outcomes.

7. RECOMMENDATIONS

The economic and environmental implications of these findings are substantial. The observed increase in biomass, coupled with the potential reduction in reliance on synthetic fertilizers, points toward a more sustainable and cost-effective approach to agriculture. However, it is crucial to acknowledge that further research is warranted to explore the long-term effects of PGPR application, optimize strain selection, and refine application methods for maximum benefits. In essence, this study contributes to the growing form of knowledge supporting the integration of PGPR, specifically *Pseudomonas* spp., into sustainable agricultural practices.

The positive outcomes in terms of enhanced crop output, cost reduction, and environmental responsibility underscore the potential of PGPR to revolutionize modern agriculture. As we move forward, continued research and refinement of these findings will be essential to ensure the successful and widespread implementation of PGPR for the benefit of global food security and agricultural sustainability. However, further research is needed to explore the long-term effects of PGPR application and optimize the choice of strains and application methods for maximum benefits.

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