

University of Bahrain

College of Science

Department of Biology



Evaluation of Plant Growth-Promoting Rhizobacteria in Bahrain and their Effects on Abiotically Stressed Plants.

A Thesis Submitted in Partial Fulfilment of the Requirements for the
M.Sc. Degree in Biological Sciences

Prepared by:

Fida Jassim Mohammed Alsaffar

ID number: 20153234

Supervised by

Dr. Salwa Althawadi (Associate Professor)

Dr. Mohammed Azeem (Assistant Professor)

University of Bahrain

Kingdom of Bahrain

May 2024

Defense committee's approval

University of Bahrain
Deanship of Graduate Studies
And Scientific Research



جامعة البحرين
عمادة الدراسات العليا والبحث العلمي

Thesis Discussion Committee Decision

Student Name: Fida Jassim Alsaffar	Student ID: 20153234
Department: Biology	College: Science
Program: M.Sc. in Biological Sciences	
Thesis Title: Evaluating of Plant Growth Promoting Rhizobacteria in Bahrain and their Effects on Abiotically Stressed Plants	
Supervisor Name: Salwa Al-Thawadi	Student GPA: 4

The Discussion Committee met on 11-Sep-24 In . Upon discussing the student whose details are stated above in public, the discussion committee recommends the following:

The student is awarded a grade Pass in the thesis subject.

☐ Without making any changes to the thesis.

☒ After making the modifications attached with this decision.

Discussion Committee Members Approval:

Name		Signature
1. Dr. Salwa Al-Thawadi	(Advisor)	Salwa Al-Thawadi
2. Dr. Ali Bin-Thani	(Internal Examiner)	Ali Bin Thani
3. Dr. Mohammed Abu-Dieyeh	(External Examiner)	Mohammed Abu-Dieyeh

6 Approved at the University Council 6th meeting, 2013
In accordance with Decision No. 736 2013 dated 14/3/2013
Modified by University Council Decision No. 2043 2013 dated 20/11/2013.

Abstract

To the best of our knowledge, this is the first study of its kind performed in Bahrain, investigating native and exotic plant growth-promoting rhizobacteria for plant growth promotion abilities. Water stress tolerance represented by different osmotic pressures (-0.15, -0.49, -0.73, -1.2 MPa) was assessed for each of the 13 morphologically distinct isolates found. The isolates obtained varied slightly in their colony characteristics (color, morphology, texture), Gram's staining and catalase testing. Screening for positive ammonia production, phosphate solubilization and salinity tolerance was then performed to assess the isolates' plant growth promotion abilities. Among the 13 isolates obtained, Isolate J demonstrated the highest PGPR potential reflected in the highest PSI (6.80 ± 0.66), high ammonia production and salinity tolerance (up to 3 M) followed by isolate B (PSI of (6.44 ± 0.47)), moderate ammonia production and salinity tolerance up to 3M) and isolate CC (PSI of 5.66 ± 0.38 and moderate results for ammonia production and salinity tolerance up to 3M).

All of the isolates were then used to prime maize seeds placed under the same varying water stresses established by PEG6000. Improvements in the germination, morphological parameters, photosynthetic pigments, and total soluble protein concentration were observed for plants raised from isolate-primed maize seeds compared to non-primed counterparts. Germination analysis highlighted the potential of isolates E, C, J respectively through the observed improved germination index and mean germination time although not significant. In the green-house experimentation, and under drought stress, the best and most significant plant morphological parameter supporting isolates found was PF followed by CC and PF2. Improvements in the growth parameters due to PGPRs were attributed to phytohormone production. Photosynthetic pigment concentration was highest for maize primed with isolate PF highlighting its potential in supporting maize growth during increasing water stress levels. The total soluble protein concentration was superior under water stress for isolate F primed maize. This study documented several observed benefits of PGPRs isolated from plants growing in Bahrain. Consequently, these isolates can be used by farmers as biofertilizers in arid and semi-arid regions with the intention of alleviating the effects of water stress. However, to achieve that, additional identification and testing of the isolates which showed the best potential is required.

Dedication

First and foremost, I dedicate this work to God almighty who has showered me in countless blessings since birth. And gave me the strength and perseverance throughout my life to reach goals beyond my expectations.

To my family, firstly, my precious mother Fatheya Alasmawi who supported my whole journey of education, taught me the value of seeking knowledge at all levels and to never stop learning.

My Father Jassim Alsaffar, who always encouraged me, gave me endless motivation, hope, and showed me that I can achieve anything I put my mind into.

And to my cherished sisters Noor, Eman, Nida, and Tasneem Alsaffar who made sure I always smiled and whose unconditional love, support, encouragement and advice I will always be grateful for.

To my beloved husband and companion, Mohammed Buhamaid, who throughout this journey believed, encouraged and supported me, provided valuable insights, and gave his unlimited help.

To my dearest friend, Rahma Aleskafi, who always understood me, and whose love and support was felt throughout my studies, and whose name fully represents who they are to me.

Finally, I dedicate this work to the many great educators I have had the luck to learn from: Mr. Khalil Alwedaei, Dr. Thuraya Almansoori, Dr. Humood Alnaser, Dr. Salwa Althawadi and Prof. Afnan Freije who I am forever grateful for as they believed and ignited the love of science in me since my undergraduate studies and always were generous in sharing their valuable knowledge and helpful advice.

Acknowledgements

I would like to thank my supervisor Dr. Salwa Althawadi and co-supervisor Dr. Mohammed Azeem for their continued and valuable support, guidance and assistance throughout the research.

Secondly, I would like to thank the biology department of the University of Bahrain for the outstanding cooperation and facilitation of my research.

Finally, I am grateful to the biology department technicians Mrs. Nawal Abdulla Hasan and Mrs. Batool Abdulwahab Ahmed for their assistance during the experimental part of this research and encouragement.

Table of Contents

Defense committee's approval.....	2
Abstract.....	3
Dedication.....	4
Acknowledgements.....	5
Table of Contents	6
Abbreviations list.....	12
Chapter 1.....	13
Introduction to the study	13
Chapter 2.....	23
Theoretical framework and Literature review	23
Plant physiological responses during water stress	24
Bacterial communities in the soil and their responses to drought	27
Mechanism utilized by PGPR to support plant growth and productivity	29
The potential of PGPRs isolated from harsh soils.	31
PGPRs potential in countries close to Bahrain's climatic conditions.	36
Other methods used in agriculture to alleviate drought stress effects on maize	37
1. Chemical fertilizers	37
2. Transgenic Maize	38
3. Different Bio stimulants	39
Current Applications of PGPRs	40
Chapter 3.....	42
Methods and procedures	42
3.1 Sample Collection	43
3.2 Isolation of Bacterial strains from the soil and root samples.....	44
3.3 Morphological and Biochemical characterization	46
3.4 Screening for water stress tolerant PGPRs:	46
3.5 Determination of isolates plant growth promotion abilities.....	47
3.6. PGPR induced water stress tolerance in maize.....	48
3.7 Statistical analysis	54
Chapter 4.....	55
Results, Discussion, and Recommendations.....	55
4.1. Isolation of rhizospheric bacteria	56
4.2. Morphological and Biochemical characterization of rhizospheric bacteria.....	57

4.3. Screening for the bacteria's ability to withstand water stress.	60
4.4. Determination of PGPR properties.....	64
4.5 PGPR induced water stress tolerance in maize.....	68
Recommendations.....	118
References	120

Table of tables

Table (1): Plant promotion mechanisms utilized by PGPRs.....	30
Table (2): PGPR Strains isolated from harsh/arid soils and their effects on plant growth.....	33
Table (3): Sources of each isolates obtained and their assigned letters.....	56
Table (4): Biochemical and morphological characteristics of isolates obtained from different sources.....	60
Table (5): Ammonia production levels and salinity tolerance for isolates investigated.....	67
Table (6): Leaf length of significantly different isolates primed maize compared to the non-primed maize.....	74
Table (7): Leaf area of significantly different isolates primed maize compared to the non-primed maize.....	75
Table (8): Root length of significantly different isolates primed maize compared to the non-primed maize.....	79
Table (9): Fresh weight of primed maize roots which are significantly higher than the non-primed maize.....	82
Table (10): Dry weights of maize roots which are significantly higher than the non-primed maize	83
Table (11): Maize shoot length for isolates exhibiting significantly higher results compared to the non-primed maize.....	88
Table (12): Maize shoot fresh weights for significantly higher isolates primed maize compared to the non-primed maize.....	90
Table (13): Maize shoot dry weights of significantly higher isolates compared to their non-primed counterparts.....	91
Table (14): Chlorophyll a concentration of significantly higher isolate primed maize compared to the non-primed maize.....	99

Table (15): Chlorophyll b concentration for significantly higher isolate primed maize compared to the non-primed maize.....	100
Table (16): Total chlorophyll for significantly higher isolate primed maize compared to non-primed maize.....	101
Table (17): Carotenoid concentration of non-primed maize compared to significantly higher isolate primed maize.....	103
Table (18): Total soluble protein concentration for maize primed isolates with significantly higher results compared to the non-primed maize.....	110
Table (19): Ranking of the best maize primed isolates compared to the non-primed maize in the parameters tested (Mean $\pm$ SE)	114
Table (20): Methods implemented by PGPRs to reduce water stress effect on plants growth.....	117

Table of figures

Figure (1): Summary of drought triggered plant responses.....	26
---	----

Figure (2): <i>Prosopis farcta</i> harvested from Karannah beach.....	43
Figure (3): <i>Cressa cretica</i> harvested from Karannah beach.	44
Figure (4): Overview of the research methodology followed.....	45
Figure (5): Maize seeds covered by isolate CC2 inoculum solution.....	49
Figure (6): (a). Placement of maize seeds (primed and unprimed) into the tissue culture growth boxes under the laminar flow. (b). Tissue culture growth boxes in the dark room (bottom).....	50
Figure (7): Time frame of plant survival and growth.....	52
Figure (8): Isolate E grown on NA media for purification purposes at 28 ± 1 C° for 24 hr.....	58
Figure (9): Morphologically Different isolates streaked on NA at 28 ± 1 C° for 24 hr. a: PF3, b: J, c: CC1 and d: PF2.	58
Figure (10): Gram testing results for different isolates viewed under the microscope. a: B, b: J, c CC2 and d: I.....	59
Figure (11): The absorbance and tolerance of various rhizospheric isolated grown in TSB with PEG6000 at different osmotic pressures (-0.15, -0.49, -0.73, -1.2 MPa)	63
Figure (12): Mean PSI measurements for phosphate solubilization positive isolates.....	64
Figure (13): Phosphate solubilization halo zones of rhizospheric isolates grown on PKV agar at 28 ± 1 C° for a week. a: Isolate J, b: isolate CC.....	65
Figure (14): Ammonia production of isolates J and CC1 compared to the control on the far left.....	66
Figure (15): Germination of primed maize seeds.....	70
Figure (16): Germination index (%) of isolates under water stress	71
Figure (17): Mean germination time (Day) for isolates under water stress.....	71
Figure (18): Maize seedlings before water stress, (a) and after water stress treatment.....	72

Figure (19): Leaf length of maize bio primed seedlings at different water stress levels in cm.....	77
Figure (20): Leaf area of maize bio primed seedlings at different water stress levels in m ²	78
Figure (21): Root fresh weight (FW) of maize bio primed seedlings at different water stress levels in g.....	85
Figure (22): Root dry weight (DW) of maize bio primed seedlings at different water stress levels in g.....	86
Figure (23): Root length of maize bio primed seedlings at different water stress levels in cm.....	87
Figure (24): Shoot length of maize bio primed seedlings at different water stress levels in cm.....	93
Figure (25): Shoot fresh weight (FW) of maize bio primed seedlings at different water stress levels in g.....	94
Figure (26): Shoot dry weight (DW) of maize bio primed seedlings at different water stress levels in g.....	95
Figure (27): Chlorophyll a concentration in leaves of maize seeds in mg/g of FW.....	104
Figure (28): Chlorophyll b concentration in leaves of maize seeds in mg/g of FW.....	105
Figure (29): Total Chlorophyll concentration in leaves of maize seeds in mg/g of FW.....	106
Figure (30): Carotenoid concentration in leaves of maize seeds in mg/g of FW.....	107
Figure (31): Total soluble protein concentration ($\pm$ SE) in leaves of maize seeds in mg/ml of FW.....	112

Abbreviations list

PGPB	Plant Growth Promoting Bacteria.
PGPR	Plant Growth Promoting Rhizobacteria.
PGPE	Plant Growth Promoting Endophytes.
PEG6000	Polyethylene glycol 6000
TSP	Total soluble proteins
PSI	Phosphate solubilization index
PVK	Pikovskaya agar
GI	Germination index
MGT	Mean germination time
NA	Nutrient agar
TSB	Tryptic soy broth
OD	Optical density
IAA	Indole acetic acid
ACC	1-aminocyclopropane-1-carboxylate deaminase
ABA	Absciscic acid
LEA	Late Embryogenesis Abundant
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Tot Chl	Total chlorophyll
Car	Carotinoids

Chapter 1

Introduction to the study

Plant stresses refer to the external factors that unfavorably affect their growth, development, and productivity (Gull & Wani, 2019). These stresses can induce altered plant responses, including changes in gene expression, growth rates, crop yield, and cellular metabolism (Ahluwalia et al., 2021). Abiotic stresses, such as drought, flooding, salinity, temperature variations, and contamination, pose significant challenges to plant growth and productivity and physiological processes (Dikobe et al., 2021; Santoyo et al., 2021; Irshad et al., 2023). They can manifest as osmotic stress, disruption of plant cell homeostasis, and malfunction in ion distribution (Gull & Wani, 2019). Plants cannot establish a biotic stress-adapted immunity even if the abiotic stress occurs recurrently (Ahluwalia et al., 2021).

During water stress or drought, various physiological and biochemical responses occur in plants, including stomatal closure, reduced photosynthesis, and metabolic adjustments (Bashir et al., 2021; Dikobe et al., 2021). The initial plant response to drought is often the suspension of growth to reduce metabolic demands (Gull & Wani, 2019). Additionally, plants synthesize protective compounds such as soluble proteins, prolines, soluble sugars, and betaine to aid in osmotic adjustment (Bashir et al., 2021; Gull & Wani, 2019).

The accumulation of stress-tolerant proteins and osmolytes enables plants to adapt molecularly and cellularly to water stress (Ali et al., 2020; Aranda et al., 2021). In addition to osmolytes, accumulation of compatible solutes such as calcium, sodium and chlorine ions in the cytosol and vacuoles allow for improved osmotic balance in the cells (Zia et al., 2021). Despite these adaptations, water stress will still impose diffusive and biochemical limitations on plants, affecting processes such as photosynthesis and growth (Pepe et al., 2022).

Maize (*Zea mays* L.) is a vital cereal crop like rice and wheat, all considered as main staple foods (Dikobe et al., 2021; Ojuederie et al., 2023;). It is commonly consumed in different forms in Asia, South America, and sub-Saharan Africa (Ojuederie et al., 2023). Due to its economic importance, maize is grown in South Africa and many other parts of the world (Dikobe et al., 2021). As a food source, maize grains are of high nutritional value as a source of fiber, sugar, protein and lipids (Agbodjato et al., 2021) as well as many phenolics, carotenoids and phytosterols (Dikobe et al., 2021). Moreover, maize is used in animal feed (Dikobe et al., 2021).

Maize follows a C₄ photosynthetic pathway and is the crop most negatively affected by osmotic stress arising from drought and salinity (Dikobe et al., 2021). This is because maize is classified as a crop of almost-high water needs (0.15-0.20 inches of water/day) (Bazrgar et al., 2023). Luckily, maize has a wide range of genotypic variability, allowing it to grow in different climate conditions and soil types (Dikobe et al., 2021).

Yet, water shortages account for 70% of potential crop yield and productivity losses worldwide (Bashir et al., 2021). Drought decreases plant yield and quality (Ali et al., 2020), thereby affecting the agricultural productivity (Ashry et al., 2022). Cell turgidity, leaf size, photosynthetic activity, stomatal conductance, water use efficiency, membrane instability, stem elongation, oxidative metabolism, and osmolyte accumulation are all processes that experience changes due to drought stress in maize (Bashir et al., 2021). Maize morphological changes due to drought in all developmental stages are measurable, especially those linked to plant metabolism (Bashir et al., 2021). Maize yield in SSA dropped in western and southern Africa to below 2 t ha⁻¹ and <1.5 t ha⁻¹, respectively, due to changes in climatic conditions (Ojuederie et al., 2023).

Future predictions of water availability indicate its imminent scarcity, which will undoubtedly affect the intensity of abiotic stresses (Gull &

Wani, 2019). As a result, the need to develop crops that are resilient to abiotic stresses is imperative to ensure food safety and security in the future (Gull & Wani, 2019).

Poor farming practices have caused severe land degradation by destroying organic matter, soil microfauna, soil flora and fauna (Agbodjato et al., 2021). Applications of chemical nitrogen fertilizers have successfully improved global food production under normal and water stressed conditions (Mazumdar et al., 2020). Yet the excessive use of fertilizer inhibits beneficial microbiota and reduces soil fertility (Santoyo et al., 2021), as fertilizers persist in the soil due to plants not using them completely (Agbodjato et al., 2021). The excessive use of nitrogen fertilizers degrades the quality of soil, water, and air, causing waterway eutrophication and soil acidification (Mazumdar et al., 2020). Another harmful outcome of excessive fertilizer use lies in the accumulation of nitrate in the crops, which threatens human health (Mazumdar et al., 2020). In addition to the previous disadvantages on the environment, synthetic fertilizers have high costs which also deters their use (Agbodjato et al., 2021).

The health and growth of plants is greatly affected by the microbial communities interacting with their tissues (Bueno et al., 2022). Microbial communities can cause disease to the plant or endorse tolerance to stresses and enhance growth (Bueno et al., 2022). Crop production can greatly benefit from bacterial inoculants with plant growth-promoting abilities (Santoyo et al., 2021).

Plant Growth-Promoting Bacteria (PGPB) assist plants in tolerating different stresses (Karnwal, 2020a). PGPB exists in many different genera such as, but not limited to, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Mycobacterium*, *Mesorhizobium*, *Flavobacterium* (Karnwal, 2020a).

When applied to plants, Plant Growth-Promoting Bacteria (PGPBs) promote plant growth and increase stress tolerance (Ashry et al., 2022). PGPBs also relieve the effects of drought stress (Ashry et al., 2022).

Based on their location, PGPB are classified into rhizospheric, endophytic or epiphytic (Bueno et al., 2022). Plant Growth-Promoting Rhizobacteria (PGPRs) resides within the rhizosphere, which is the area of soil around the roots of plants (Karnwal, 2020a; Santoyo et al., 2021). Examples of PGPRs include *Microbacterium*, *Azobacter*, *Achromobacter*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Burkholderia*, *Azospirillum*, and *Serratia* (Jeyanthi et al., 2018). PGPE, or plant growth promoting endophytes are microorganisms that reside inside the plant tissues without causing plant disease or needing differentiated plant structures like nodules (Bueno et al., 2022).

PGPRs display various mechanisms to colonize, compete, and establish themselves in the rhizosphere (Santoyo et al., 2021). Root exudates and deposits by plants like photosynthesis products, amino acids, sugars, organic acids, and many other molecules act as chemo attractants that attract specific PGPRs, which then colonize them (Santoyo et al., 2021). Different plants and plant species produce different root exudates and, therefore different microenvironments, have different microbes colonizing their rhizosphere (Santoyo et al., 2021). The microbiome of the plant is closely associated to its genotype (Santoyo et al., 2021; Soltani et al., 2023), soil properties, developmental stage, climatic conditions and different abiotic and biotic factors (Bueno et al., 2022). Soil properties include pH, water availability, temperature, and nutrient content (Ahmed et al., 2022). Each different microenvironment has its physicochemical properties and inhabits unique microorganisms (Ahmed et al., 2022; Santoyo et al., 2021).

The rhizosphere is an area of great ecological richness and diversity (Santoyo et al., 2021). Subsequently, the rhizosphere becomes an area of high competition for nutrients by microorganisms (Santoyo et al., 2021). Phytopathogenic microorganisms are eliminated by PGPRs through specialized secretion systems that produce antibiotics and lytic enzymes (Santoyo et al., 2021). The evolution of secretion systems in bacteria facilitates proteins release (by ejection) into the extracellular space around them or directly into hosts and other cells (Hernandez et al., 2020). There are many secretion systems, however type (IV) has the potential to alter various diverse microbial communities (Hernandez et al., 2020). Type IV secretion system (T6SS) is used by bacteria in competitive situations to deliver toxic proteins into neighboring bacterial cells (Bueno et al., 2022). Competition is not the only interaction between bacteria in the plant's rhizosphere, synergistic cooperation in the form of quorum sensing can also occurs (Bueno et al., 2022). Quorum sensing regulates the responses of bacteria in a community through secretion of distinct signalling molecules (Bueno et al., 2022).

PGPRs, when used as bio-inoculants, are an eco-friendly alternatives to reduce the use of chemicals in agriculture (Santoyo et al., 2021), especially nitrogen fertilizers (Mazumdar et al., 2020).

Many studies have linked PGPRs to improved plant systemic response to abiotic stress due to their induction of plant physical and chemical changes (Etemadi et al., 2020). Additionally, through horizontal gene transfer (HGT), bacteria can transfer their abilities in the form of genetic information, to the rest of the phylogenetically distant bacterial community allowing them to gain novel function that enhance their adaptation to the environment (Bueno et al., 2022).

The plant promotion abilities of PGPRs are varied and assist plant growth in many perspectives (Scagliola et al., 2021). Through direct and indirect

mechanisms, PGPRs improve plants' growth (Scagliola et al., 2021). Direct mechanisms of growth support include the production of phytohormones or by increasing the rhizosphere nutrient bioavailability (Scagliola et al., 2021). Firstly, the fixation of nitrogen into forms that are more accessible to plant uptake (Bueno et al., 2022; Mazumdar et al., 2020). Secondly, the production of utilizable siderophores from iron in the soil (Mazumdar et al., 2020). To produce siderophores, iron chelation is required to convert insoluble iron hydroxides and oxyhydroxides into soluble complexes (Jeyanthi et al., 2018). However, through this, rhizobacteria limits the access of phytopathogens to iron and at the same time makes iron available for plant uptake by active transport (Jeyanthi et al., 2018). Siderophore production is especially advantages to plants in calcareous soils (Khan et al., 2020a). Therefore, indirectly, PGPRs limit nutrients like iron which are available in the soil to other pests and pathogens (Karnwal, 2020a). To defend against phytopathogens, PGPRs employ different mechanisms that include the production of harmful hydrogen cyanide (HCN), chitinase enzyme, antibiotics (Mazumdar et al., 2020), and hydrolytic enzymes (Karnwal, 2020a). Additionally, PGPRs act as decomposers that allow better absorption and detoxification (Karnwal, 2020a).

A proposed mechanism for improved growth during stress due to PGPR priming centers around growth regulators or phytohormones by PGPRs (Lin et al., 2020). Growth regulators allow the roots to absorb water and nutrients more efficiently from the soil (Lin et al., 2020). Additionally, plant phytohormones endorse cell division and growth (Ashry et al., 2022). Phytohormones also enhance water uptake of plants by improving the formation of lateral roots and absorption surface area (Ashry et al., 2022). Phytohormone synthesis such as 1-aminocyclopropane-1-carboxylate deaminase (ACC) synthesis (Mazumdar et al., 2020), which directly supports host plant growth (Scagliola et al., 2021). Plants naturally

synthesize Indole acetic acid (IAA) hormone for apical dominance, shoot elongation, root initiation, tropism, and induction of cambial cell proliferation (Mazumdar et al., 2020). Bacteria that produce IAA naturally compose 80% of rhizospheric bacteria (Jochum et al., 2019). Bacteria produce IAA for many hypothesized reasons such as tryptophan storage, microbe-microbe signaling, and to form relationships with plants (Jochum et al., 2019). External IAA application to plants allows for the same hormonal effects, especially when produced by PGPRs due to their ongoing slow release of phytohormones (Mazumdar et al., 2020). IAA-producing rhizobacteria work in harmony with the naturally synthesized IAA to increase the size of adventitious roots and their numbers to allow for improved nutrient absorbance and hence plant growth (Mazumdar et al., 2020).

Plant growth cannot be completed without phosphate as it is a vital nutrient involved in photosynthesis, energy transfer, macromolecule biosynthesis, and respiration (Mazumdar et al., 2020). Soils have shortages of organic and inorganic forms of phosphates as they are insoluble and strongly complexed (Mazumdar et al., 2020). For plants which can only uptake inorganic forms of phosphate, PGPR solubilization of organic forms of phosphates is vital in supporting their growth (Mazumdar et al., 2020). PGPRs are able to solubilize phosphates by producing mineral dissolving compounds such as phosphate hydrolyzing enzymes, protons, anions, and organic acids (Mazumdar et al., 2020). Finally, PGPRs enhance plant growth by increasing their tolerance to abiotic stressors (Scagliola et al., 2021).

The use of water stress-tolerant PGPRs as bio-inoculants is cheap and eco-friendly (Ojuederie & Babalola, 2023). Additionally, Halo-tolerant PGPR application to stressed soils allows for sustainable development of the soil systems (Alexander et al., 2020). Beneficial microorganisms, which are

naturally associated with plants, are alternatives to chemical fertilizers with no secondary effect on the environment (Santoyo et al., 2021). PGPRs improve plant growth without genetic modifications or external fertilizer applications (Mazumdar et al., 2020), while also being a highly accessible solution to farmers in developing and developed countries (Mazumdar et al., 2020). Thus, exploitation of new sources and strains is highly required. Bahrain's environment is characterized as an arid to extremely arid environment (Sadeq et al., 2020). As rainfall is erratic with mean annual temperatures of 26.8°C and mean annual precipitation of 99.8mm (Sadeq et al., 2020). Aridity prevails all year long with evaporation rates of 3.5mm to 15.6mm (Sadeq et al., 2020). The soil in Bahrain is alkaline and poorly defined in structure and shallow underlined with sand of gypsic or calcic horizon (Sadeq et al., 2020).

The flora of Bahrain is comprised of six types based on the habitat factors and dominant species (Biodiversity baseline assessment report, 2015; Abbas, 2006). These types are sabkha and salt pans, sand dunes, coastal plains, stone pavements, and rocky habitats (Biodiversity baseline assessment report, 2015; Abbas, 2006). Sand dune vegetation is characterized by having xerophytic (desert-adapted) flora, while halophytic (salt-adapted) plants reside in sabkha and salt pans (Biodiversity baseline assessment report, 2015).

Native plants in Bahrain include 327 species such as *Prosopis fracta*, *Cressa cretica*, *Lycium shawii*, and *Calligonum polygonoids* (Biodiversity baseline assessment report, 2015). *P. fracta* and *C. cretica* are halophytic plants with many folk medicinal uses (Abbas, 2006). *C. cretica* can be found occasionally on compact coastal saline soils (Norton et al., 2009) and in hyper arid deserts (Naz et al., 2024). *C. cretica* was traditionally used as an expectorant (Norton et al., 2009). *P. fracta* is a desert-adapted plant used medicinally as an antimicrobial and antioxidant (Salimi-Sabour et al.,

2022). Also, residing in the kingdom of Bahrain are plants of the *Bougainvillea* genus, which tolerate drought, salinity, and are wind resistant (Karnwal, 2020a).

Harsh environments are inhabited by unique species with different capacities (Gaete et al., 2020). As a result, native microorganisms found in arid and semi-arid regions have the advantage of drought tolerance and may help crops alleviate drought stress (Shirinbayan et al., 2019). To develop biofertilizers suitable for use in arid and semi-arid regions, it is essential to identify efficient native strains of microorganisms that are adapted to and tolerant of dehydration (Shirinbayan et al., 2019).

The aim of this research is to investigate the bacterial populations associated with plants growing in the kingdom of Bahrain for their plant growth promotion abilities. These plant-growth promoting rhizobacteria may have the potential to ameliorate different levels of water stress in crops like maize by enhancing plant germination and growth under stress.

Chapter 2

Theoretical framework and Literature review

Drought stress significantly affects the food and agriculture industries (Ashry et al., 2022), with notable impacts on maize agriculture. The advantages of using PGPRs on crops has been widely documented (Ulah & Bano, 2015). PGPRs provide an eco-friendly and cost-effective solution for farmers to combat the detrimental effects of abiotic stress on crops (Ashry et al., 2022). They mitigate water stress through changes in the root architecture, production and accumulation of osmolytes, phytohormones and antioxidants (Ahmad et al., 2022).

This literature review discusses the different plant responses to water stress and the current knowledge of bacterial communities' drought related responses. The literature review then discusses PGPR's plant promotion mechanisms and highlights their abilities when obtained from harsh environments, such as those found in Bahrain and in countries close to Bahrain with similar climatic conditions. Additionally, Different agricultural methods used to increase plant productivity under drought stress are discussed. Finally, an overview of the PGPRs currently applied is discussed.

Plant physiological responses during water stress

During drought conditions, photosynthesis is affected by stomatal and non-stomatal limitations (Ahluwalia et al., 2021). In effective CO₂ fixation is an example of stomatal limitation, while non-stomatal limitations occur due to reductions in chloroplast activity, Rubisco enzyme activity, leaf nitrogen, photosystem (I and II), ATP availability (Ahluwalia et al., 2021).

Plant species exhibit varying degrees of tolerance and adaptation to water stress, influenced by both genetic factors and environmental conditions (Pepe et al., 2022). Some species demonstrate improved water use efficiency through adjustments in stomatal aperture, while the severity and

duration of water stress further dictate the extent of these adaptations (Pepe et al., 2022).

Natural cellular processes such as energy production, apoptosis, cellular signaling, and gene expression give rise to free radicals (Lourenço et al., 2019). Free radicals are highly reactive species with an unpaired electron in the valence shell, which can be donated or accepted along with another electron from other molecules (Flieger et al., 2021). Examples of free radicals include reactive oxygen species (ROS) like superoxide radical (O_2^-), hydroxyl radical ($OH^\cdot$), and hydrogen peroxide (H_2O_2) (Flieger et al., 2021; Ahmad et al., 2022;). These reactive free radicals cause oxidative damage to macromolecules exposed to them, such as proteins, carbohydrates, DNA, and lipids in the cells (Flieger et al., 2021; Ahmad et al., 2022), leading to enzyme deactivation, DNA damage, and protein degradation (Dikobe et al., 2021). However, as these ROS arise from natural conditions, plants can mitigate their effects through the action of enzymatic and non-enzymatic antioxidants (Dikobe et al., 2021).

Plants perform stomatal closure in an effort to stop water loss, concurrently, by closing the stomata the plants limit CO_2 fixation and elevate the production of ROS species which causes oxidative damage (Dikobe et al., 2021). Oxidative damage by ROS species will affect cellular components causing enzyme deactivation, DNA damage and protein degradation (Dikobe et al., 2021).

During abiotic stress, organelles such as chloroplasts and mitochondria experience hindrances in the electron transport chain (Bazragar et al., 2023). These hindrances allow molecular oxygen (O_2) to bind with free electrons and accumulate as reactive oxygen species (ROS), which damage cell integrity (Bazragar et al., 2023) and macromolecule structure (Flieger et al., 2021).

The damage to macromolecule structure impairs proper physiological functioning and leads to the buildup of cytotoxic compounds like malondialdehyde in the cells (Flieger et al., 2021). Additionally, plants perform stomatal closure in an effort to stop water loss; however, this leads to limited CO₂ fixation and elevated ROS production, causing further oxidative damage (Dikobe et al., 2021). Depending on the severity and duration of osmotic stress along with the crop growth stage, the adverse effects vary (Ahmed et al., 2022).

Another plant growth process severely affected by drought stress is phytohormone production. Plant hormone Cytokinins synthesis is often halted during water stress, leading to leave senescence (premature death) (Zia et al., 2021). Ethylene production, which restricts growth, is another plant hormone associated with senescence and stomatal closure (Zia et al., 2021). Finally, abscisic acid (ABA) also restricts plant growth by signalling drought related processes leading to growth disturbances (Zia et al., 2021). Droughts effects on plants can be summarized in figure (1) shown below.

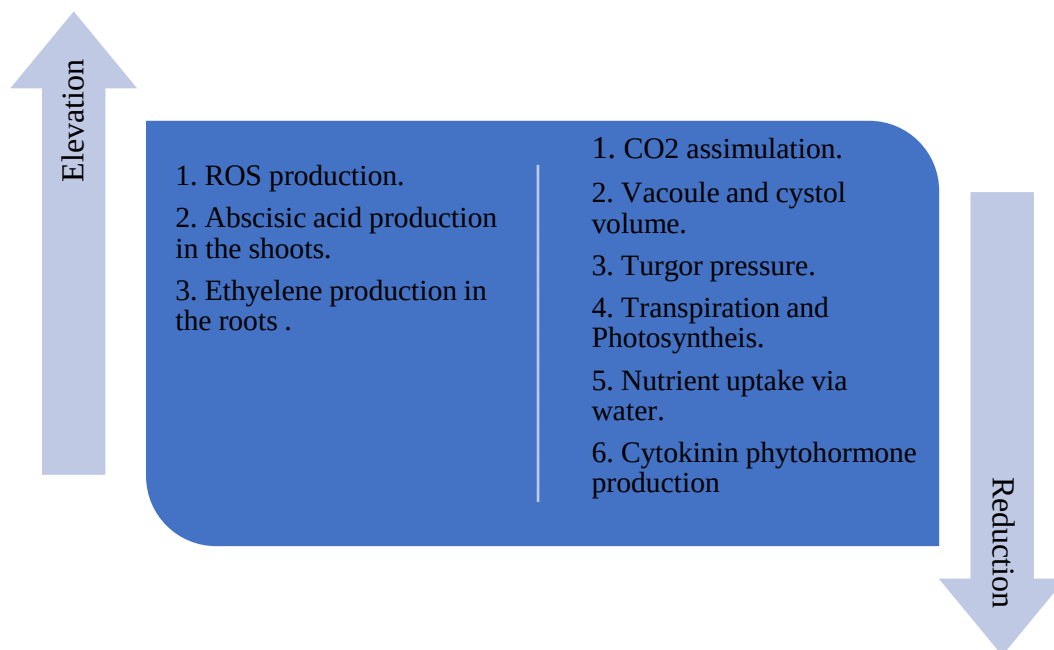


Figure (1): Summary of drought triggered plant responses.

Owing to above mentioned factors summarized in table (1), the agronomical crop productivity is severely compromised (Santoyo et al., 2021) which poses a serious threat to food security as 90% of the nutrition worldwide is sourced from 12 crop varieties and 14 livestock species (Karnwal, 2020b). This a biotic stress leads to the loss of major crops worldwide (Gull & Wani, 2019). This issue is further exacerbated by global climate change, which alters the frequency (Bashir et al., 2021) and extent of this stress (Bashir et al., 202; Soltani et al., 2023). The productivity of crops is undeniably affected by the increase in drought-prone areas (Ahmed et al., 2022).

Bacterial communities in the soil and their responses to drought.

Drought greatly influences microbial communities and their activities (Bogati & Walczak., 2022). Microbial communities are altered indirectly when plants face altered climate conditions and change their communities' structure and function in response or directly due to drought or invasions by other plants microbiota (Fahey et al., 2020). Plants dictate their surrounding microbiota through their exudates and root deposits (Zia et al., 2021). As a result, even non-native invasive plant species can directly influence the microbial communities in soil (Fahey et al., 2020). A second implication of non-native invasive plant species is that they can reduce the number of plants for microbial colonization by competing with and reducing plant diversity (Fahey et al., 2020).

Drought's increased frequency and severity is linked to significant changes in soil microbiota (Fahey et al., 2020) which include changes in community size, structure and activity (Bogati & Walczak., 2022). In addition, water stress will decrease the total bacterial biomass due to nutrient limitations (Naylor & Coleman-Derr, 2018). Drought stress will decrease bacterial

richness more than the reductions due to foreign bacterial invasions by exotic plants (Fahey et al., 2020). Bacterial richness causes more decomposition of organic matter and nutrient mineralization (Naylor & Coleman-Derr, 2018). Moreover, due to drought, microbial abundance will decrease along with carbon and nitrogen cycling in the soil (Bogati & Walczak., 2022).

Antibiotics are found in excess during drought in the soil, due to the bacteria's attempt to outcompete surrounding bacteria for resources or in an attempt to signal drought response pathways in the community (Naylor & Coleman-Derr, 2018).

However, some bacteria can response to the altered water potential by changing their membrane structure and increasing water and nutrient uptake (Khan et al., 2020a). The PGPR *Azospirillum* forms a cyst around the roots and produces melanin and polyhydroxybutrate to maintain water under dehydration (Khan et al., 2020a). Microbes can also form spores to survive the drought for longer such as *Myxobacteria* and *Bacillus* or synthesize more soluble proteins, Osmo protectants and siderophores (Bogati & Walczak., 2022). Additionally, plants will also increase DNA manufacturing in the cells to protect proteins structures and hence function (Bogati & Walczak., 2022).

Instead of gram-negative bacteria in the rhizosphere, drought causes the rhizosphere to be mostly inhabited by gram-positive bacteria such as *Actinobacteria* due to their thicker peptidoglycan layer which provides protection (Bogati & Walczak., 2022; Hartman & Tringe, 2019). Gram positive bacteria can also store osmolytes to elevate their drought tolerance (Naylor & Coleman-Derr, 2018; Hartman & Tringe, 2019). Gram positive bacteria possess the ability to use enzymes produced from inorganic nitrogen to decompose complex organic compounds such as recalcitrant

carbon in drought-affected, nutrient poor soils (oligotrophs); compared to the copitrophic gram negatives that grow in water and nutrient rich soils (Naylor & Coleman-Derr, 2018). Lastly, many of the gram-positive bacteria sporulate while gram negatives cannot due to evolutionary losses of this trait (Naylor & Coleman-Derr, 2018). These advantages allow better competition of gram-positive strains against gram negatives (Hartman & Tringe, 2019).

While drought causes unfavorable effects on soil microbiota, it can also trigger their adaptations, which may culminate into the production of novel functional groups that may benefit agricultural practices (Bogati & Walczak., 2022).

Mechanism utilized by PGPR to support plant growth and productivity.

Bacteria must display three important characteristics in order to be classified as PGPRs: root colonization, survival and replication in competition with other microbes in the rhizosphere (Jeyanthi et al., 2018). Secondly, they must survive long enough to be able to exude their plant promotion abilities and finally they must measurably promote plant growth (Jeyanthi et al., 2018).

PGPRs demonstrate many mechanisms used to promote plant growth directly and indirectly (Mataranyika et al., 2022) as observed in table (1). Through the production of various substances by PGPRs such as nitrogenase, phosphatase, IAA and cytokinin's, siderophores, phenazines

Table (1): Plant promotion mechanisms utilized by PGPRs.

	Mechanism	Substances that are produced by PGPR	References
Directly by providing the plant with nutrients from the soil or by secreting them.	Fixation of N ₂	Nitrogenase enzyme	Jeyanthi et al., 2018; Mazumdar et al., 2020; Ahluwalia et al., 2021; Mataranyika et al., 2022; Bueno et al., 2022.
	Phosphate solubilization	Phosphatase enzyme and organic acids that lower the pH.	Jeyanthi et al., 2018; Shirinbayan et al., 2019; Mataranyika et al., 2022; Bueno et al., 2022; Gupta et al., 2022.
	Secretion of Siderophores	Carboxylates, catecholates pyoverdines and hydroxamate.	Jeyanthi et al., 2018; Mazumdar et al., 2020.
	Secretion of phytohormones	IAA, gibberellins, cytokines, abscisic acid and ethylene.	Jeyanthi et al., 2018; Scagliola et al., 2021;
	Increasing the availability of iron.	Production of siderophores	Jeyanthi et al., 2018; Mazumdar et al., 2020;
Indirectly due to competition with other microbes	Production of antibiotics	Phenazines, hydrogen cyanide, cyclic lipopeptides and pyoluteorin.	Jeyanthi et al., 2018; Mazumdar et al., 2020;
	Production of lytic enzymes	Production of glucanases, lipases, and proteases.	Jeyanthi et al., 2018; Karnwal, 2020a;
	Induction of plant systemic resistance	Hormones (Jasmonate and ethylene) which stimulate plant defence responses against pathogens.	Jeyanthi et al., 2018; Etemadi et al., 2020;

and proteases many of the harmful effects brought on by water stress can be mitigated (Jeyanthi et al., 2018; Shirinbayan et al., 2019; Karnwal,

2020a; Etemadi et al., 2020; Mazumdar et al., 2020 Scagliola et al., 2021; Ahluwalia et al., 2021; Bueno et al., 2022; Gupta et al., 2022; Mataranyika et al., 2022). These mechanisms are of great importance when discussing PGPR effectiveness, as they surpass the importance of PGPR concentrations around the root in the effects on plant growth and productivity (Bueno et al., 2022).

The potential of PGPRs isolated from harsh soils.

Desert adapted or stress native PGPRs assist plants survival during water stresses (Zia et al., 2021) and therefore may help crops in tolerating stress (Shirinbayan et al., 2019). In table (2), different abilities of PGPRs isolated from harsh environments were investigated for enhanced plant growth. Gao et al., (2019) researched the PGPRs associated with the plant *Caragana microphylla* in north China. In their study, Gao et al., (2019) found 5 isolates that demonstrated the best plant growth promotion abilities of nitrogen fixation, siderophore production, ACC production, phosphate solubilization and IAA production. *Arabidopsis* seedlings inoculated with these strains experienced elevated root and shoot growth (Gao et al., 2019). While the study was well structured, concise, and investigated many plant growth promotion abilities qualitatively, the authors recommend using these isolates for desert revegetation and management without the screening of them in water stress either in vitro or in vivo.

Ashry et al., (2023) researched drought-tolerant bacteria from harsh soils in Egypt. The isolates were exposed to water stress using PEG6000 to create multiple osmotic pressures (-0.15, -0.49, -0.73, -1.2 MPa) (Ashry et al., 2022). Two isolates identified in the research, *Bacillus cereus* (DS4) and *Bacillus albus* (DS9), were highlighted as potential candidates to

reduce drought stress effects on maize (Ashry et al., 2022). These isolates produced the highest amounts of phytohormones, IAA (1.61 and 0.9 µg/ml) and gibberellins (49.95 µg/ml for DS9). Co-inoculation of both isolates increased the seedling length (4.10 cm), germination rate (100%) and the vigour index (410) under water stress compared to individual inoculations (Ashry et al., 2022).

While the previous study investigated many important seedling stress and growth indicative parameters like antioxidants and phytohormones, a major limitation of this study pertains to the functional limitations and photosynthetic pigment concentration measurements. Photosynthetic pigment concentrations respond rapidly to changes in the environment (Pepe et al., 2022) allowing for plant productivity estimations under stress. Additionally, the study was performed in laboratory settings and did not explore the inoculates' effect in the field, where soil bacteria interactions may affect the results, furthermore, authors didn't mention the characteristics of the soil (Pepe et al., 2022).

Kour et al., (2020) research using drought adaptive *Pseudomonas libanensis* found that the drought tolerant strains studied achieved higher phosphorus solubilization during water stress compared to non-water stressed conditions. In their study Kour et al., (2020) found significant increases in wheat shoot and root length, fresh and dry weight. The advantages provided by *P. libanensis* were attributed to the effects of ACC deaminase, which will decompose ethylene that reduces growth (Kour et al., 2020). The latter study was performed on the model plant tomato; however, crops such as maize and wheat have different nutritional and bioactive composition and therefore, might not show the same results.

Table (2): PGPR Strains isolated from harsh/arid soils and their effects on plant growth.

PGPR strain	Source	Water stress tolerance by the PGPR	Method of PGPR application	Effects on plant growth	Reference
1. Pyllobacterium brassicacearum (DC-12)	Arid desert shrubs in north China	none	Seedlings were treated with bacterial suspensions on half strength Murashige and Skoog media (MS)	In vitro testing on <i>Arabidopsis thaliana</i> seedlings revealed enhancements in shoot fresh weight ($3.84 \pm 0.54\text{mg}$ to $5.32 \pm 0.65\text{mg}$), lateral root formation ($8.95 \pm 1.64/\text{plant}$ to $16.31 \pm 2.75/\text{plant}$) and overall root surface area.	Gao et al., 2019
2. Arthrobacter nicotinovorans (EC-3)					
3. Acidovorax radices (DR-3)					
4. Pseudomonas migulae (HR-4)					
5. Methylobacterium brachiatum (IR-10)					
1. Bacillus cereus (DS4)		Isolates were exposed to water stress using		In maize the highest amounts of phytohormones IAA (1.61 and 0.9	

2. <i>Bacillus albus</i> (DS9)	Harsh soils in Egypt.	PEG6000 to create multiple osmotic pressures. (-0.15, -0.49, -0.73, -1.2 MPa)	Seed inoculation with PGPRs	µg/ml) and gibberellins (49.95 µg/ml for DS9) were observed for isolates. Co-inoculation of both isolates increased the seedling length (4.10 cm), germination rate (100%) and the vigour index (410) under water stress compared to individual inoculations.	Ashry et al., 2022
<i>Pseudomonas libanensis</i> .	Baru Sahib region of Himachal Pradesh in india	Isolate was exposed to 5%, 6%, 7% and 8% PEG8000 induced water stress.	Seed inoculation by dipping	Strain demonstrated phosphate solubilization, ammonia, ACC hydrogen cyanide production. In wheat, Shoot and root length (25 and 15 cm respectively) as well as the fresh plant weight (0.663 g) were improved. Additionally, the concentration of osmolytes (proline and glycine betaine) was increased.	Kour et al., 2020
Consortium of three strains related to <i>Bacillus subtilis</i>	Atacama Desert in Chile	None	Seed and seedling inoculation using	Consortium demonstrated phosphate solubilization (1.80 cm), auxin	Astorga-Eló et al., 2021).

			rhizobacterial suspension.	production (122.6 µg/mL and ACC deaminase activity (KB/mg of protein). In tomato's facing drought stress, improved seed germination and seedling growth.	
Ochrobactrum sp. (NBRISH6)	Extreme environment close to volcanos in Nicobar and Andaman Islands in India.	Strain demonstrated drought tolerance created by PEG6000 up to 60%	Seeds mixed with bacterial cell suspension	Strain indicated improved maize growth under water stress demonstrated by enhanced morphological parameters such as shoot (94.66 cm) and root length (50 cm), fresh and dry weights (18.9 and 2.97g for shoots, 14.4 and 1.53 g for roots), leaf diameter (4.56 cm), mineral nutrient concentration (nitrogen 4.6 %, phosphorus 560 µg/gm and iron 121 ppm) and photosynthetic rates (chlorophyll a 1.25 mg/g. FW, chlorophyll b 1.31 mg/g. FW and total chlorophyll 6.50 mg/g. FW).	Mishra et al., 2020.

In the Atacama Desert of Chile resides the native plant *Cistanthe longiscapa* which was utilized by Astorga-Eló et al., (2021) to isolate PGPRs and further screen them for water stress tolerance abilities on tomato plants. In the study, tomato plants inoculated with PGPR consortium were exposed to varying water shortages (24, 72 and 120 hours) and showed improved seed germination, height and weight of seedlings (Astorga-Eló et al., 2021). While the study by Astorga-Eló et al., (2021) was informative, it did not perform any water stress screening on the PGPRs harvested prior to application on tomato seeds, and only included plant growth promotion abilities for the consortium of strains.

Mishra et al., (2020) identified a *Ochrobactrum sp.* strain isolated from volcanos in Nicobar and Andaman Islands in India to be plant growth promoting due to its role in supporting maize growth under deficit water stress. In their study, Mishra et al., (2020) compared plant growth indicative parameters such as root and shoot lengths along with their weights (fresh and dry), photosynthetic rates (through chlorophyll a and b), antioxidant, phytohormone and osmolytes concentrations after water stress of halted irrigation for 12 days and found significantly higher results due to inoculation with PGPRs. Mishra et al., (2020) study was highly detailed and offered a comprehensive investigation into the molecules and processes affected by drought which can be alleviated by PGPRs.

PGPRs potential in countries close to Bahrain's climatic conditions.

Desert soil contains the most diverse microbial biomes compared to other biomes (Duar et al., 2018). As when used to boost the production of crops, microbes from arid and semi-arid regions exert many outstanding benefits (Duar et al., 2018). There are many studies performed in countries close to Bahrain using native plants associated rhizobacteria that

demonstrate their excellent potential in mitigating water stress. BiBi et al., (2023) performed a study on PGPRs isolated from 10 native plant species in Qatar, two of the isolates, identified as of *Bacillus subtilis* species, had the potential to inhibit the growth of fungi pathogens (*Fusarium oxysporum* and *Botrytis cinerea*). Additionally, in their study BiBi et al., (2023) found that the strains had numerous plant growth promoting characteristics and significantly improved tomato plants biomass (both above and below ground). A different study performed by Duar et al., 2018 in Saudia Arabia isolated 17 *Bacillus*, *Acinetobacter* and *Enterobacter* strains from the rhizospheric soil of local plants residing in Hada Al Sham. In that study, *Acinetobacter pittii* (JD-14) coated alfalfa experienced the highest improvements in photosynthetic pigment concentration, relative water content, nitrogen, potassium and phosphate concentrations, additionally, morphological parameters such as plant fresh and dry weight and height.

Other methods used in agriculture to alleviate drought stress effects on maize.

1. Chemical fertilizers

Chemical fertilizer application in agriculture allows for an increase of 50% in production (Bueno et al., 2022). However, the use of chemical fertilizers continuously and excessively caused detrimental effects to soil chemical and biological properties leading to nutrient and biodiversity losses (Scagliola et al., 2021). Microorganisms play a significant role in preserving the biological and physiochemical balances in the soil through biotic mechanisms (Scagliola et al., 2021).

Nitrogen fertilizers are usually applied to soils as ammonium (NH_4^+) and nitrate (NO_3^-) (Mazumdar et al., 2020). However, as crops only absorb 30-

65% of the fertilizer nitrogen, the sustainability of this agricultural practice is questionable (Mazumdar et al., 2020). Moreover, nitrogen pollution is more challenging than carbon pollution due to the many reactive N chemical forms (Mazumdar et al., 2020).

Phosphorus is another important fertilizer often used in agriculture. Nascimento et al., (2020) conducted research pertaining to mineral fertilization and strains *Bacillus pumilus* and *Bacillus amyloliquefaciens* performance on plant development parameters. In that research, it was found that without mineral fertilization, the inoculated plants root and shoot phosphorus concentration increased more than the minerally fertilized plants (Nascimento et al., 2020).

In a previous study by Lin et al., (2020), PGPR inoculation enhanced leaf area compared to unprimed maize plants fertilized with inorganic fertilizers (Lin et al., 2020).

2. Transgenic Maize

A well-studied strategy used by plants to deter water stress effects include late embryogenesis abundant (LEA) proteins, which impede water stress activated protein aggregation (Mishra et al., 2020). With the purpose of generating drought tolerant maize, Liu et al., (2024) investigated the overexpression of LEA proteins, known to increase the osmotic stress tolerance. After imposing drought stress on the transgenic maize using PEG6000, improvements in germination rates, root length and radicle length, along with elevated concentrations of antioxidants like catalase and superoxide dismutase. The overexpression of LEA proteins enhanced the drought resistance of maize plants (Liu et al., 2024).

While genetic modification, biotechnology, or classical breeding to introduce drought-tolerant crops appear successful, a technology

applicable to many crops in many areas is more desirable (Antonucci et al., 2021). Additionally, genetically modified crops (GM) have many drawbacks to their use, such as unintentional gene flow from the engineered plant to its wild relatives (Kumar et al., 2020). Moreover, antibiotic resistance genes which are inserted into the engineered crop may transfer to other microbes in their surroundings (Kumar et al., 2020).

3. Different Bio stimulants

Bio stimulants offer a sustainable solution to agricultural difficulties caused by abiotic stress (Ashry et al., 2022; Agbodjato et al., 2021; Antonucci et al., 2021). These agronomic tools include organic and inorganic products that contain bioactive substances and/ or microorganism, which enhance plant growth, productivity, and stress tolerance through improvements in nutrient absorption, photosynthetic activity, and biosynthetic pathways (Franzoni et al., 2022). The quality of plant products is also enhanced regardless of their nutrient content (Franzoni et al., 2022; Agbodjato et al., 2021). Additionally, Bio stimulates often contain osmolytes that mitigate water stress in plants (Antonucci et al., 2021). Despite their benefits, bio stimulants effectiveness can vary between crop species, and the extraction or production method (Baltazar et al., 2021). Another factor that affects the use of bio stimulants is their varied effects on the same species (Baltazar et al., 2021).

Seaweeds and plant extracts are the first group of bio stimulants, followed by humic substances, microorganisms including PGPRs, hydrolysed proteins and nitrogen containing compounds and lastly inorganic compounds with bio stimulant action (Franzoni et al., 2022). Bio stimulants can contain a mixture of these substances and microorganisms (Agbodjato et al., 2021). Microbial inoculants have demonstrated biofertilizer,

biopesticide, bioherbicide and biocontrol agents' effects and are a reliable replacement to chemical fertilizers (Agbodjato et al., 2021).

Biofertilizers which are microorganism based are an essential agricultural component to enhance crop productivity (Bueno et al., 2022). In a study by Agbodjato et al., (2021), the effects of *Psuedomonas putida* bio stimulants on maize growth was used to recommend its use in agriculture. The highest recorded growth parameters (height, leaf area and stem diameter) and maize grain yield were observed for *Psuedomonas putida* bio stimulant and half a dose of urea and NPK (Agbodjato et al., 2021). The aforementioned study highlighted the possibilities for conjugative use of microorganisms and chemical fertilizers to reduce the latter effects on the environment. However, the potential of the rhizobacteria as the sole treatment was not measured in comparison.

Current Applications of PGPRs

Commercial applications of PGPRs to plants currently involve species such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Pseudomonas putida* (Ahmad et al., 2022), *Azosprillum brasilense*, *Serratia entomophilia* and *Azobacter chroococcum* (Jeyanthi et al., 2018). When PGPRs are not studied with Environmental factors are in vitro, their in vivo application results may differ (Jeyanthi et al., 2018). Additionally, the method of PGPR application to crops can modulate their effectiveness (Jeyanthi et al., 2018).

Current PGPR delivery methods include seedling dipping, soil application, foliar spray, biopriming and seed treatment (Ahmad et al., 2022). In the US, 60-75% of the maize, cotton, soya bean and peanuts are produced with the help of PGPR *Bacillus subtilis* which protects the crops from phytopathogens such as *Rhizoctonia* and *Fusarium* (Jeyanthi et al., 2018).

Applications of multiple PGPR strains are more effective than single strains, as multiple stresses can be tackled at once (Ahmad et al., 2022). However, the formulation of PGPRs used as fertilizer must include several features such as satisfactory water solubility, a long shelf life, compatibility with other agrochemicals (Ahmad et al., 2022), safety, stability and broad-spectrum action (Jeyanthi et al., 2018). However, the method used to deliver PGPR to the plants is imperative to obtain satisfactory results (Ahmad et al., 2022).

Chapter 3

Methods and procedures

3.1 Sample Collection

In December 2023, native plants *Prosopis farcta*, *Cressa cretica* were collected from Karannah beach (26°14'09.4"N 50°30'31.0"E) as shown in figures (3 and 4) while exotic plant *Bogenvillia spp* was harvested from AlMalikyah area (26°05'23.7"N 50°29'14.4"E). The roots and adjacent soil from each plant were collected in polyethylene bags and transported to the Microbiology laboratory of the Department of Biology, University of Bahrain, Sakhir, Kingdom of Bahrain where immediately subjected for Rhizobacteria isolation methodologies. Figure (2) shows the general procedure used to isolate and screen rhizobacteria (including rhizospheric, endophytic and epiphytic bacteria) and evaluate their potential as plant growth promoters.



Figure (2): *Prosopis farcta* collected from Karannah beach.



Figure (3): *Cressa cretica* collected from Karannah beach.

3.2 Isolation of Bacterial strains from the soil and root samples.

The first step in this analysis as shown in figure (2) involves the isolation of bacteria from the samples collected. Therefore, following the method of Karnwal, (2017) with minor modifications, 10g of the sample (roots and soil) were macerated using a mortar and pestle, and then added to 90mL sterilized distilled water. The mixture was then placed in an orbital shaker for 5 minutes at 150rpm. Serial dilution in 9mL of sterilized distilled water with 1ml of previous dilution followed until the dilution 10^{-6} was reached. 100 μ l aliquots from 10^{-6} dilution were plated onto nutrient agar (NA) Petri plates using a spreader. The plates were incubated at 28 ± 1 for 24 hr. following their growth in NA plates, the isolates were subcultured into new NA plates to isolate morphologically dissimilar colonies until pure colonies were obtained.

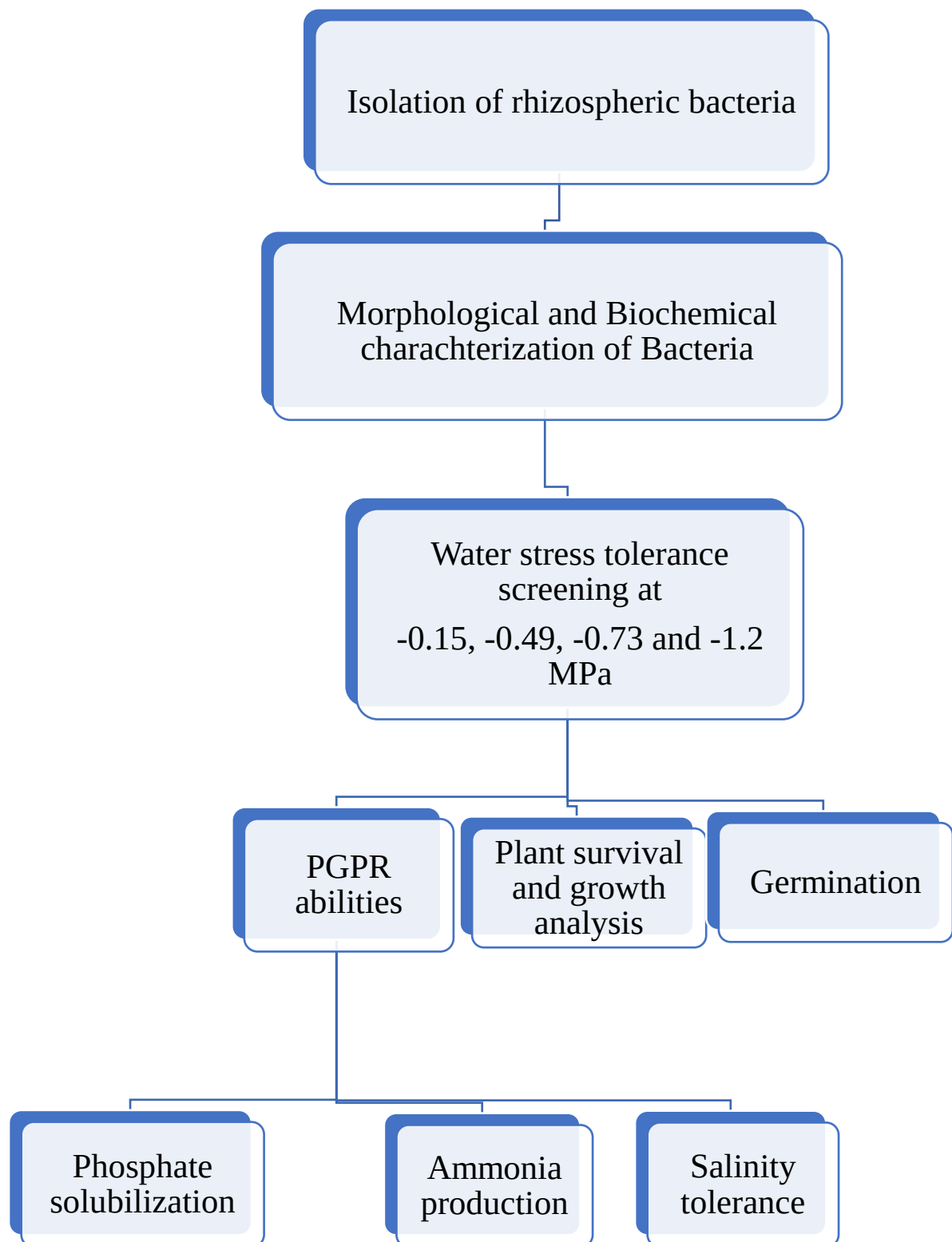


Figure (4): Overview of the research methodology followed.

3.3 Morphological and Biochemical characterization

Pure morphologically distinct colonies were identified by given letters and/or numbers. Each isolate was described based on macroscopic colony characteristics (colony color, colony shape, colony texture). Microscopic examination was also conducted to identify the cell morphology and its gram reaction using a microscope at 400X.

3.3.1 Gram staining

To identify the gram pattern of the isolates, Gram staining was performed following the method of Tripathi & Sapra, (2020) (Tripathi & Sapra 2020), and the slides were viewed under the microscope.

3.3.2 Catalase test

To assess the isolates oxygen needs, a catalase test was performed following the method of Kumar et al., (2021). Briefly, using a dropper, 3% hydrogen peroxide (H_2O_2) was dropped on isolates placed on microscopic slides. The bubbling of H_2 gas was observed for a minute and results were documented.

3.4 Screening for water stress tolerant PGPRs:

To determine the bacterial ability to tolerate drought stress, the method of Ashry et al., (2022) was followed. Tryptic soy broth (TSB) test tubes were prepared containing increasing Polyethylene Glycol 6000 (PEG6000) concentrations which are equivalent to different water stress potentials (osmotic pressure) (-0.15, -0.49, -0.7, -1.2 MPa). TSB media was dissolved in 500mL of distilled water, then depending on the desired water potential, PEG6000 was added. The solution was then heated to 50-55C° in a water bath to dissolve the PEG6000 completely before dispensing 5mL into each test tube and autoclaving. The test tubes were inoculated with one colony's worth of bacteria in triplicates for each isolate and placed in a shaking water

bath at 28C° and 150 rpm for 24 hr. The absorbance was measured at 600nm using an uninoculated TSB and PEG6000 solution at different concentrations as controls for each water stress. The triplicate mean, and standard deviation values were calculated for each isolate to determine the final isolates' ability to tolerate water stress.

Absorbance mean results were collectively used to determine the level of tolerance for each isolate: Optical density (OD) less than 0.3 indicates highly drought sensitive isolates, OD of 0.3-0.39 are sensitive isolates, 0.4 to 0.5 are tolerant and OD higher than 0.5 indicates completely/ highly tolerant isolates (Ashry et al., 2022).

3.5 Determination of isolates plant growth promotion abilities

After performing the water screening test, all of the isolates abilities to promote plant growth were measured using the following essays:

3.5.1 Phosphate solubilization potential

For quantitative phosphate solubilization analysis, the method of (Rehman et al., (2023) was followed with modifications. Pikovskaya agar (PKV) plates were prepared and streaked with isolates grown on NA in triplicates and kept in at 28 ± 1 C° for a week. Positive results were indicated by the growth of bacteria around the streaked colony (Halo-zone). The halo-zone around the bacterial colonies and the bacterial colony diameter was measured in (cm) using a ruler and the Pikovskaya formula for phosphate solubilization index (PSI) (Rehman et al., 2023) was applied:

$$PSI = \frac{(\text{Colony diameter (cm)} + \text{halo zone diameter (cm)})}{\text{colony diameter (cm)}}$$

3.5.2 Ammonia production

Ammonia production was measured qualitatively and quantitatively following the method of Kumar et al., (2021). Isolates grown on NA for 72 h were inoculated into peptone water tubes (10 mL) as triplicates and incubated at 28 ± 1 C° for 72 h in a shaking water bath at 120 rpm. Following their growth in peptone water, 1ml of Nessler reagent was applied to each of the test tubes. Positive results are indicated by the appearance of reddish brown to yellow color upon the addition of Nessler's reagent within 10-30 minutes.

3.5.3 Screening for salinity tolerant PGPRs:

For salinity tolerant PGPR screening, the method used by Soltani et al., (2023) (Soltani et al., 2023) was followed. Pure isolates were streaked onto NA plates with various concentrations of NaCl (1M, 2M, 3M) previously prepared with pH of 6.8 ± 0.2 . The plates were incubated at 28 ± 1 C° for 24- 48 hr. and the bacterial isolate's ability to grow was recorded as positive results.

3.6. PGPR induced water stress tolerance in maize.

3.6.1 Seed biopriming with isolates inoculum

The method of Ullah & Bano (2015) was followed with modifications. Bacterial strains were grown in 100mL of Nutrient broth in an orbital shaker at 28 ± 1 and 120 rpm for 24 h. After their growth, the strains were centrifuged at 5000 rpm for 40 minutes to obtain the pellet. The pellet was then re-suspended in distilled water to yield an optical density (OD) of about 1 at 600 nm as evident by the turbidity measured using distilled water as a control. Maize seeds were previously sterilized by 10% Clorox (prepared from 5-6% sodium hypochlorite) for 3 min and then 95% ethanol

for 3 minutes and finally by distilled water three times and completely air dried under the laminar flow. As shown in figure (5), sterilized seeds were placed in the inoculum solution for 2hr in an orbital shaker at 150 rpm and 28 ± 1 C to get bio primed seeds.



Figure (5): Maize seeds covered by the isolate CC2 inoculum solution.

3.6.2 Maize germination

The bio primed seeds prepared in part 3.6.1 were distributed into autoclaved plant tissue culture growth boxes filled with tissues using sterilized forceps in triplicates under the laminar flow as shown in figure (6) a. After completely drying under the laminar flow, the primed and non-primed Maize seeds were irrigated with respective 5mL of the following treatments: T non stressed (using sterilized distilled water), T-0.15 MPa, T -0.49 MPa, T -0.73 MPa, T-1.2 MPa

The seed filled boxes were then left in a dark room to germinate for a week at temperatures 22 ± 3 as shown in figure (6) b. The seeds were checked daily for seed germination.

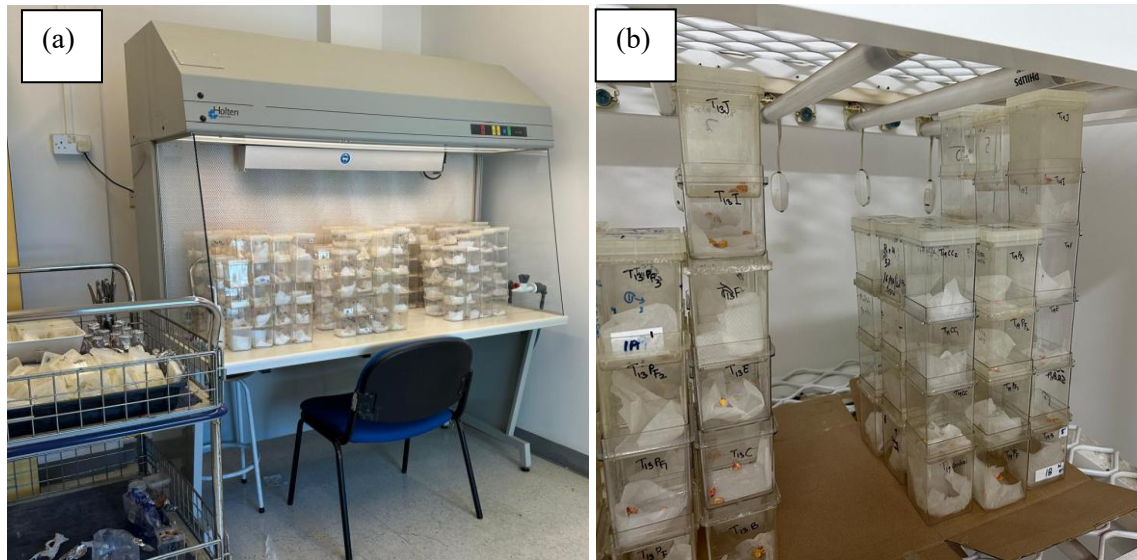


Figure (6): maize germination analysis steps. a: Placement of maize seeds (primed and unprimed) into the tissue culture growth boxes under the laminar flow. b: Tissue culture growth boxes in the dark room (bottom).

The Germination index (GI) was determined using the following equation:

$$\text{Germination index} = (10 \times n_1) + (9 \times n_2) + \dots + (1 \times n_{10}) \quad (\text{Kader, 2005})$$

While mean germination time (MGT) was calculated using the following equation:

$$\text{Mean germination time} = \text{MGT} = \frac{\sum n_i \times d_i}{N} \quad (\text{Abbasi Khalaki et al., 2019}).$$

3.6.3 Plant survival and growth under water stress

The study was conducted in the University of Bahrain green house, Sakhir from February to April 2024. Bio-primed Maize seeds were planted using sterilized forceps into plastic seedling nursery trays filled with a mixture of soil and perlite (4:1) and irrigated with tap water for 2 weeks. The trays were lined and secured with plastic bags for effective irrigation from below, and the plants were irrigated until the top layer of soil was moist. Universal neutralized potting soil (MIKSKAAR; Estonia) was used. Soil characteristics include Ash content: Max 15%, Organic matter content Min 85%, pH of 5.5-6.5, EC 0.7 mS/cm, and NaCl amount less than 2%. The soil used is also free from insects, fungal diseases and all types of bacteria, fungi, and pathogens. Following the method of Ullah & Bano (2015) (Ullah & Bano (2015), after plant growth for 2 weeks, application of the designated treatment [T non stressed, T-0.15 MPa, T-0.49 MPa, T-0.73 MPa, T-1.2 MPa] for primed and non-primed seeds commenced with triplicates for each. After one week of treatment exposure, the plants were irrigated with tap water for 30 days as indicated by figure (7). The plants were then harvested, processed and kept in the fridge for the duration of the analysis. Morphological, photosynthetic pigments and total soluble protein analyses of maize were recorded.

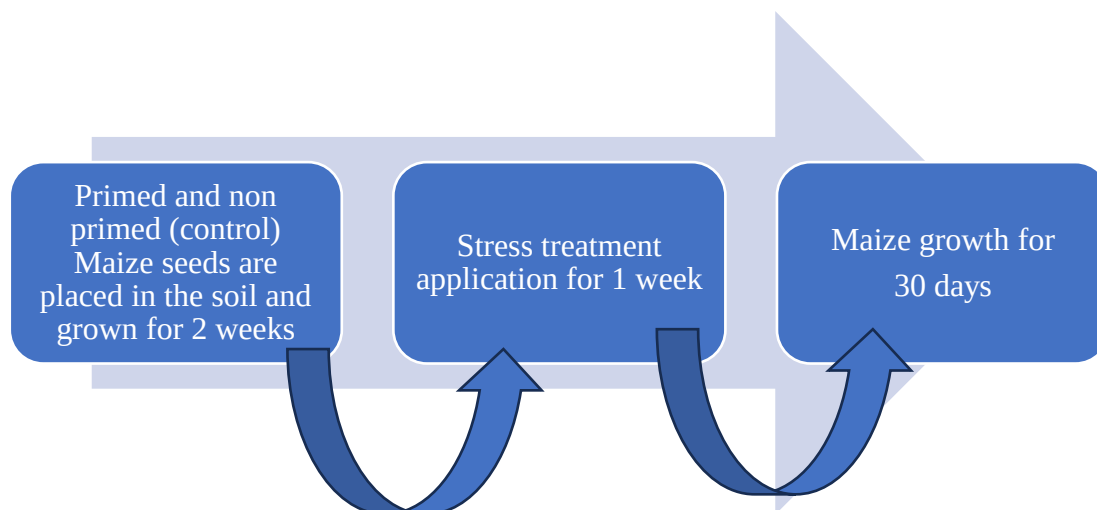


Figure (7): Time frame of plant survival and growth.

3.6.2.1 Measured Parameters

Following the method of Rehman et al., (2023) (Rehman et al., 2023) with some modifications, Maize plants' leave width, and length was recorded and the leaf area was calculated prior to harvesting. After harvesting, the leaves were kept fresh in the fridge for 3 days inside paper bags until chemical analysis was performed. The shoot and roots of maize seedlings were harvested, their lengths were measured using a ruler while their fresh weights were measured using a balance after clearing from soil particles using water and drying. Shoot and root dry weights were obtained by drying the samples in the oven at 60 C° inside paper bags for a week until a constant weight was reached.

3.6.2.2 Photosynthetic pigment determination

The estimation of photosynthetic pigments in the leaves was measured on the day of harvesting following the method of Rehman at al., (2023). Briefly, 0.1 g of fresh leaf material was ground thoroughly in 80% acetone (10 mL) using a mortar and pestle. After grinding and obtaining the extract, the absorbance was measured spectrophotometrically at wavelengths 663,

645, and 480 nm. The formulas given below were used for the estimation of photosynthetic pigments and leaf carotenoids (Rehman et al., 2023):

$$\text{Chlorophyll a} = [12.7(A_{663}) - 2.69(A_{645})] \times V/1000 \times W$$

$$\text{Chlorophyll b} = [22.9(A_{645}) - 4.68(A_{663})] \times V/1000 \times W$$

$$\text{Total Chlorophyll} = [20.2(A_{645}) - 8.02(A_{663})] \times V/1000 \times W$$

V = volume of the extract (mL)

W = weight of the fresh leaf tissue (g)

$$\text{Carotenoids} = A_{\text{car}}/E_m \times 100\% \times 100$$

$$A_{\text{Car}} (\text{carotenoid}) = (OD_{480}) + 0.114(OD_{663}) - 0.638(OD_{645})$$

E_m = Emission

3.6.2.3 Plant Soluble Protein Content

Following the method of Rehman et al., (2023). 0.5g of fresh plant material was homogenized in 10 mL of freshly prepared Potassium phosphate buffer using a mortar and pestle on ice. 500 μ L of the plant extract was then transferred to Eppendorf tubes and centrifuged at 10,000 rpm for 10 min. Following the centrifugation 100 μ L of the supernatant was added to 2mL deionized water and 0.5mL of Bradford reagent. The mixture was left for 5 minutes, and the absorbance was measured spectrophotometrically at 595 nm. To blank the spectrophotometer prior to taking measurements a blank was prepared following the same steps but without the addition of the supernatant. Total soluble proteins were estimated in mg/mL by comparison with a standard curve of bovine serum albumin (BSA) constructed using concentrations (0.05, 0.1, 0.25, 0.5, 0.75 mg/mL). The unit was then correlated to mg/g FW.

3.7 Statistical analysis

IBM SPSS statistics version 26 was used to perform statistical analysis. ANOVA was used to identify significance differences between the means of each treatment. Mean differences were deemed significant if p value < 0.05. Results that were significant in ANOVA were then tested using post hoc Tukey's analysis to find the significant differences within the treatments between the primed and non-primed maize. Values are reported as mean $\pm$ standard error (SE).

Chapter 4

Results, Discussion, and Recommendations

4.1. Isolation of rhizospheric bacteria

This study represents the first study performed on PGPRs in the kingdom of Bahrain. In total, 13 morphologically different isolates were obtained from different sources as observed in table (3). Individual letters were assigned to isolates from *Bougainvillea spp*, while PF denotes isolates from rhizospheric soil surrounding *Prosopis farcta*. Similarly, CC indicates isolates obtained from rhizospheric soil surrounding *Cressa cretica*.

Table (3): Sources of each isolates obtained and their assigned letters.

	Sample source (Plant species)	Isolate
1	<i>Bogenvilla spp</i>	B
2	<i>Bogenvilla spp</i>	C
3	<i>Bogenvilla spp</i>	E
4	<i>Bogenvilla spp</i>	F
5	<i>Bogenvilla spp</i>	J
6	<i>Bogenvilla spp</i>	I
7	<i>Prosopis farcta</i>	PF
8	<i>Prosopis farcta</i>	PF1
9	<i>Prosopis farcta</i>	PF2
10	<i>Prosopis farcta</i>	PF3
11	<i>Cressa cretica</i>	CC
12	<i>Cressa cretica</i>	CC1
13	<i>Cressa cretica</i>	CC2

4.2. Morphological and Biochemical characterization of rhizospheric bacteria

The diversity of isolates was reflected in the variation of characteristics observed in table (4). Isolate E appeared in greenish yellow color shown in figure (8), while PF1 appeared in two colors orange and white as can be seen in figure (9) a. Excluding isolates E and PF1, the rest of the isolates appeared white in color as can be observed from figure (9) b, c and d. The texture of isolates ranged from fuzzy for CC1 to rough for C and F and smooth for all of the remaining isolates as can also be observed from figure (9). Most of the colonies found were round except for F, J and CC1 which appeared irregular. Under the optical microscope, the isolates cell shape was discerned, the majority of the isolates were coccus except for J and CC2 which were short bacilli as can be seen in figure (10). Gram testing indicated that only isolate E is gram negative, while the rest were gram positive. These results have been demonstrated previously when plants face drought stress shifting the microbiota to gram positive instead of gram negative (Bogati & Walczak., 2022).

Anaerobic respiration mode was determined for the majority of isolates found (9): C, E, F, I, PF, PF1, PF2, PF3, CC2). While isolates B and CC1 show low levels of catalase production, isolates J and CC demonstrated higher levels of catalase activity by the vigorous production of bubbles. It is also important to note that the catalase test is not specific for facultative bacteria which could be the isolates being investigated. Differences in gram and catalase tests results further reflect the heterogeneity of soil bacteria which encounter different microenvironments. PGPRs can produce catalase to mitigate hydrogen peroxide produced by stressed plants (Gupta et al., 2022).



Figure (8): Isolate E grown on NA media for purification purposes at 28 ± 1 C° for 24 hr.

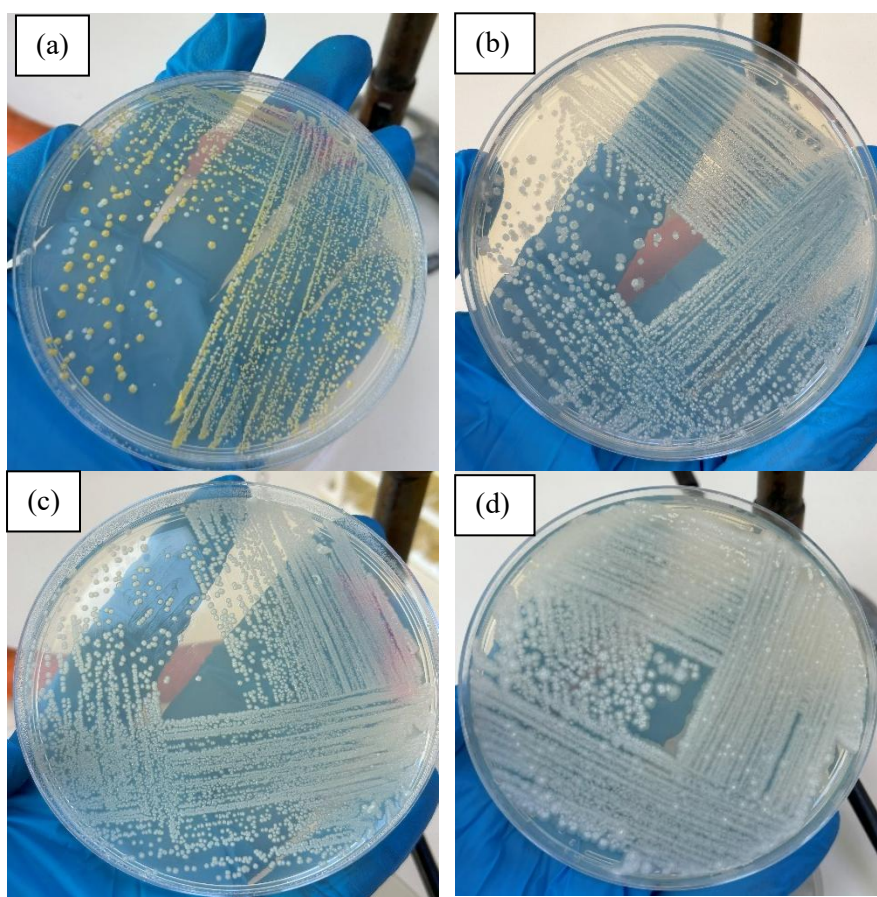


Figure (9): Morphologically different isolates streaked on NA at 28 ± 1 C° for 24 hr. a: PF3, b: J, c: CC1 and d: PF2.

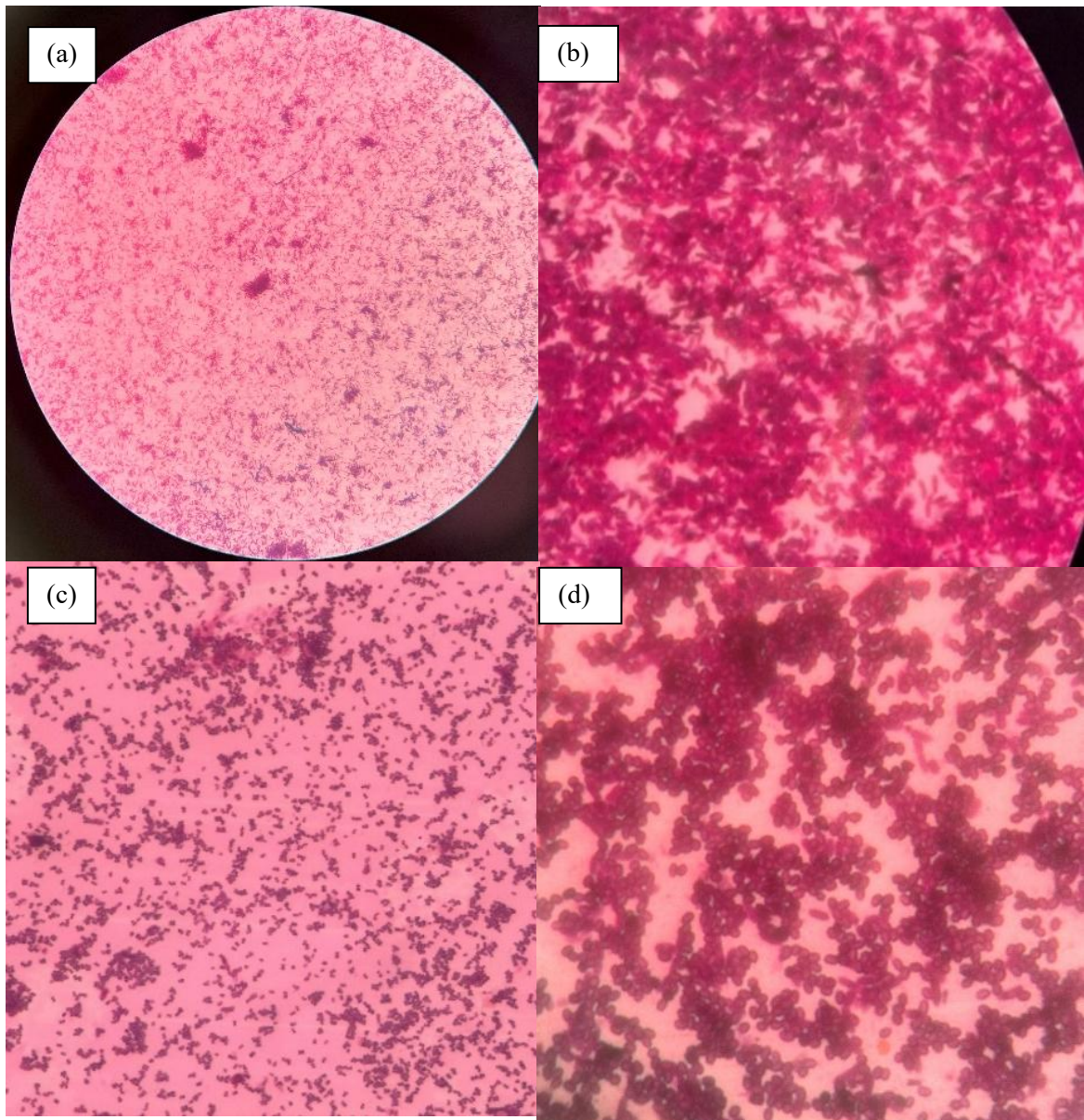


Figure (10): Gram testing results for different isolates viewed under the light microscope at 400X magnification. a:Isolate B, b: Isolate J, c: Isolate CC2 and d:Isolate I .

Table (4): Biochemical and morphological characteristics of isolates obtained from different sources.

<i>Sample source (Plant species)</i>	<i>Biochemical characterization</i>			<i>Colony characteristics</i>			
	Isolate	Gram test	Catalase test	Colony colour	Colony texture	Colony shape	Cell shape
<i>Bogenvilla sp</i>	B	(+)	(+)	White	Smooth	Round	Coccus
<i>Bogenvilla sp</i>	C	(+)	(-)	White	Rough	Round	Coccus
<i>Bogenvilla sp</i>	E	(-)	(-)	Greenish Yellow	Smooth	Round	Coccus
<i>Bogenvilla sp</i>	F	(+)	(-)	White	Rough	Irregular	Coccus
<i>Bogenvilla sp</i>	J	(+)	(++)	White	Smooth	Irregular	Short bacillus
<i>Bogenvilla sp</i>	I	(+)	(-)	White	Smooth	Round	Coccus
<i>Prosopis farcta</i>	PF	(+)	(-)	White	Smooth	Round	Coccus
<i>Prosopis farcta</i>	PF1	(+)	(-)	White	Smooth	Round	Coccus
<i>Prosopis farcta</i>	PF2	(+)	(-)	White	Smooth	Round	Coccus
<i>Prosopis farcta</i>	PF3	(+)	(-)	Orange and white	Smooth	Round	Coccus
<i>Cressa cretica</i>	CC	(+)	(++)	White	Smooth	Round	Coccus
<i>Cressa cretica</i>	CC1	(+)	(+)	White	Fuzzy	Irregular	Coccus
<i>Cressa cretica</i>	CC2	(+)	(-)	White	Smooth	Round	Short bacillus

4.3. Screening for the bacteria's ability to withstand water stress.

The addition of PEG6000 in the growth medium establishes osmotic pressure (Shirinbayan at al., 2019). And the absorbance at 600 nm reflects bacterial growth under drought stress (Shirinbayan at al., 2019). The isolates' ability to withstand water stress was varied, as observed in figure

(11). After finding significance in ANOVA analysis differences between the treatments, Tukey post hoc comparisons were performed to find the differences within the treatments for the different isolates.

Overall, tolerance decreased with the increase of water stress. PF3 was able surpass all the rest of the isolates in withstanding the increases in water stress levels significantly, except at -1.2 MPa. In contrast, isolates B and PF2 recorded the lowest values of absorbance overall. At water stress of -0.15 MPa, PF3 recorded the highest mean of absorbance (0.92 ± 0.014), followed by PF (0.418 ± 0.021), PF1 (0.369 ± 0.032), J (0.361 ± 0.021) and finally, CC2 (0.353 ± 0.033). Isolates B (0.144 ± 0.002) and E (0.200 ± 0.01) showed the lowest absorbance at -0.15 MPa.

When isolates were grown in water stress of -0.49 MPa, PF3 again surpassed the rest of the isolates in absorbance (0.581 ± 0.042). Isolate F (0.410 ± 0.026) succeeded PF3, followed by isolates J (0.323 ± 0.011) and CC1 (0.24 ± 0.040). While isolates I (0.053 ± 0.014) and C (0.0593 ± 0.003) recorded the lowest absorbances.

PF3 also surpassed all the isolates at water stress of -0.73 MPa (0.465 ± 0.013). Followed by F (0.259 ± 0.014), CC1 (0.244 ± 0.023) and CC (0.127 ± 0.018). Conversely, isolates C (0.011 ± 0.005), CC2 (0.0190 ± 0.007) and I (0.066 ± 0.009) recorded the lowest absorbances.

Finally, at water stress of -1.2 MPa, the highest tolerability was observed for isolates F (0.294 ± 0.021) and PF3 (0.291 ± 0.04) followed by CC1 (0.154 ± 0.019) and CC (0.109 ± 0.022) respectively. However, in Tukey pos hoc comparisons, the difference between isolates PF3 and F was not significant. Isolates C, CC2 and PF2 were unable to survive the high-water stress level of -1.2 MPa and showed no growth as indicated by the absorbance. Therefore, the ranking of the isolates based on water stress

tolerance ability is PF3, F and CC1. After comparing the means, ANOVA analysis results indicated significant difference ($P < 0.05$) between the isolates and the values of tolerance in each water stress level.

The cumulative results in figure (10) further infer the number of isolates which are sensitive or tolerant to water stress. Isolates B, C, E, I, CC, CC1, CC2, PF, PF1 and PF2 were highly sensitive to water stress, isolates F and J were sensitive, while only isolate PF3 appears to be completely tolerant to water stress.

Soil drought consequences on rhizospheric bacteria includes dissolution, transport, and diffusion of substrate supply reductions (Ahmad et al., 2022). As a result, bacteria residing in soil must adjust their osmotic conditions to retain water and maintain hydration by accumulating solutes (Ahmad et al., 2022).

In an investigation performed by Ashry et al., (2022), 20 different isolates were obtained from Egypt's harsh soils. Out of the 20 isolates obtained, 9 isolates were found to be highly sensitive and only three isolates were classified as highly tolerant to water stress of -1.2 MPa by having OD larger than 0.5 (Ashry et al., 2022).

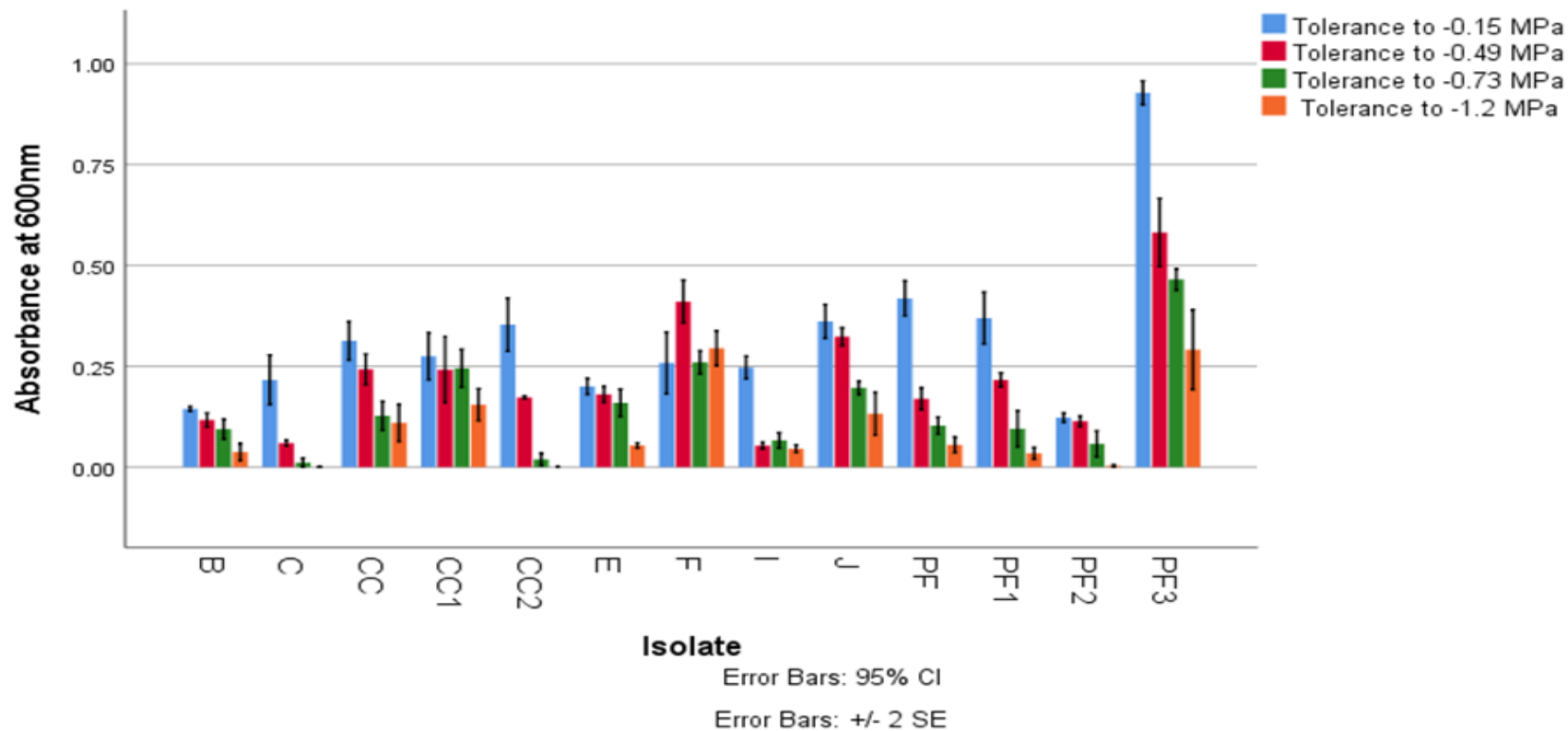


Figure (11): The absorbance and tolerance (mean $\pm$ SE) of various rhizospheric isolated grown on TSB culture with PEG6000 at different osmotic pressures (-0.15, -0.49, -0.73, -1.2 MPa).

4.4. Determination of PGPR properties

4.4.1 Phosphate solubilization

Three isolates were able to solubilize phosphate into its utilizable form as demonstrated in figure (12). Isolate J recorded the highest PSI (6.80 ± 0.668), followed closely by B (6.44 ± 0.47), CC (5.66 ± 0.38) and finally CC1 (5.16 ± 0.30). Isolates J and CC PSI formation is shown in figure (13). ANOVA analysis revealed a statistically significant difference between the means of isolates. In Tukey's post hoc analysis, isolate J was found significantly higher than isolate B only.

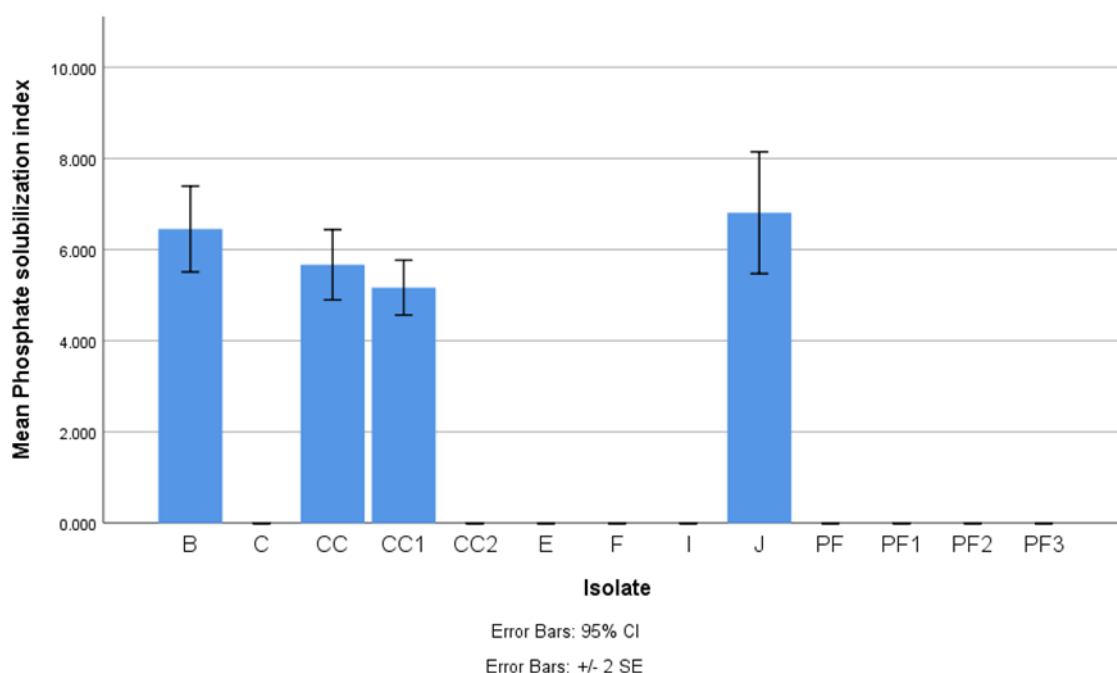


Figure (12): PSI (mean $\pm$ SE) for phosphate solubilization positive isolates. Phosphate solubilizing rhizobacteria serves many functions depending on the different environmental variables they are interacting with (Gupta et al., 2022). Soil pH and composition, temperature, salinity, and cation exchange capacity are all variables that determine phosphate solubilizing rhizobacteria functions (Gupta et al., 2022). Soils have high contents of

phosphorus; however, the available phosphorus is in insoluble phosphate (Pi , PO_4^{3-}) form, especially in alkaline soils, hindering plants absorption of phosphorus (Shirinbayan et al., 2019). Plants require phosphorus as an essential component of ATP, DNA and RNA as well as in phospholipids (Mataranyika et al., 2022). These molecules are closely involved in root elongation and photosynthesis (Mataranyika et al., 2022). PGPRs, which possess the potential to solubilize phosphate into soluble forms (HPO_4^{2-} and H_2PO_4) by producing protons and organic acids, decrease the surrounding pH and increase the bioavailability of phosphorus to plants (Mataranyika et al., 2022; Shirinbayan et al., 2019).

Bahrain is characterized by having an alkaline soil (7.9-8.1) (Sadeq et al., 2020) due to the limited rainfall and presence of calcium rich (calcareous) stones. Hence, the phosphorus in the soil is thought to be mostly unavailable for plant absorption. Therefore, the presence of these bacteria in the soil imparts great benefit to the associated plants.

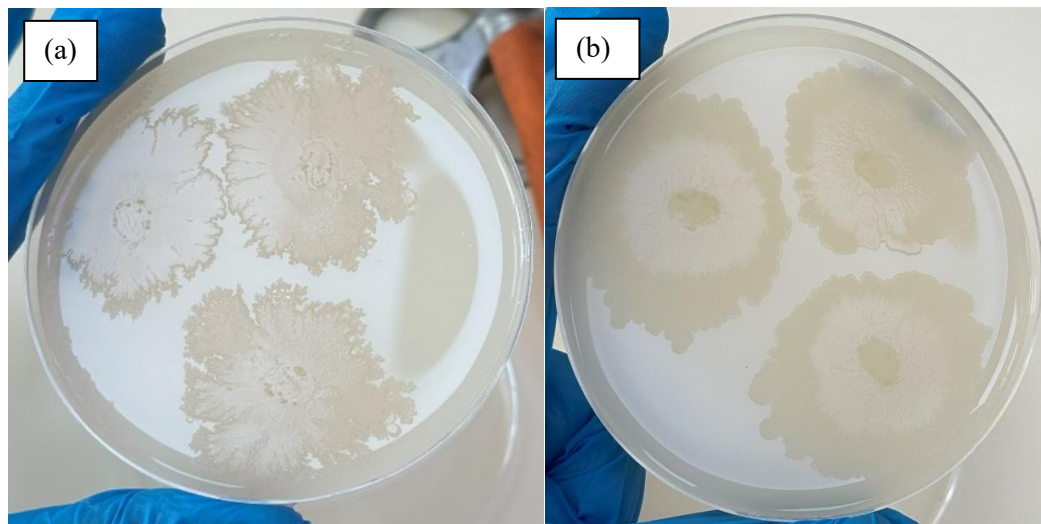


Figure (13): Phosphate solubilization halo zones of rhizospheric isolates grown on PKV agar at $28 \pm 1 \text{ C}^\circ$ for a week. a: Isolate J, b: isolate CC.

Strains of *Psuedomonas fluorescens*, *P. putida*, *Rhizobium leguminosarum*, *Enterobacter agglomerans* and *Xanthomonas maltophilia* are examples of PGPRs which have previously demonstrated phosphate solubilization potential (Mataranyika et al., 2022).

4.4.2 Ammonia production

The value in ammonia production by bacteria lies in the fact that nitrogen is an essential element required for plant growth (D Agustiyani et al., 2022). When present in excess amounts, ammonia also prevents colonization of some pathogens (D Agustiyani et al., 2022). Nessler reagent contains iodide and mercury ions which react with ammonia in alkaline conditions to produce a reddish-brown complex (Zhao et al., 2019).

Ammonium production was observed for 10 isolates in varying levels. Isolate C surpassed all the others in ammonia levels as shown in table (5), followed by isolates J, F, E, B, PF3 and CC. Isolates I, PF and PF2 were unable to produce ammonium. In figure (14), The positive color change is compared to the control.



Figure (14): Ammonia production of isolates J and CC1 compared to the control on the far left.

Table (5): Ammonia production levels and salinity tolerance for isolates investigated.

Isolate	Ammonia production	Salinity tolerance (1,2,3 M NaCl)
B	++	3M
C	+++	3M
E	++	1M
F	++	3M
J	++	3M
I	-	3M
PF	-	3M
PF1	+	3M
PF2	-	3M
PF3	++	3M
CC	++	3M
CC1	+	3M
CC2	+	3M

Positive results of ammonia production are indicated by (+) for low, (++) moderate, and (+++) for the highest color change after the addition of Nessler reagent. While (-) denotes no color change.

4.4.3. Salinity tolerance potential

Positive results of salinity tolerance were observed in varying concentrations of NaCl (1, 2, 3 M) for the isolates as mentioned in table (5). All 13 strains were able to withstand 1M NaCl. However, in 2 and 3 M concentrations of NaCl, all but isolate E were able to survive. In the study performed by Mishra et al., (2020), their investigated strain *Ochrobactrum* sp. (NBRISH6) demonstrated many PGPRs attributes such as phosphate solubilization and IAA production however, its NaCl tolerance was up to 2 M only.

From the screened isolates, isolate J demonstrated the best combined PGPR abilities reflected in PSI (6.80 ± 1.15), positive ammonia production and salinity tolerance (up to 3 M). Isolate B followed isolate J in total PGPR abilities by having PSI of (6.44 ± 0.81), positive ammonia production and salinity tolerance up to 3M. Isolate CC was observed with PSI of 5.66 ± 0.66 , positive results for ammonia production and salinity tolerance up to 3 M. Finally, isolate CC1 with a PSI of 5.16 ± 0.52 and positive ammonia production.

4.5 PGPR induced water stress tolerance in maize.

4.5.1 Maize Germination

Germination was observed for primed and non-primed seeds under different water stress levels daily as seen in figure (15) and the results were analysed. Under no water stress, the non-primed seeds had GI of 66% and MGT of 0.76 days as shown in Figure (16) and (17). In comparison, isolates E and PF3 elevated the maize seed germination to 100% GI and MGT remained the same at 0.76 days surpassing the results of non-primed seeds. In -0.15 MPa water stress, isolate E, F and I primed seeds showed similar GI (100%) to unprimed seeds but higher MGT (unprimed is 1.74 days, isolate E, F and I are 1.99 days). Water stress of -0.49 MPa caused lower GI 33% and MGT 0.14 days for non-primed maize seeds compared to isolate B, C, E, J, PF, CC and CC1 primed maize seeds (66% GI and MGT is 1.38, 0.31, 1.38, 1.13, 1.38, 1.38 and 0.76 days respectively) primed seeds. Isolate F supported maize seed germination the best with the highest values of GI (100%) and MGT 1.74 days in the same water stress.

In water stress of -0.73 MPa, non-primed maize seeds germinated with a GI of 66% and MGT of 1.38 days. Isolates PF and CC2, surpassed the non-primed with GI of 100% and MGT of 1.74 and 1.99 respectively. Most

notably, under -1.2 MPa water stress, isolate E and C appeared to support maize seed germination the best with 66 % GI and 1.38 days MGT. Similarly, Isolates J and PF3 also showed estimable results on maize seeds germination with 33% GI and MGT of 0.76 and 0.52 days respectively. Inversely, under the same water stress, non-primed maize showed no germination. It can be deduced from the aforementioned results that the isolates with the best potential of supporting maize germination are isolates E followed by C, J, PF and PF3 respectively. However, after performing ANOVA analysis, significance between the treatments was not established.

Bueno et al., (2022) studied the impact of bacterial inoculation concentration on maize growth and found that the inoculum concentration did not majorly affect the colonization and plant growth promotion results. The study showed that, the individual abilities of bacterial species were more prominently compelling than inoculum concentration for plant growth promotion (Bueno et al., 2022). Successful root colonization by PGPRs, PGPRs activity related traits consistent expression and the inherent plant differences for water stress drought further infer PGPRs ability to deter water stress disturbances to plant growth effectively (Jochum et al., 2019).

Drought is the extended water supply shortage; agricultural drought is the lack of rainfall which hinders water availability necessary for plants development and growth (Zia et al., 2021). Germination rates have been shown to decrease during water stress in maize (Ahmed et al., 2022). In addition to germination rates, seedling growth is also reduced (Mataranyika et al., 2022). This occurs simply because the seeds are not imbibed with sufficient water to germinate (Zia et al., 2021)

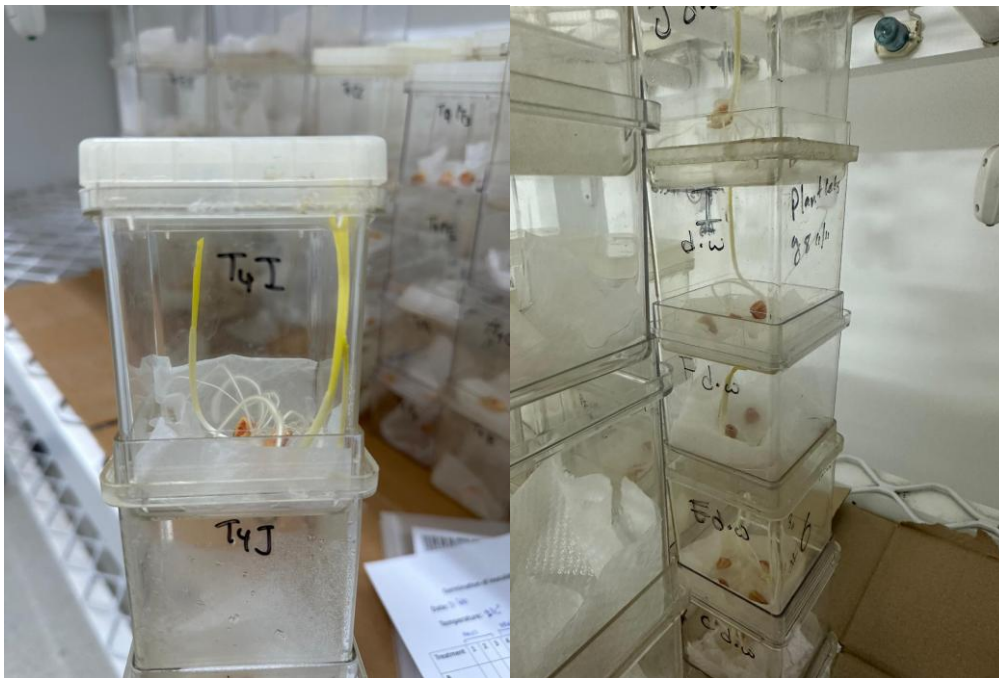


Figure (15): Germination of primed maize seeds.

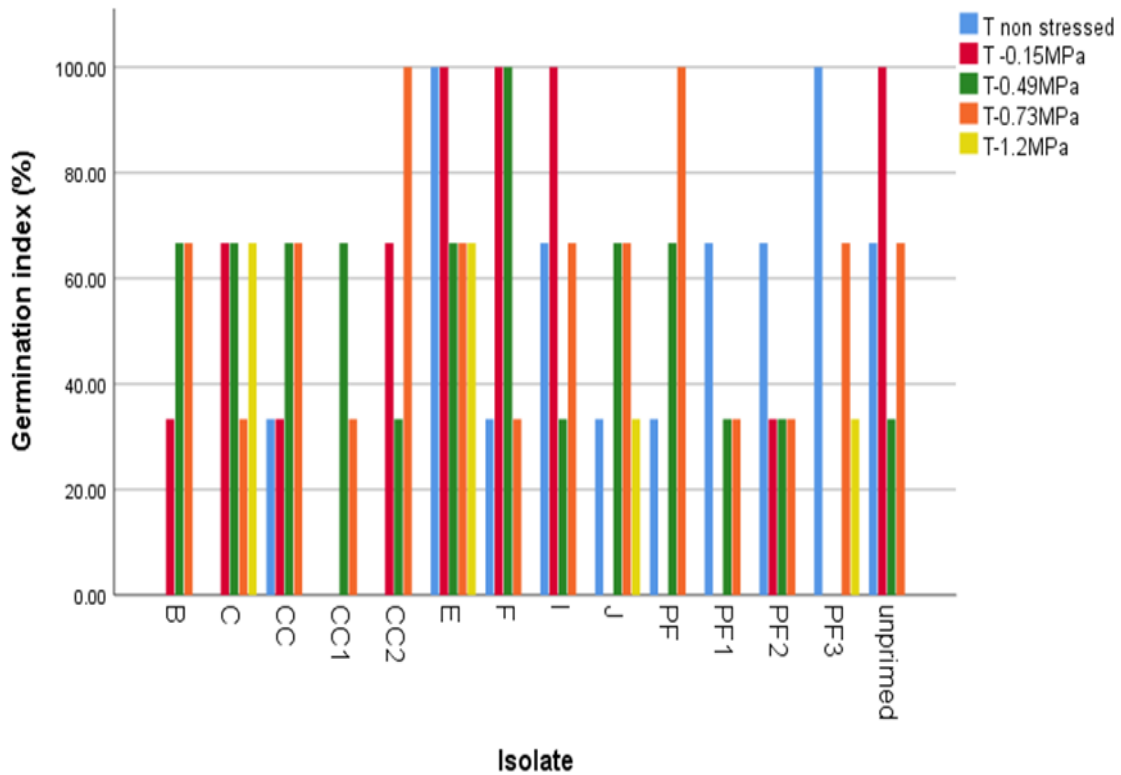


Figure (16): Germination index (%) of isolates under water stress. incubated at 21C° with no lights.

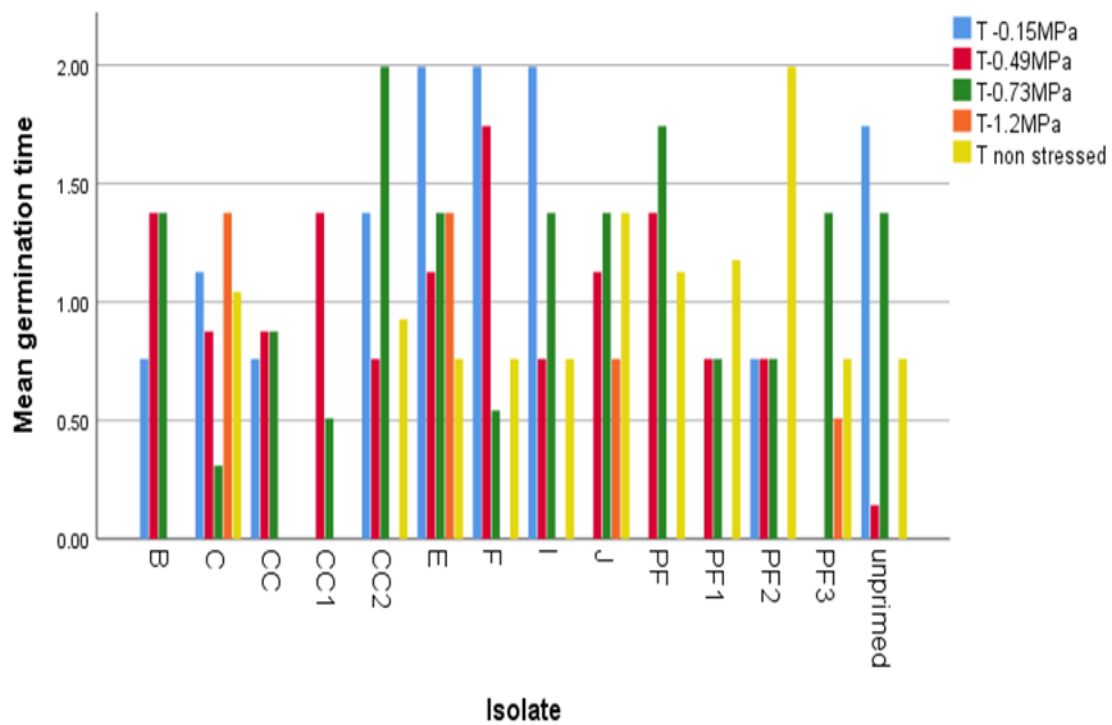


Figure (17): Mean germination time (Day) for isolates under water stress incubated at 21C° with no lights.

4.5.2 Plant survival and growth.

The response of bacteria in the plant rhizosphere to water stress depends on the stress intensity, duration, plant species and development stage (Ahmad et al., 2022). Nonetheless, when plants undergo water stress their roots, shoots and leaves experience reduced growth, leaf water potential, transpiration rates and turgor pressure due to the reduced water uptake causing damage to plant cells (Ahmed et al., 2022). In figure (18) a, maize seedlings were grown using tap water allowing the plant to develop normally, while in figure (18) b, water stress was applied causing different observable effects.

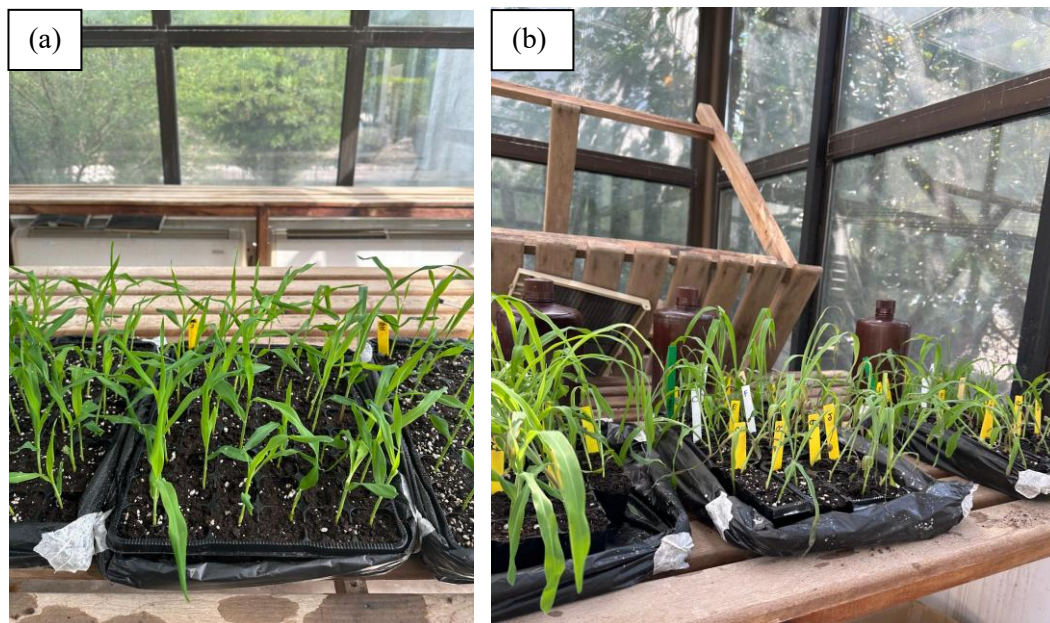


Figure (18): Maize seedlings before water stress, (a) and after water stress treatment (b).

4.5.2.1 *Morphological parameters*

Plants engage many mechanisms in response to water stress (Pepe et al., 2022). Leaf's specifically, respond promptly to environmental conditions (Pepe et al., 2022). Root and shoot growth are also halted in response to water stress (Bashir et al., 2021). However, A leaf structure will better reflect the effects of water stress than the stem or the root (Pepe et al., 2022). Drought stress decreases many plant growth parameters such as leaf area (Ahmed et al., 2022) which leads to decreased yield (Lin et al., 2019).

A. Leaf Length

When seedlings were grown without water stress, leaf length (cm) mean was highest for isolates J, PF1 and PF primed maize seedlings compared to non-primed maize seedlings length shown in figure (19) this difference was statistically significant as shown by Tukey post hoc multiple comparisons shown in table (6). Even when water stress was applied in varying levels, primed maize seedlings surpassed non primed seeds in leaf length. In water stress of -0.15 MPa, isolates CC2, PF1, and E primed maize measured longer than the non-primed maize. Similarly in -0.49 MPa, isolates E, I and CC1 primed maize were longer than the non-primed leaves. Water stress of -0.73 MPa caused the non-primed maize leaf length to be 29.76, while isolate primed maize leaf lengths were far higher, in particular, isolates I, F and CC. Although in water stress of -1.2 MPa, isolate CC2 was the only isolate with significant improvements in leaf length compared to the non-primed maize. Whilst CC primed maize leaf length was higher than the non-primed maize, this improvement was not significant as shown by the Tukey test.

Table (6): Leaf length of significantly different isolates primed maize compared to the non-primed maize.

Treatment	Non-primed maize leaf length mean $\pm$ SE	Isolate primed maize leaf length mean $\pm$ SE	P value
No water stress	57.67 $\pm$ 0.33	J (65.0 $\pm$ 0.57) PF1 (65 $\pm$ 0.00) PF9 (63 $\pm$ 0.88)	0.00 0.00 0.00
-0.15MPa	33.33 $\pm$ 0.33	CC2(54.33 $\pm$ 0.667) PF1(53.67 $\pm$ 0.88) E (49.67 $\pm$ 0.88)	0.00 0.00 0.00
-0.49MPa	39.0 $\pm$ 0.57	E (51.67 $\pm$ 0.88) I (47.67 $\pm$ 0.33) CC1 (47 $\pm$ 0.57)	0.00 0.00 0.00
-0.73 MPa	29.76 $\pm$ 0.34	I (49.33 $\pm$ 1.20) F (38.33 $\pm$ 0.66) CC (36 $\pm$ 0.57)	0.00 0.00 0.00
-1.2MPa	31.67 $\pm$ 0.33	CC2(36.67 $\pm$ 0.67)	0.00

Figure (19): Leaf length of maize bio primed seedlings at different water stress levels in cm.

B. Leaf Area

The Leaf area (m²) mean presented in figure (20) under no water stress was found to be the highest and statistically significant for maize primed with isolate CC followed by PF1, PF and CC2 compared to the much lower non-primed maize leaf area. When exposed to -0.15 MPa stress, the highest leaf area was found for isolate CC2 followed by PF1 and E. This difference was statistically significant compared to the non-primed maize leaf area as shown in table(7). Similarly for -0.49 MPa, isolate E primed maize had significantly larger leaf area followed by CC2, and I compared to the much lower non-primed seedlings area. Isolate I primed maize scored the highest significant leaf area at water stress of -0.73 MPa, followed by isolate CC2 and F, compared to the non-primed maize which had much lower mean of area. Water stress of -1.2 MPa showed the largest and significant leaf area

for maize primed with isolate CC compared to the non-primed maize. Similarly, in a study performed by Lin et al., 2019, when maize seedlings were supplemented with inorganic fertilizers and irrigated every 12 days, PGPR primed maize demonstrated increased leaf area ($1141 \pm 43.0 \text{ cm}^2$) compared to non-primed maize ($986 \pm 18.9 \text{ cm}^2$).

The leaf length and area were higher for primed maize seedlings than non-primed maize seedlings under no water stress. Moreover, even when water stress was administered, primed maize seedlings again surpassed the non-primed. In this study, plants primed with isolates CC2 followed by CC and lastly E showed the best leaf parameters out of the rest of the isolates.

Table (7): Leaf area of significantly different isolates primed maize compared to the non-primed maize.

Treatment	Non-primed Maize leaf area (m²) mean $\pm$SE	Isolate primed maize leaf area (m²) mean $\pm$SE	P value
No water stress	115.33 $\pm$ 0.66	CC (182 $\pm$ 2.00) PF1 (162 $\pm$ 0.00) PF (158.33 $\pm$ 2.20)	0.000 0.000 0.000
-0.15MPa	(33.33 $\pm$ 0.57)	CC2 (108.66 $\pm$ 1.33) PF1 (107.33 $\pm$ 1.76) E (99.33 $\pm$ 1.76)	0.000 0.000 0.000
-0.49MPa	(58.50 $\pm$ 1.50)	E (103.33 $\pm$ 1.76) CC2 (100 $\pm$ 2.30) I (95.33 $\pm$ 0.66)	0.000 0.000 0.000
-0.73 MPa	(29.66 $\pm$ 0.57).	I (74 $\pm$ 1.80) CC2 (67.33 $\pm$ 0.67) F (57.50 $\pm$ 1.00)	0.000 0.000 0.000
-1.2MPa	31.667 $\pm$ 2.08	CC (50.50 $\pm$ 0.50)	0.000

Water stress exerts many morphological effects on plants. Reductions in the leaf relative water content due to water stress affect the mass flow of water from the soil to the root xylem (Hafez et al., 2021). This reduction

will occur due to the change in differences of water potential between the soil and plant cell sap to a lower soil osmotic pressure (Hafez et al., 2021). Following this change is the eminent reduction in water adsorption by the roots which will directly lower the relative water content and turgor pressure of leaf cells (Hafez et al., 2021). Plants will therefore possess a limited flow of nutrients, and a limited mass flow of water-soluble nutrients needed for growth such as calcium, magnesium, silicon, sulfate, and nitrate (Mataranyika et al., 2022; Lin et al., 2020). Moreover, Plants will experience reductions in the leaf area, stem diameter, plant height and eventually yield when water is deficient (Lin et al., 2020).

Plants which encounter drought stress will also have a reduced yield due to several water stress effects which interfere with each other (Bazragar et al., 2023). Due to the reduced leaf area, reduction in the amount of photosynthetically active radiation (PAR) absorbed will occur followed by reductions in dry mass accumulated (Bazragar et al., 2023). The decrease in CO₂ exchange is another important aspect which will influence the yield. As the exchange in CO₂ per unit of absorbed light is reduced inevitably during water stress (Bazragar et al., 2023).

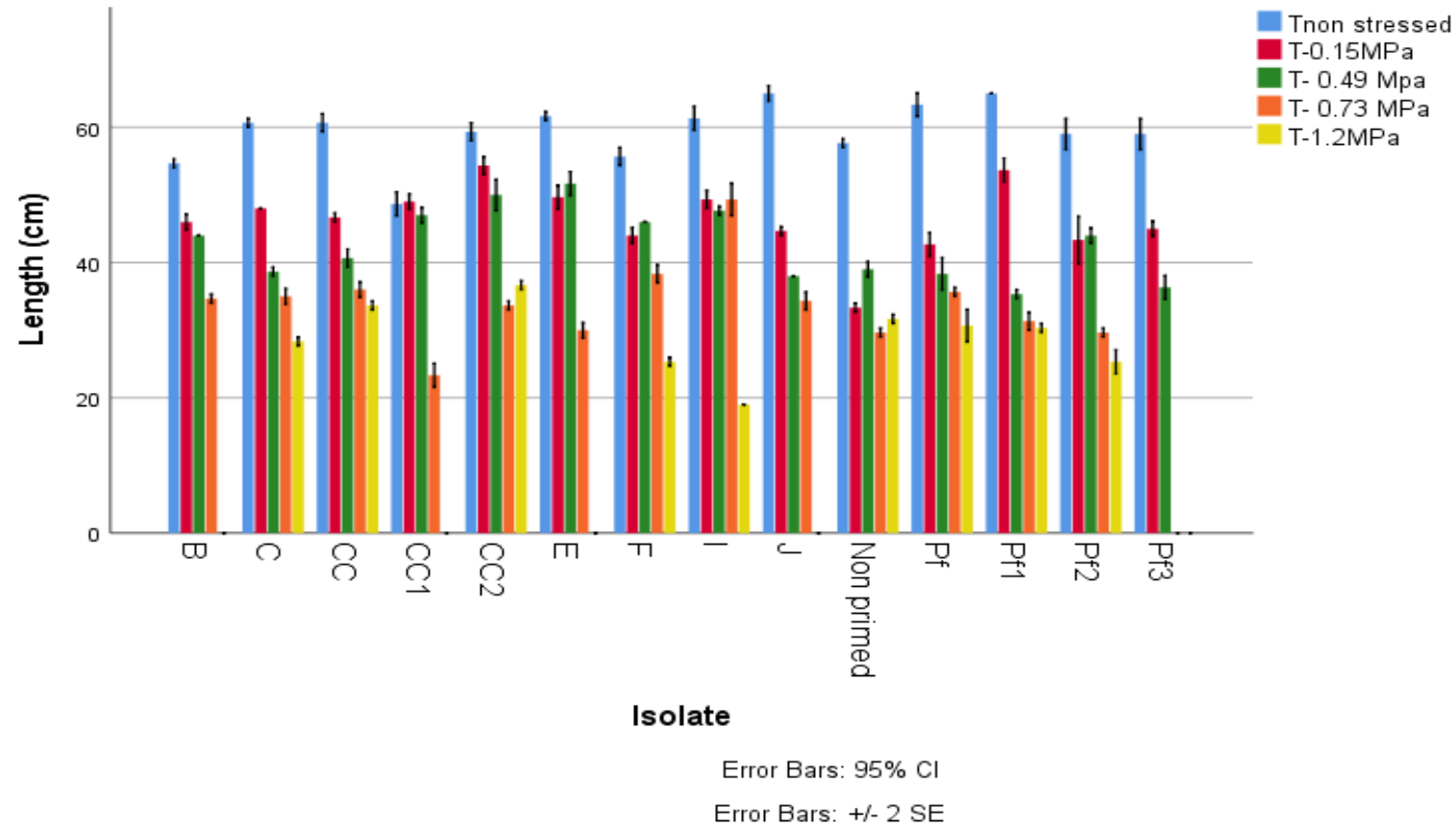


Figure (19): Leaf length of maize bio primed seedlings at different water stress levels in cm (mean $\pm$ SE).

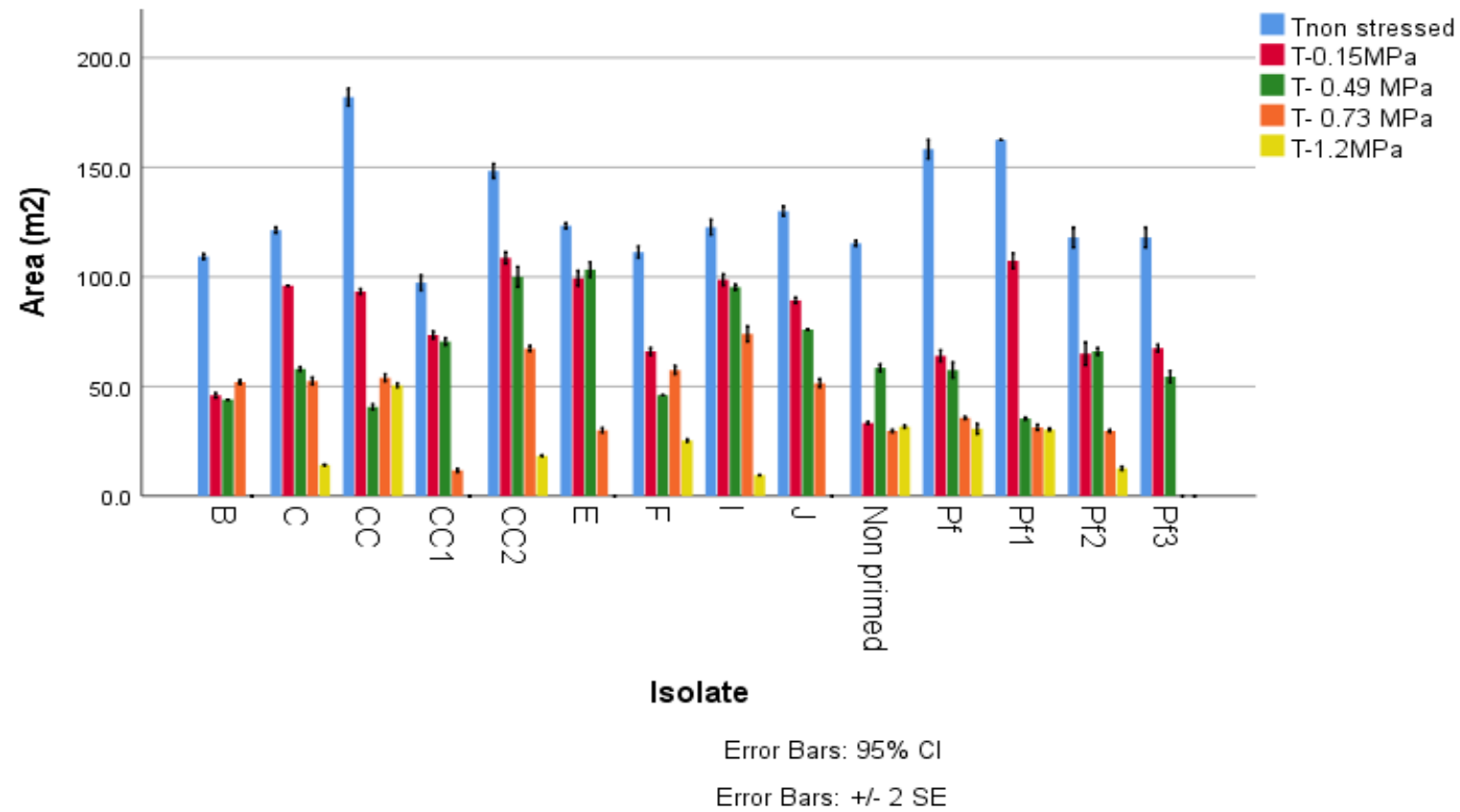


Figure (20): Leaf area of maize bio primed seedlings at different water stress levels in m²

A. Root Length

As seen in figure (23) and in table (8), without the application of water stress (T non stressed) root length mean (cm) was observed to be the significantly higher for primed maize seedlings such as those primed with isolate CC, PF2 and F. When water stress of -0.15 MPa was applied, maize seedlings primed with isolates B, J, PF1, I, CC, F but were not statistically significant, however isolates CC2 and C were significantly longer root length when compared to the non-primed maize. At water stress of -0.49 MPa, the only isolate with significant increases was CC2. At water stress of -0.73 MPa, E, I and CC1 primed maize seedlings had significantly longer roots compared to the non-primed maize seedlings. In the highest water stress applied (-1.2 MPa), the longest and most significant root length compared to non-primed maize was observed for CC primed maize seedlings.

Table (8): Root length of significantly different isolates primed maize compared to the non-primed maize.

Treatment	Non-primed maize root length (cm) mean $\pm$ SE	Isolate primed maize root length (cm) mean $\pm$ SE	P value
No water stress	57.67 $\pm$ 0.33	CC (60.67 $\pm$ 0.67) PF2 (59.00 $\pm$ 1.15) F (55.67 $\pm$ 0.66)	0.000 0.000 0.000
-0.15MPa	33.33 $\pm$ 0.33	CC2 (54.33 $\pm$ 0.66) C (48.00 $\pm$ 0.000)	0.020 0.030
-0.49MPa	39 $\pm$ 0.60	CC2 (50.00 $\pm$ 1.15)	0.010
-0.73 MPa	29.67 $\pm$ 0.32	E (30.00 $\pm$ 0.60) I (49.33 $\pm$ 1.20) CC1 (23.33 $\pm$ 0.88)	0.001 0.014 0.000
-1.2MPa	31.67 $\pm$ 0.33	CC (33.67 $\pm$ 0.34)	0.001

Root parameters analysis allows for better understanding of the plant responses as roots are the first line of defense against abiotic stresses (Gull & Wani, 2019). For if the soil surrounding the plant is healthy and biologically diverse, its chances of survival are elevated (Gull & Wani, 2019). Root morphology is altered significantly by plants to manage water stress as long and extensive root architecture permits better uptake of water from the surrounding soil (Ahmad et al., 2022). Depending on the soil chemistry, changes in the abiotic factors, and plant phenotype and genotype, the occurrence of soil microbes is varied (Ahmad et al., 2022). Additionally, bacterial communities demonstrate different patterns at specific root depths (Ahmad et al., 2022). Plant productivity and health is directly affected by the root length, surface area and biomass as improved nutrient and water uptake occurs when roots are better developed (Ahmad et al., 2022). Increased root length translates into larger surface area for water absorption which is an advantage to plants facing water stress (Mataranyika et al., 2022).

In alignment with our study, Mishra et al., (2020) also observed water stress root length increases in PGPR *Ochrobactrum sp.* primed maize (50 cm) compared to water stressed control (39 cm). Lin et al., (2019) found that the root length significantly increased after PGPR inoculation (6473 ± 379 cm) after irrigation every 12 days compared to non-primed (5883 ± 150 cm) even when inorganic fertilizers were added due to PGPRs phytohormones production that modifies root system growth. Calvo et al. (2017) also reported similar results of improved root length of PGPR inoculated maize (1004.1 cm) compared to the control (682.1 cm) which was linked to hormone production aided by the additional improvements in plant mineral availability. Furthermore, a study performed by Jochum et al., (2019) also found improvements in maize root length by *Bacillus sp.*

and *Enterobacter sp.* to 323.94 and 370.16 cm respectively compared to the control root length of 271.31 cm under water stress. Jochum et al., (2019) correlated the increase in root length to the strains ability to produce IAA. Other strains that produce IAA to support root length growth include *Azospirillum brasiliense* and *Pseudomonas putida* (Mataranyika et al., 2022).

B. Root Fresh Weight (FW)

To affirm the significance of PGPRs on maize root fresh weights, comparisons were made between them and non-primed maize using Tukey post hoc multiple comparisons as seen in table (9). Root fresh weight (g) which was presented in figure (21) without water stress application was found highest for maize seedlings primed with isolates CC, F and CC2. When water stress of -0.15 MPa was applied, maize seedlings primed with isolates E, CC and CC2 were higher compared to the non-primed maize seedling root weight. After increasing the water stress to -0.49 MPa, maize seedlings primed with isolates J and CC2 were the highest amongst all the tested maize including the non-primed. Water stress of -0.73 MPa observed the highest root weight for maize seedlings primed with CC. while isolate CC2 and C were also higher than the non-primed maize, their differences were found not significant. At -1.2 MPa, PF primed maize seedlings showed higher growth (0.69 ± 0.8 g) however this was not found to be significant.

Table (9): Fresh weight of primed maize roots which are significantly higher than the non-primed maize.

Treatment	Non-primed maize root fresh weight (g) mean $\pm$ SE	Isolate primed maize root fresh weight (g) mean $\pm$ SE)	P value
No water stress	0.95 $\pm$ 0.12	CC (3.25 $\pm$ 0.25 g), F (3.07 $\pm$ 0.32 g) CC2 (2.30 $\pm$ 0.20 g).	0.000 0.000 0.001
-0.15MPa	0.71 $\pm$ 0.03	E (1.87 $\pm$ 0.15) CC (1.87 $\pm$ 0.18 g) CC2 (1.79 $\pm$ 0.21)	0.000 0.000 0.000
-0.49MPa	0.80 $\pm$ 0.07 g	J (1.43 $\pm$ 0.11) CC2 (1.35 $\pm$ 0.12 g)	0.001 0.005
-0.73 MPa	0.57 $\pm$ 0.11	CC (0.93 $\pm$ 0.12)	0.013
-1.2MPa	0.67 $\pm$ 0.00		

C. Root Dry Weight (DW)

Root dry weight (g) shown in figure (22) under no water stress treatment was the higher in isolate primed maize seedlings. To confirm the significance of these results, Tukey post hoc comparisons were performed as seen in table (10). Maize primed with isolates CC2, CC and PF were higher than non-primed maize seedlings without water stress. This pattern was recurrent for water stress of -0.15 MPa, where maize primed with isolate CC, CC1 and CC2 were significantly heavier than the non-primed maize. At water stress of -0.49 MPa, maize primed with isolates CC, J and CC2 were higher than the non-primed maize. At water stress of -0.73 MPa, the highest dry root weight was observed for maize primed with CC. Finally, water stress of -1.2 MPa revealed the maximum dry root weight for maize primed with isolates F (0.095 $\pm$ 0.016), PF (0.091 $\pm$ 0.004 g).

Table (10): Dry weights of maize roots which are significantly higher than the non-primed maize (g).

Treatment	Non-primed maize dry weight (g) mean $\pm$ SE	Isolate primed maize dry weight (g) mean $\pm$ SE	P value
No water stress	0.067 $\pm$ 0.004	CC2 (0.496 $\pm$ 0.004)	0.000
		CC (0.357 $\pm$ 0.003)	0.000
		PF (0.312 $\pm$ 0.005)	0.000
-0.15MPa	0.052 $\pm$ 0.001	CC (0.352 $\pm$ 0.004)	0.000
		CC1 (0.143 $\pm$ 0.002)	0.000
		CC2 (0.138 $\pm$ 0.004)	0.000
-0.49MPa	0.0517 $\pm$ 0.002	CC (0.165 $\pm$ 0.002)	0.000
		J (0.125 $\pm$ 0.002)	0.000
		CC2 (0.105 $\pm$ 0.011)	0.000
-0.73 MPa	0.041 $\pm$ 0.004 g.	CC (0.09 $\pm$ 0.002)	0.000
-1.2MPa	0.049 $\pm$ 0.001 g	F (0.095 $\pm$ 0.016)	0.000
		PF (0.091 $\pm$ 0.004)	0.000

In agreement with our results, Mishra et al., (2020) also observed increases in root fresh and dry weights (14.44 and 1.53 g) of maize under water stress compared to water stressed control (8.86 g and 0.93 g respectively) due to PGPR priming using *Ochrobactrum sp.*, these effects were also attributed to phytohormone production by PGPRs. Plant hormones allow better adsorption of nutrients and water from the soil by the roots (Lin et al., 2020). However, in a study performed by Calvo et al. (2017) root dry weight of PGPR inoculated maize (0.060 g) was similar to the control (0.068 cm).

Bacteria have the ability to change the root cell membrane elasticity during water stress, significantly enhancing the plant tolerance (Ahmad et al., 2022). Additionally, plants support root growth over shoot growth during water stress by assigning more nutrients to it (Mishra et al., 2020). Root

growth is favoured during water stress and for swift osmotic adjustment and partial turgor pressure recovery (Ahmed et al., 2022). This will allow for the restoration of water uptake osmotic gradients (Ahmed et al., 2022) and curb the deadly effects of cell turgidity (Mishra et al., 2020). Additionally, as the root surface area increases, nutrient and water uptake are enhanced leading to better plant growth (Gao et al., 2019).

Moreover, PGPB can reduce the effects of drought-induced ethylene accumulation which obstructs root elongation through the production of amino cyclopropane -1-carboxylate deaminase (ACC) (Mataranyika et al., 2022; Zia et al., 2021). *Arthrobacter*, *Bacillus*, and *Microbacterium* are all examples of bacteria that produce ACC deaminase during water stress (Mataranyika et al., 2022)

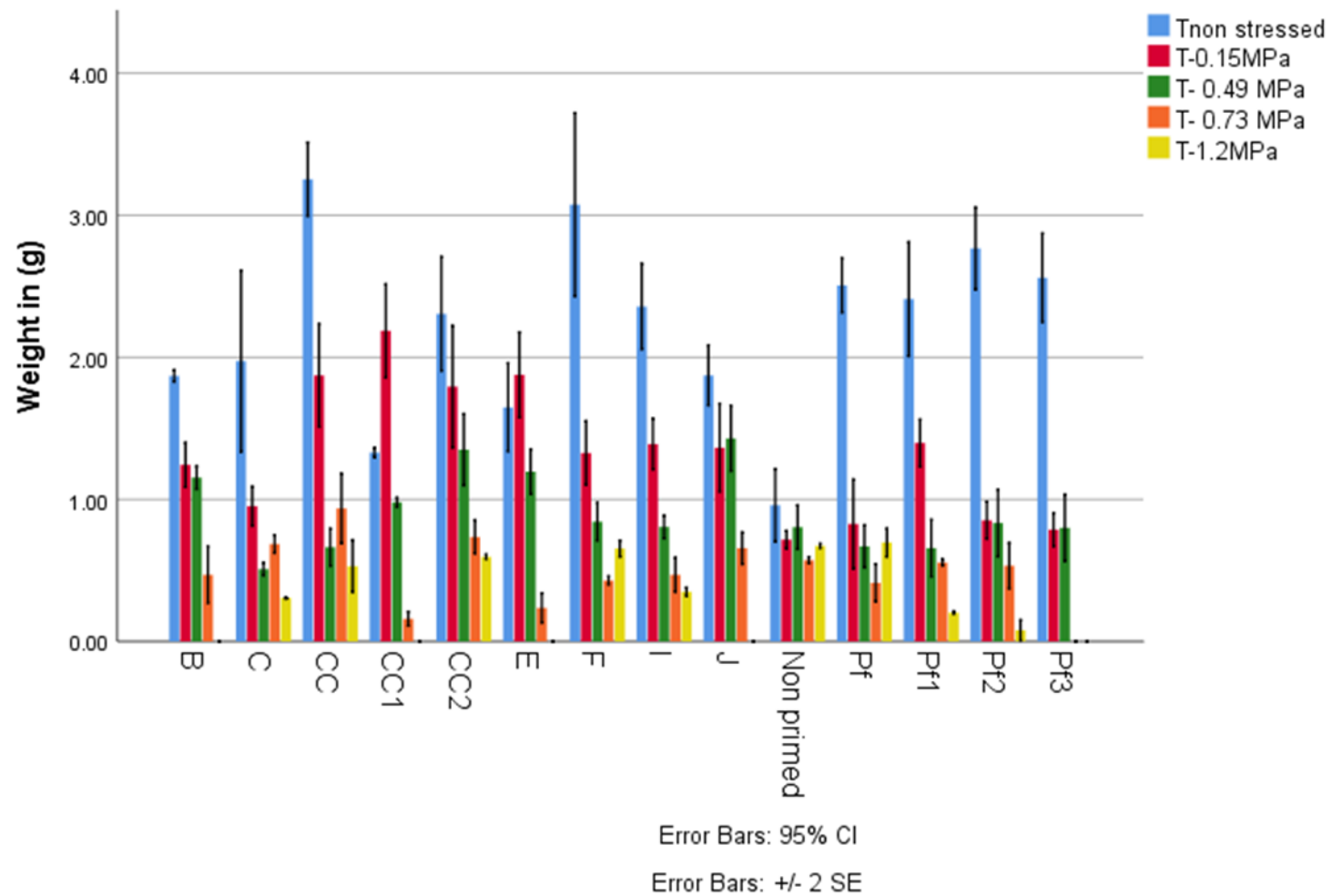


Figure (21): Root fresh weight (FW) of maize bio primed seedlings at different water stress levels in g.

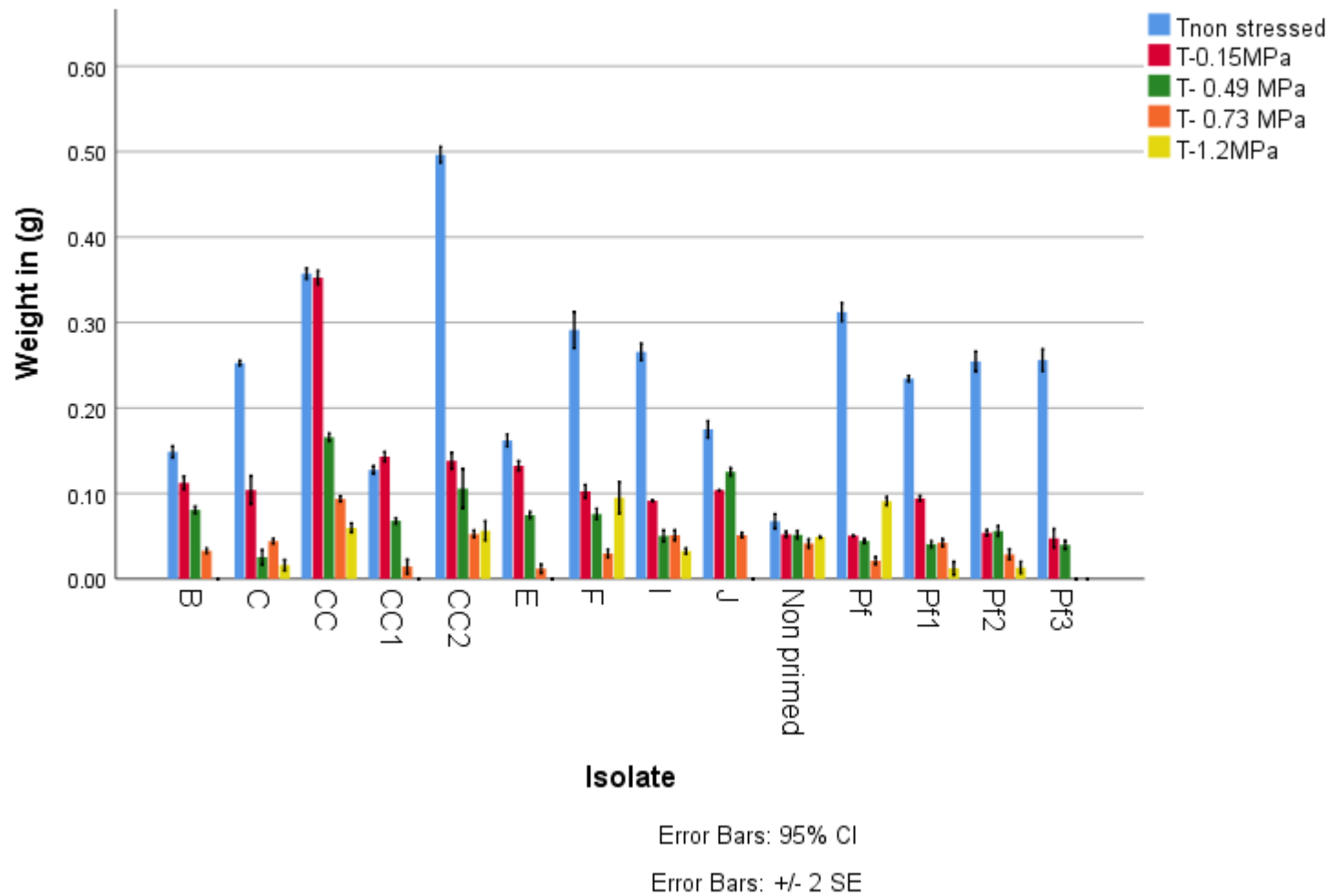


Figure (22): Root dry weight (DW) of maize bio primed seedlings at different water stress levels in g.

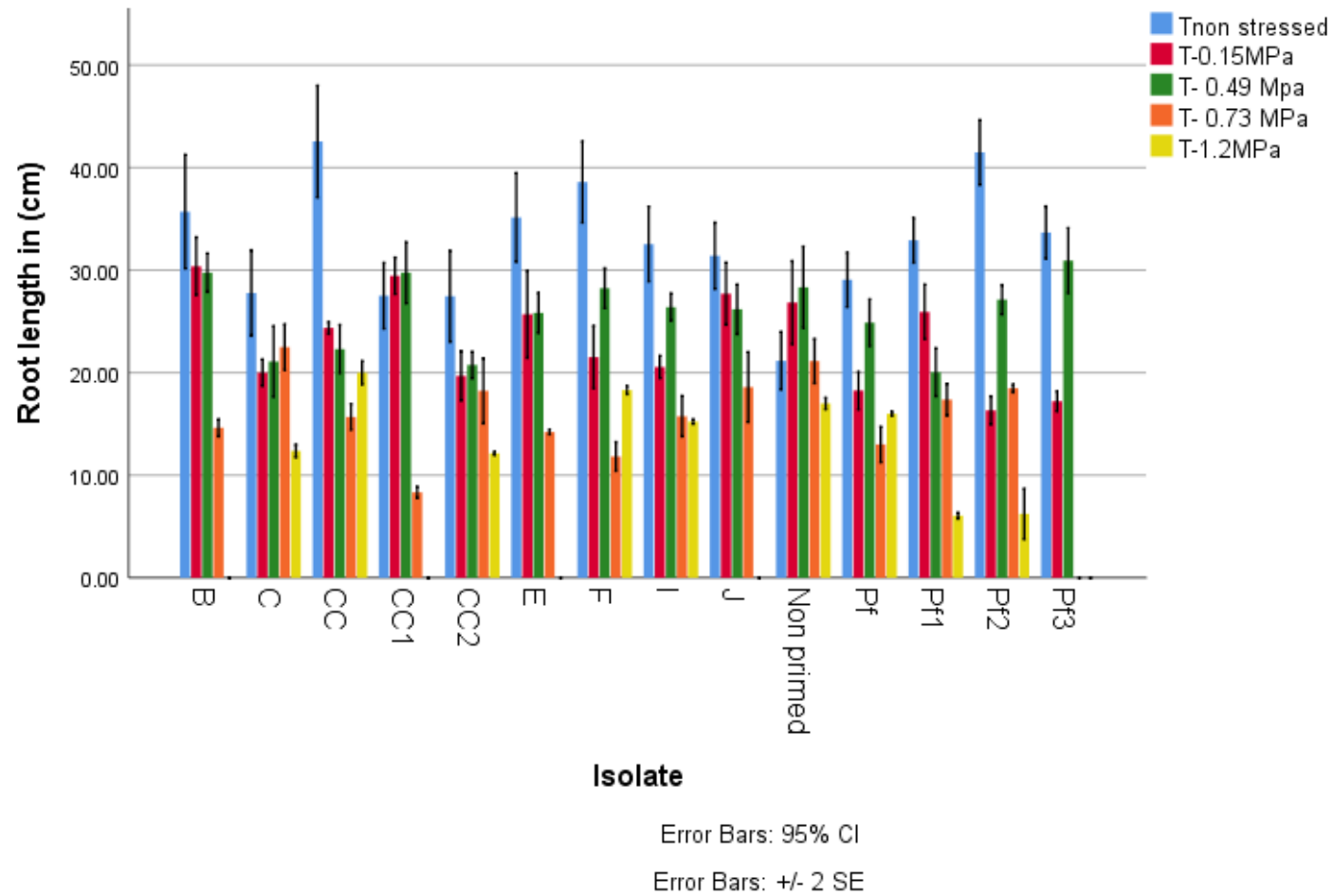


Figure (23): Root length of maize bio primed seedlings at different water stress levels in c

D. Shoot Length

Shoot length (cm) displayed in figure (24) without imposed water stress was found to be significantly higher for Maize seedlings primed with isolate CC, I and PF as can be seen in table (11). When water stress of -0.15 MPa was administered, non-primed maize shoot length was only 14.43 ± 1.7 cm compared to the higher maize shoot length for isolates E (20.53 ± 2.05 cm), F (19.0 ± 0.52 cm) and CC2 (18.76 ± 0.84 cm). At -0.49 MPa water stress, non-primed maize shoot length mean was found to be 12.33 ± 1.91 cm which is lower than that for maize primed with isolate B (18.633 cm). Again in -0.73 MPa water stress, non-primed shoot length (11.34 ± 0.96 cm) was lower than that of maize primed with isolates B (15.34 ± 1.62 cm) and I. At the highest water stress of -1.2 MPa, non-primed maize shoot length mean was lower than that for maize primed with isolates F and PF, however this difference was not found significant in Tukey post hoc multiple comparisons.

Table (11): Maize shoot length for isolates exhibiting significantly higher results compared to the non-primed maize.

Treatment	Non-primed maize shoot length (cm) mean $\pm$ SE	Isolate primed maize shoot length (cm) mean $\pm$ SE	P value
No water stress	15.033 ± 1.00	CC (28.5 ± 1.32) I (26.00 ± 1.15) PF1 (25.88 ± 1.25)	0.000 0.000 0.000
-0.15MPa	14.43 ± 0.98	E (20.53 ± 1.18) F (19.0 ± 0.30) CC2 (18.76 ± 0.48)	0.001 0.017 0.027
-0.49MPa	12.33 ± 1.10	E (18.03 ± 0.56)	0.001
-0.73 MPa	11.34 ± 0.55	B (15.34 ± 0.93) I (14.93 ± 0.56)	0.011 0.031
-1.2MPa	12.2 ± 0.15		

Low plant growth and overall size is to be expected when plants face water stress (Mataranyika et al., 2022). accordingly, the study by (Lin et al., 2020) showed an increase in maize height for PGPRs (78.8 ± 2.93 cm) compared to control (77.6 ± 2.18 cm) in imposed water stress. Stem diameter increase was used as an indicator of PGPR strengthening effects on plant stems facing drought stress (Lin et al., 2020). Overall, Drought stress triggers plants to prioritize root growth rather than shoot growth, extreme drought causes plants to halt both processes (Ahmad et al., 2022).

E. Shoot Fresh Weight (FW)

In the present study, shoot fresh weights (g) seen in figure (24) without water stress application were highest for maize primed with isolates CC, CC2 and PF1, compared to the lower non primed shoot weight significantly as can be seen in table (12) below.. At water stress of -0.15 MPa, shoot weight was highest for isolates E and CC1 compared to their non-primed counterparts. At water stress of -0.49 MPa, the primed shoot weight for isolates CC2 , E and I were again higher than their non primed counterparts. However, in water stress of -0.49MPa, only CC2 was close to being significantly different than the non-primed maize ($p=0.055$). At water stress of 0.73MPa, primed shoots of isolate I were higher than the non-primed. Finally, water stress of -1.2 MPa revealed higher weights for maize primed with isolates CC, F and PF Although these differences were not statistically significant in Tukey post hoc comparisons. Plant's fresh weight is often reduced by water stress due to the reduced influx of water and nutrients (Mataranyika et al., 2022). However, PGPRs can increase the biomass by increasing photosynthesis (Zia et al., 2021).

Table (12): Maize shoot fresh weights for significantly higher isolates primed maize compared to the non-primed maize.

Treatment	Non-primed maize shoots fresh weights (g) mean $\pm$ SE	Isolate primed maize shoots fresh weights (g) mean $\pm$ SE	P value
No water stress	0.98 $\pm$ 0.04	CC (5.39 $\pm$ 0.21) CC2(4.06 $\pm$ 0.12) PF (3.60 $\pm$ 0.28)	0.000 0.000 0.000
-0.15MPa	0.63 $\pm$ 0.08	E (1.73 $\pm$ 0.07) CC1(1.40 $\pm$ 0.13)	0.000 0.001
-0.49MPa	0.723 $\pm$ 0.10	CC2(1.23 $\pm$ 0.12)	0.055
-0.73 MPa	0.44 $\pm$ 0.01	I (0.86 $\pm$ 0.13)	0.004
-1.2MPa	0.43 $\pm$ 0.00		

F. Shoot Dry Weight (DW)

As presented in figure (26), shoot dry weights (g) without water stress were higher for maize primed with isolates CC, PF and PF1 compared to the non-primed maize. This difference was significant as shown in table (13) below. Similarly, when water stress of -0.15 MPa was administered, maize primed with isolate E, CC2 and F were higher. At water stress of -0.49 MPa, non-primed maize shoot dry weight was lower than that found in primed maize. Maize primed with isolates J and CC2 had the highest weights after drying. Water stress of -0.73 MPa, fresh weight was higher for maize primed with isolates PF2, I and CC although these differences were not considered significant in Tukey post hoc multiple comparisons. In the highest water stress of -1.2 MPa, maize seedlings primed with isolate F had a higher weight than the non-primed seedlings.

Table (13): Maize shoot dry weights of significantly higher isolates compared to their non-primed counterparts.

Treatment	Non-primed Maize shoot dry weight (g) mean $\pm$ SE	Isolate primed maize shoot dry weight (g) mean $\pm$ SE	P value
No water stress	0.059 $\pm$ 0.001	CC (0.50 $\pm$ 0.005)	0.000
		PF (0.45 $\pm$ 0.011)	0.000
		PF1 (0.39 $\pm$ 0.009)	0.000
-0.15MPa	0.041 $\pm$ 0.002	E (0.14 $\pm$ 0.003)	0.000
		CC2 (0.12 $\pm$ 0.006)	0.000
		F (0.11 $\pm$ 0.012)	0.000
-0.49MPa	0.0417 $\pm$ 0.001	J (0.11 $\pm$ 0.012)	0.000
		CC2(0.077 $\pm$ 0.001)	0.006
-0.73 MPa	0.022 $\pm$ 0.002		
-1.2MPa	0.0347 $\pm$ 0.001	F (0.10 $\pm$ 0.007)	0.000

While in the study by (Lin et al., 2020) no significantly different results of increased biomass (shoot and root) due to PGPR priming were documented. A general trend of biomass increase was observed for PGPR primed plants (Lin et al., 2020). Similar results were documented by Mishra et al., (2020) which found increases in maize shoot length (94cm) compared to control (62cm), fresh and dry weights (18.94 and 2.97 g) compared to the water stressed control (14 and 1.13 cm) under water stress due to PGPR priming using *Ochrobactrum sp.*, these effects were also attributed to phytohormone production by PGPRs. Calvo et al. (2017) found similar results of both *Bacillus sp.* PGPR inoculated maize (0.123 g) and the control (0.126 g). Shoot growth improvements are thought to be induced by PGPRs secretion of phytohormones that mitigate the water

stress growth inhibitory effects (Ahluwalia et al., 2021; Mishra et al., 2020).

Generally, PGPR primed plants experience increased growth rate in green house trials (Getahun et al., 2020). In a study performed by Lin et al., (2020) found no significant difference of increased biomass (shoot and root) due to PGPR priming, however a general trend of biomass increase was observed for PGPR primed plants (Lin et al., 2020).

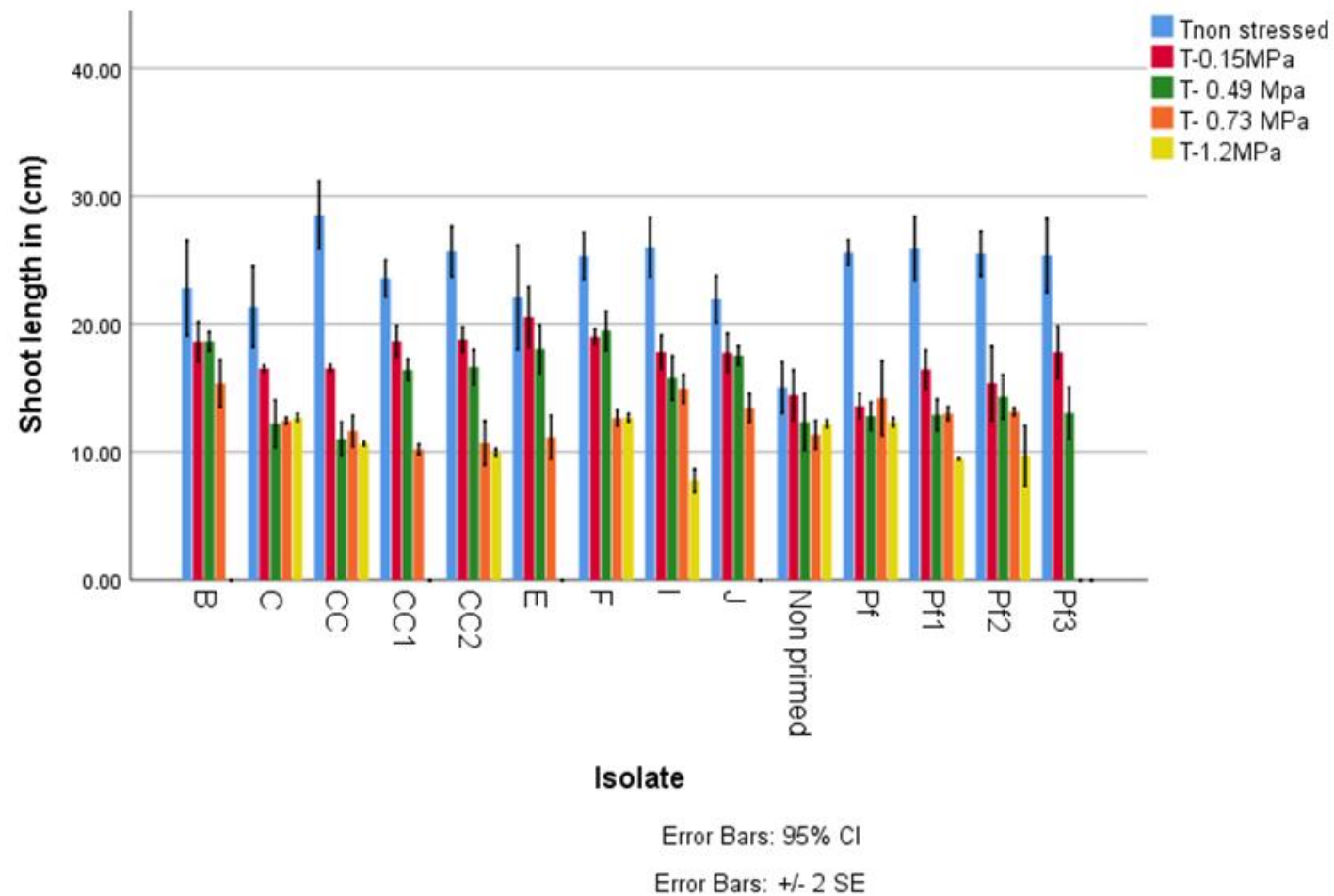


Figure (24): Shoot length of maize bio primed seedlings at different water stress levels in cm.

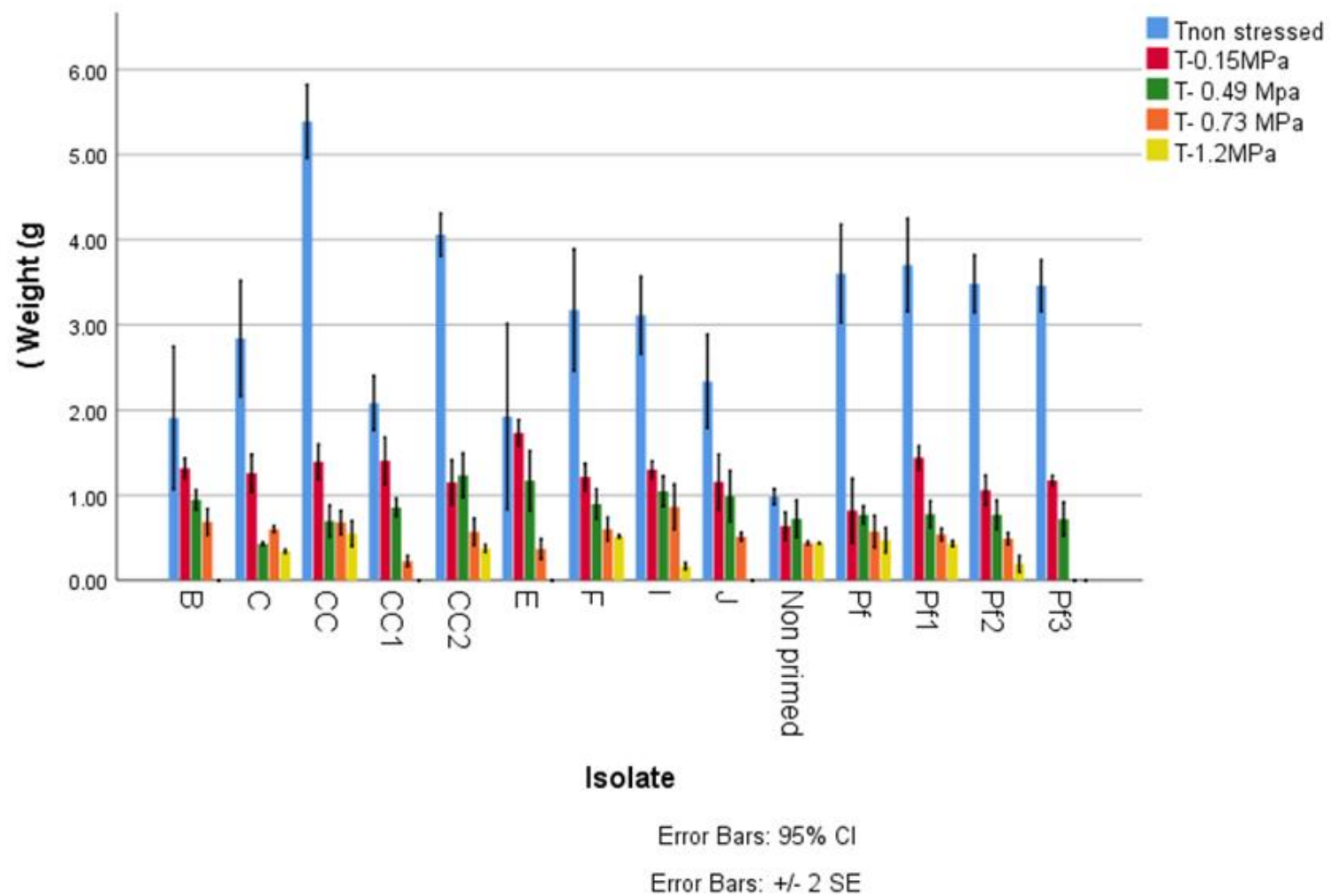


Figure (25): Shoot fresh weight (FW) of maize bio primed seedlings at different water stress levels in g.

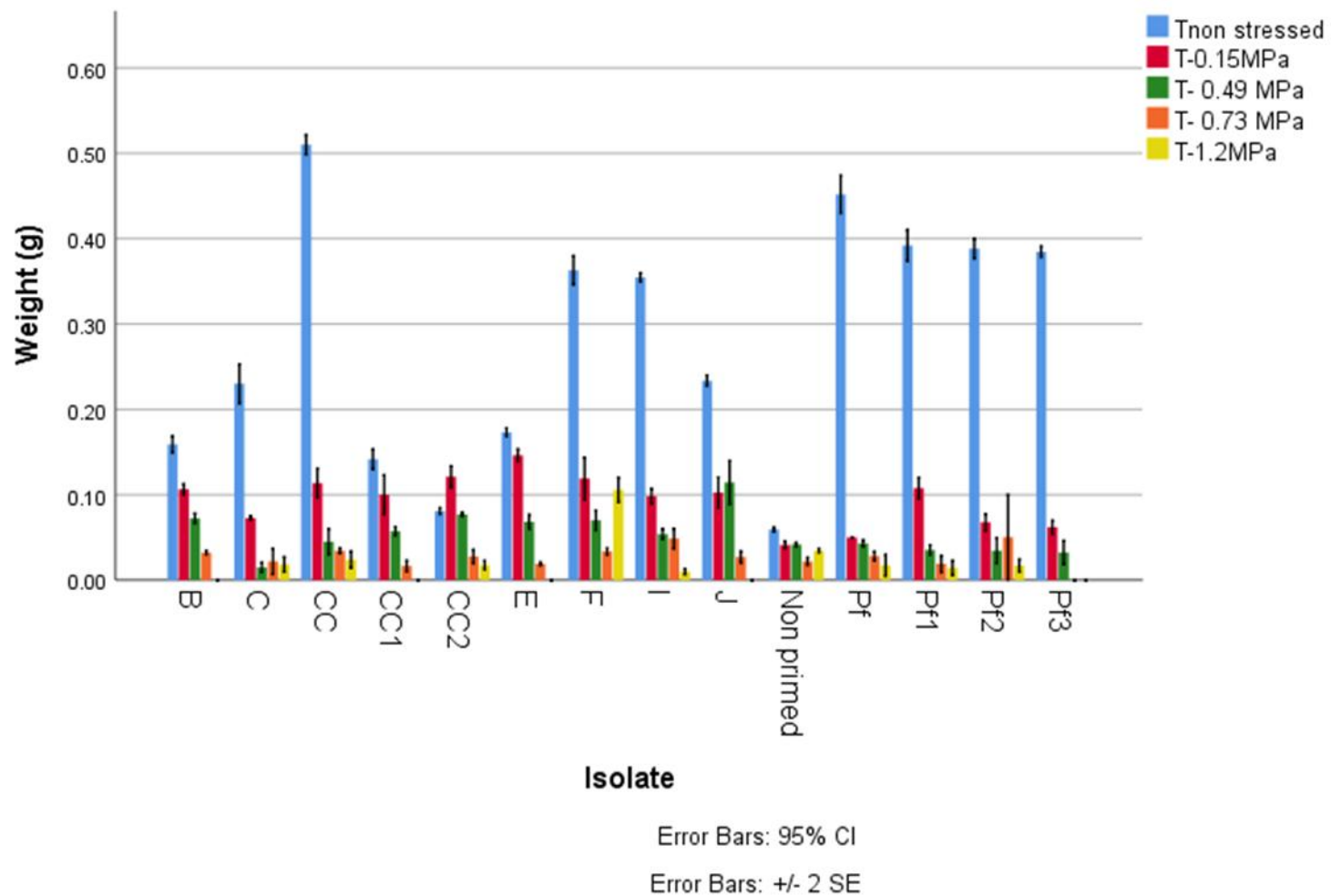


Figure (26): Shoot dry weight (DW) of maize bio primed seedlings at different water stress levels in (g).

. Morphological parameter review

For all of the tested morphological parameters (leaf length, leaf area, shoot length, shoot FW, shoot DW, root length, root FW and root DW) significant differences between the isolate means for each treatment were found using ANOVA. Therefore, it can be derived from the results that the priming of maize seeds was beneficiary for leaf length, leaf area, shoot length, shoot FW, shoot DW, root length, root FW and root DW under no water stress and with water stress. As in all treatments studied (T non stressed, T -0.15 MPa, -0.49 MPa, -0.73 MPa, -1.2 MPa), the primed maize seedling measured parameters were higher than the non-primed maize. In many cases, the primed maize seedlings demonstrated higher parameters than non-primed for more than one isolate.

Isolate CC primed maize experienced the greatest growth promotion as evident by the morphological parameters' findings. However, many of isolates included in this study have also shown remarkable growth parameters without the stress application indicating plant growth promotion abilities but no tolerance for water stress effects.

The reason for improved maize growth under water stress conditions by PGPRs can be explained by many mechanisms such as the increase in relative water content and production of osmolytes (Lin et al., 2020). Increased concentration of osmolytes such as proline, amino acids, soluble sugars in the leaves during water stress help in mitigating the oxidative stress that arises (Zia et al., 2021; Getahun et al., 2020; Lin et al., 2020). Furthermore, PGPRs have been shown to diminish the harmful effects of water deficiency on maize plants and enhance plant development by producing plant hormones which in turn allow for better adsorption of nutrients and water from the soil by the roots (Lin et al., 2020).

Concurrently, PGPRs improve the root system growth by phytohormones, maintain water availability and use efficiency and assist in nutrient absorption for plants surviving water stress (Getahun et al., 2020; Lin et al., 2020). Other possible mechanisms discussed for improved drought tolerance and growth after PGPR inoculation include regulations of plant stress response genes (Getahun et al., 2020).

4.5.2.2 Photosynthetic pigments

Photosynthesis is another prime metabolic process severely affected by water stress (Pepe et al., 2022; Bashir et al., 2021). As a variable which is highly responsive to environmental changes, photosynthesis is often used to measure the functional limitations imposed by a given stress (Pepe et al., 2022). In plants experiencing water stress, chlorophyll content is often measured as a reliable indicator of metabolic imbalances due to stress (Franzoni et al., 2022; Mishra et al., 2020). As visible water stress effects mainly include yellowing of the leaves and loss of turgor as a result of chlorophyll degradation (Franzoni et al., 2022).

The process of photosynthesis depends on interactions with multiple physiological and morphological plant traits which elicit growth (Pepe et al., 2022). Therefore, photosynthesis can be utilized to measure a plant's ability to adapt to the local environment (Pepe et al., 2022). During water stress, photosynthesis is arrested by plants (Ahmed et al., 2022; Bashir et al., 2021) which will affect the survival, yield and growth of crops (Ahmed et al., 2022). As drought reduces stomatal conductance and CO₂ diffusion and fixation, the activity of ribulose biphosphate decreases and CO₂ metabolism is arrested (Ahmed et al., 2022). Additionally, the plants reduced uptake of water and dissolved nutrients reduces photosynthesis (Mataranyika et al., 2022).

A. Photosynthetic pigments: Chlorophyll a (Chl a)

In this study, Chlorophyll a content (mg/g of FW) demonstrated in figure (27) for non-primed maize under no water stress was 13.53 ± 0.73 mg/g of FW which is lower than that found for primed maize. Maize primed with isolates I (32.06 ± 1.94 mg/g of FW), CC1 (28.56 ± 0.39 mg/g of FW) and J (26.14 ± 1.95 mg/g of FW) showed significantly higher concentrations of chlorophyll a than the non-primed as can be seen in table (14). When water stress of 0.15MPa was applied, non-primed maize concentration of chlorophyll a was 10.64 ± 0.38 mg/g of FW. When chlorophyll a concentration of non-primed maize is compared to that of maize primed with isolates PF (28.78 ± 1.35 mg/g of FW), CC2 (24.91 ± 2.29 mg/g of FW) it is evident that primed surpasses the non-primed concentration. At water stress of -0.49 MPa, non-primed maize chlorophyll a concentration was found to be 22.2 ± 0.91 mg/g of FW, while maize primed with isolate C (22.75 ± 0.67 mg/g of FW) was higher it was not found statistically significant. Water stress of -0.73 MPa revealed that the non-primed maize concentration of chlorophyll a was 24 ± 1.28 mg/g of FW, which was lower than that found for maize primed with isolate B (28.11 ± 0.41 mg/g of FW). Finally, at -1.2 MPa water stress, the concentration of Chlorophyll A for non-primed maize was determined to be 21.66 ± 0.19 mg/g of FW. The non primed concentration of chlorophyll A was lower than maize primed with isolate PF (34.35 ± 0.75 mg/g of FW), PF2 (32.48 ± 0.47 mg/g of FW) and CC (30.33 ± 0.49 mg/g of FW).

Table (14): Chlorophyll a concentration of significantly higher isolate primed maize compared to the non-primed maize.

Treatment	Non-primed maize chlorophyll a concentration (mg/g of FW) mean $\pm$ SE	Isolate primed maize chlorophyll a concentration (mg/g of FW) mean $\pm$ SE	P value
No water stress	13.53 $\pm$ 0.42	I (32.06 $\pm$ 1.09) CC1 (28.56 $\pm$ 0.22) J (26.14 $\pm$ 1.12)	0.000 0.000 0.000
-0.15MPa	10.64 $\pm$ 0.22	PF (28.78 $\pm$ 0.78) CC2 (24.91 $\pm$ 1.32)	0.000 0.000
-0.49MPa	22.2 $\pm$ 0.52		
-0.73 MPa	24 $\pm$ 0.74	B (28.11 $\pm$ 0.24)	0.000
-1.2MPa	21.66 $\pm$ 0.11	PF (34.35 $\pm$ 0.43) CC (30.33 $\pm$ 0.28) PF2 (27.81 $\pm$ 0.32)	0.000 0.000 0.000

B. Photosynthetic pigments: Chlorophyll b (Chl b)

Chlorophyll b concentration (mg/g of FW) displayed in figure (28) for non-primed maize under no water stress was found to be significantly lower than that for maize primed with isolates I, PF3 and CC1 as shown in table(15). After water stress of -0.15 MPa was administered, chlorophyll b concentration for non-primed maize was again significantly lower when compared to the primed maize isolates PF, B and PF2. At water stress of -0.49 MPa, many isolate primed maize were higher than the non-primed such as isolates: PF,C,PF1 and I, however, this difference was not found to be significant in the Tukey post hoc multiple comparisons. At -0.73 MPa water stress, non-primed chlorophyll b concentration was lower than that for maize primed with isolates CC2, B and CC1. Finally, at water stress of -1.2 MPa, non-primed chlorophyll b content was significantly lower than that found in maize primed with isolates PF, CC2 and PF2.

Table (15): Chlorophyll b concentration for significantly higher isolate primed maize compared to the non-primed maize.

Treatment	Non-primed maize chlorophyll concentration (mg/g of FW) mean $\pm$ SE	Isolate primed maize chlorophyll b concentration (mg/g of FW) mean $\pm$ SE	P value
No water stress	12.74 $\pm$ 0.27	I (30.07 $\pm$ 1.37) PF3 (29.17 $\pm$ 1.61) CC1 (25.46 $\pm$ 0.40)	0.000 0.000 0.000
-0.15MPa	10.46 $\pm$ 0.20	PF (30.14 $\pm$ 1.41) B (25.38 $\pm$ 1.67) PF2 (23.12 $\pm$ 0.82)	0.000 0.000 0.000
-0.49MPa	20.08 $\pm$ 1.53		
-0.73 MPa	20.9 $\pm$ 1.89	CC2 (28.52 $\pm$ 0.32) B (26.74 $\pm$ 0.43) CC1 (26.33 $\pm$ 0.21)	0.000 0.000 0.001
-1.2MPa	20.93 $\pm$ 3.70	PF (37.07 $\pm$ 0.78) CC2 (34.39 $\pm$ 0.52) PF2 (30.04 $\pm$ 0.58)	0.000 0.000 0.000

C. Photosynthetic pigments: Total Chlorophyll (Tot Chl)

As shown in figure (29) and table (16), total chlorophyll concentration (mg/g of FW) for non-primed maize under no water stress was lower than that for maize primed with isolates I, PF3 and CC1. However, at water stress of -0.15 MPa, only isolate B recorded significantly higher results than the non-primed. At water stress of -0.49 MPa, non-primed maize concentration of total chlorophyll was significantly lower than that for maize primed with isolates C, PF1 and I. At water stress of -0.73 MPa, non-primed maize content of total chlorophyll was again significantly lower than that found for maize primed with isolates CC2, B and CC1. Lastly, at water stress of -1.2 MPa, non-primed maize seedling concentrations of total chlorophyll was lower than that of maize primed with isolates PF, CC2 and PF2.

Table (16): Total chlorophyll for significantly higher isolate primed maize compared to non-primed maize.

Treatment	Non-primed maize total chlorophyll (mg/g of FW) mean $\pm$ SE	Isolate primed maize total chlorophyll (mg/g of FW) mean $\pm$ SE	P value
No water stress	11.23 $\pm$ 0.24	I (26.5 $\pm$ 1.21) PF3 (25.72 $\pm$ 1.42) CC1 (22.45 $\pm$ 0.36)	0.000 0.001 0.000
-0.15MPa	9.22 $\pm$ 0.18	B (22.38 $\pm$ 1.47)	0.046
-0.49MPa	17.70 $\pm$ 1.35	C (20.10 $\pm$ 0.61) PF1 (19.23 $\pm$ 0.44) I (18.59 $\pm$ 0.25)	
-0.73 MPa	18.43 $\pm$ 1.66	CC2 (25.15 $\pm$ 0.28) B (23.58 $\pm$ 0.38) CC1 (23.22 $\pm$ 0.19)	0.000 0.000 0.001
-1.2MPa	18.46 $\pm$ 3.26	PF (32.65 $\pm$ 0.69) CC2 (30.33 $\pm$ 0.46) PF2 (26.49 $\pm$ 0.52)	0.000 0.000 0.000

PGPRs mitigate photosynthesis disturbances due to stomatal closure through the secretion of the phytohormone cytokinin's which influences cell division and differentiation in the leaves and increasing vascular bundles (Ahluwalia et al., 2021). Previous research has indicated the improvement of chlorophyll content in plants grown with complex high microbial population soil as opposed to low microbial population soil (Shirinbayan et al., 2019). In a study conducted by Shirinbayan et al., (2019), the *Azobacter* primed maize plants had an elevated chlorophyll content (up to 25) in 80% field capacity irrigation level (Shirinbayan et al., 2019). Mishra et al., (2020) study on maize inoculated with *Ochrobactrum* sp. (NBRISH6) under water stress found results in accordance with the previously mentioned ones. Where chlorophyll a increased to 1.25 mg/g of FW compared to water stressed control 0.77 mg/g of FW, and chlorophyll b increased to 1.31 mg/g of FW compared to water stressed control 1.17 mg/g of FW, total chlorophyll increased to 6.50 mg/g of FW compared to water stressed control 5.45 mg/g of FW.

Lin et al., (2019) performed a study in which the chlorophyll content was measured using SPAD. In their study, Lin et al., (2019) estimated the chlorophyll content in maize grown with PGPRs under water stress of irrigation every 12 days with inorganic supplementation and found significant increases (38.5 ± 1.27) compared to the control (37.3 ± 0.79). similarly, SPAD readings in Calvo et al., 2017 research found increases in PGPR primed maize chlorophyll content (39.85) compared to the control (35.16).

D. Photosynthetic pigments: Carotenoids (Car)

As presented in figure (30), carotenoid concentration (mg/g of FW) mean for non-primed maize without water stress was lower than that found for maize primed with isolates CC1, F and I. this difference was significant as can be seen in table (17). At water stress of -0.15 MPa, non-primed maize carotenoid content was again lower than maize primed with isolates B, PF and C. At water stress of -0.49 MPa, maize primed with isolates PF, PF1 and CC2 were higher than the non-primed maize. Similarly, at water stress of -0.73 MPa, the non-primed maize carotenoid concentration lower than that for maize primed with isolates CC1, CC2 and CC. Lastly, at water stress of -1.2 MPa, maize primed with isolates PF, PF2 and C were significantly higher than non-primed maize concentration of carotenoids. In accordance with our study, Khan et al., 2020b, found increased carotenoid content in maize after co inoculation of *Psuedomonas* sp and *Bacillus pumilus*.

Carotenoids are secondary metabolites with many functions. These non-polar molecules can act as photoprotective and antioxidant molecules (Franzoni et al., 2022). Carotenoids act as photoprotective molecules by preventing the overexcitation of photosystem II (Antonucci et al., 2021),

and as antioxidants by attacking toxic ROS, stabilizing structures and dissipating excess energy (Ahluwalia et al., 2021; Antonucci et al., 2021). Through these roles, carotenoids enhance the thylakoid and cell membrane rigidity (Ahluwalia et al., 2021). Lastly, carotenoids are precursors to abscisic acid (ABA) and many other phytohormones (Ahluwalia et al., 2021).

Table (17): Carotenoid concentration of non-primed maize compared to significantly higher isolate primed maize.

Treatment	Non-primed maize carotenoid content (mg/g of FW) mean ±SE	Isolate primed maize carotenoid content (mg/g of FW) mean ±SE	P value
No water stress	0.029±0.000	CC1(0.057±0.000)	0.000
		F (0.056±0.001)	0.000
		I (0.055 ±0.001)	0.000
-0.15MPa	0.021 ±0.000	B (0.072 ±0.001)	0.000
		PF (0.0723 ±0.001)	0.000
		C (0.069 ±0.000)	0.000
-0.49MPa	0.040±0.001	PF (0.085±0.000)	0.000
		PF1(0.085±0.000)	0.000
		CC2(0.075±0.000)	0.000
-0.73 MPa	0.050±0.003	CC1(0.087±0.000)	0.000
		CC2(0.0827±0.000)	0.000
		CC (0.076±0.000)	0.000
-1.2MPa	0.0421±0.005	PF (0.081±0.000)	0.000
		PF2(0.080±0.000)	0.000
		C (0.068±0.000)	0.000

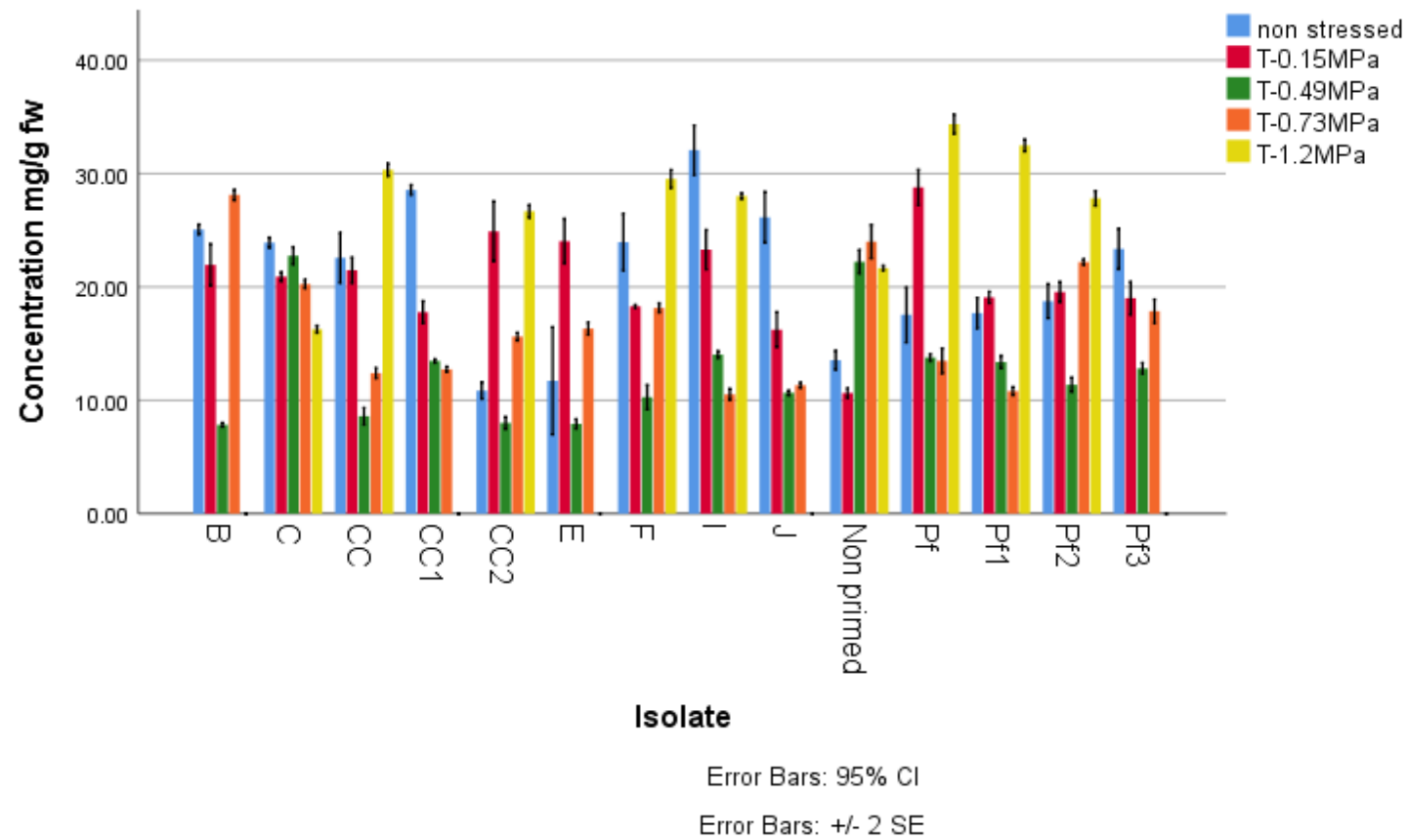


Figure (27): Chlorophyll a concentration in leaves of maize seeds in mg/g of FW (mean $\pm$ SE).

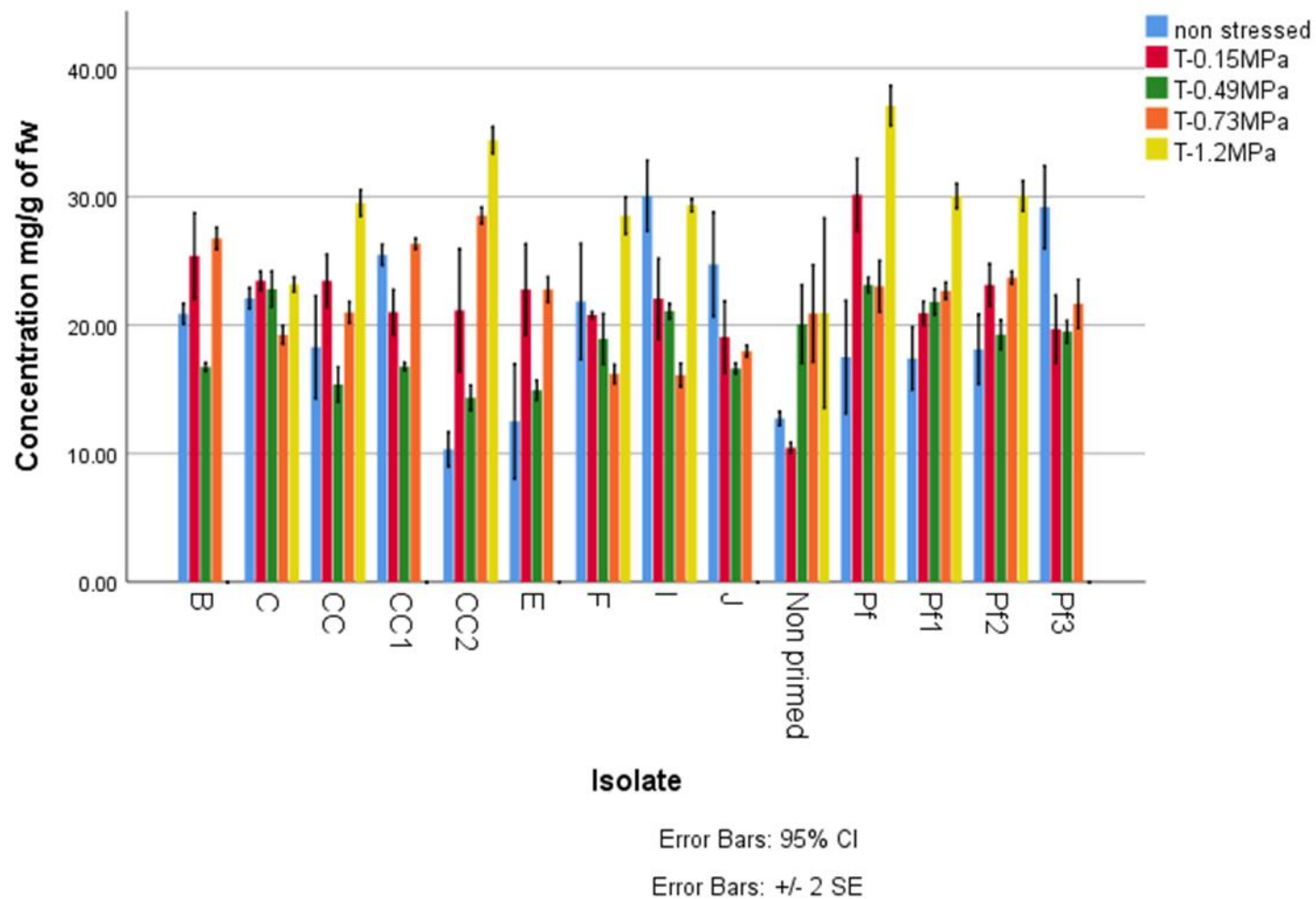


Figure (28): Chlorophyll b concentration in leaves of maize seeds in mg/g of FW (mean $\pm$ SE).

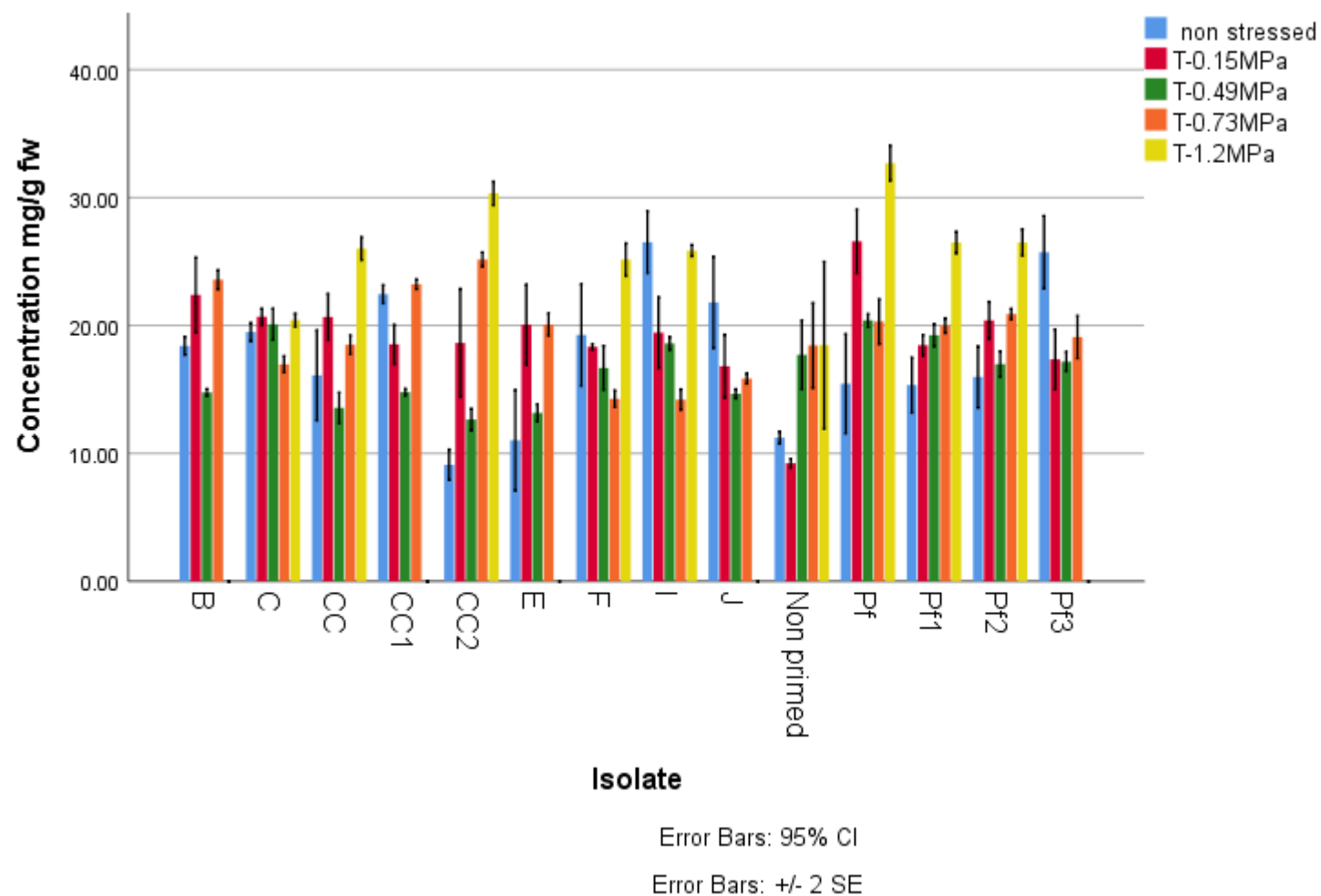


Figure (29): Total Chlorophyll concentration in leaves of maize seeds in mg/g of FW (mean $\pm$ SE)..

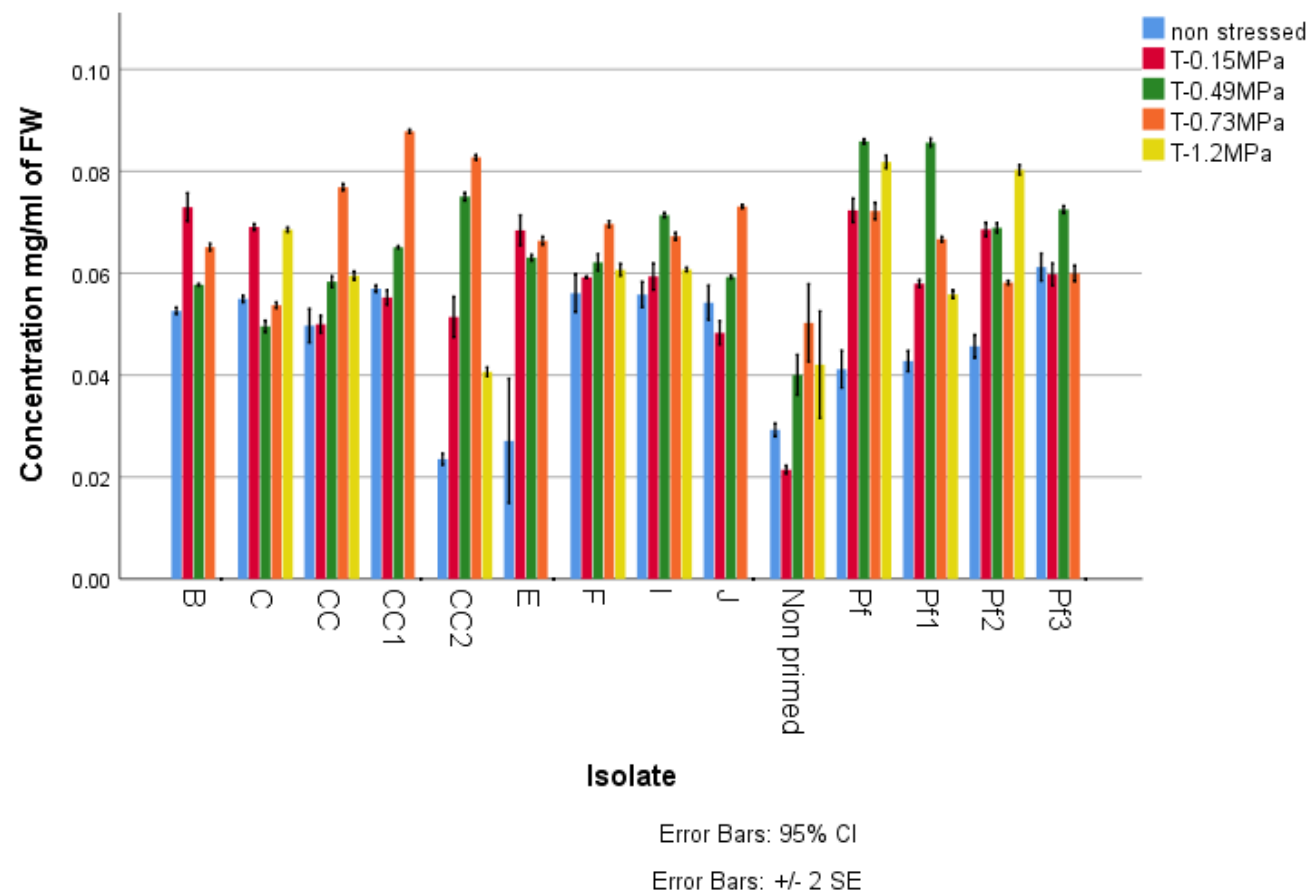


Figure (30): Carotenoid concentration in leaves of maize seeds in mg/g of FW (mean $\pm$ SE).

E. Photosynthetic pigments review

ANOVA statistical analysis performed on the means of isolates in each treatment found significant differences between them in all of the tested photosynthetic pigments. Therefore, the results of the study indicate that when it pertains to photosynthetic pigment concentrations (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids), priming maize seeds was more efficient than no priming under both conditions, no water stress and under water stresses of -0.15, -0.49, -0.73 and -1.2 MPa. Consistently, maize primed with isolates PF and PF2 have shown higher photosynthetic pigmentation concentration under the highest water stress. The primed maize photosynthetic pigment concentrations were higher than the non-primed maize in all of the treatments. In many cases, the primed maize seedlings had higher concentrations than non-primed parameters for more than one isolate. Without water stress, maize primed with isolates I and CC1 showed the best photosynthetic pigment concentration.

4.5.2.3. Total soluble proteins

As demonstrated in figure (31), without water stress application, TSP concentration mean (mg/ml of FW) determined for non-primed maize seedlings was 0.24 ± 0.015 mg/ml of FW, while primed maize seeds concentration were significantly higher, especially for isolates I, PF and CC1 as shown in table (18). The increase of TSP even without water stress indicates the beneficial effects of PGPR priming. When water stress of -0.15 MPa was applied, non-primed maize TSP concentration was lower than that found for isolates I, J and C. At water stress of -0.49 MPa, non-primed maize TSP was again lower than that found for isolates PF3, PF1 and CC, while isolate F's result was suggestive ($P=0.062$). Water stress of -0.73 MPa showed that the TSP concentration of non-primed maize was lower than that primed with isolates E, J and CC2. Lastly, at water stress of -1.2 MPa, isolates F, CC and PF were higher than the non-primed maize seedlings. In a study conducted by Ahmad et al., (2019), PGPRs *Pseudomonas putida* and *P. fluorescens* mitigated the decreasing concentration of TSP under 6 days of drought stress significant

Table (18): Total soluble protein concentration for maize primed isolates with significantly higher results compared to the non-primed maize.

Treatment	Non-primed maize TSP (mg/ml of FW) mean $\pm$SE	Isolate primed maize TSP (mg/ml of FW) mean $\pm$SE	P value
No water stress	0.24 $\pm$ 0.015	I (0.78 $\pm$ 0.038)	0.000
		PF (0.76 $\pm$ 0.005)	0.000
		CC1 (0.68 $\pm$ 0.02)	0.000
-0.15MPa	0.447 $\pm$ 0.02	I (0.78 $\pm$ 0.030)	0.000
		J (0.75 $\pm$ 0.006)	0.000
		C (0.62 $\pm$ 0.006)	0.000
-0.49MPa	0.48 $\pm$ 0.000	PF3 (0.796 $\pm$ 0.003)	0.000
		PF1 (0.756 $\pm$ 0.015)	0.000
		CC (0.71 $\pm$ 0.004)	0.000
-0.73 MPa	0.047 $\pm$ 0.02	E (0.822 $\pm$ 0.012)	0.000
		J (0.797 $\pm$ 0.001)	0.000
		CC2 (0.593 $\pm$ 0.006)	0.000
-1.2MPa	0.454 $\pm$ 0.01	F (0.807 $\pm$ 0.018)	0.000
		CC (0.659 $\pm$ 0.004)	0.000
		PF (0.61 $\pm$ 0.008)	0.000

One of the plants specialized responses to drought stress pertains to the Total Soluble Protein (TSP) content, which will decrease in concentration depending on the stress duration and severity (Vaidya et al., 2015; Ahmad et al., 2019). The reduction in TSP might occur due to the increased rate of protein breakdown by proteases and oxidative stress consequences (Ahmad et al., 2019; Ghaffar et al., 2023). Therefore, improved tolerance of water stress is demonstrated by higher TSP concentration through the accumulation of proteins (dehydrin) (Ghaffar et al., 2023). These dehydrin proteins are highly hydrophilic (water loving) and act as Osmo protectants by enhancing the water retention potential of the plant, which will preserve the photosynthetic machinery and enhance the accumulation of solutes (Ghaffar et al., 2023) eventually leading to better growth.

From the present study it can be derived that the priming of maize using isolates obtained in this study elevated osmoregulation through the total soluble proteins under no water stress and with it (-0.15 MPa, -0.49 MPa, -0.73 MPa, -1.2 MPa). As observed in all of the treatments investigated, primed maize TSP concentrations were significantly higher than those for non-primed maize. In all the treatments, the primed maize had higher concentrations than non-primed for more than one isolate.

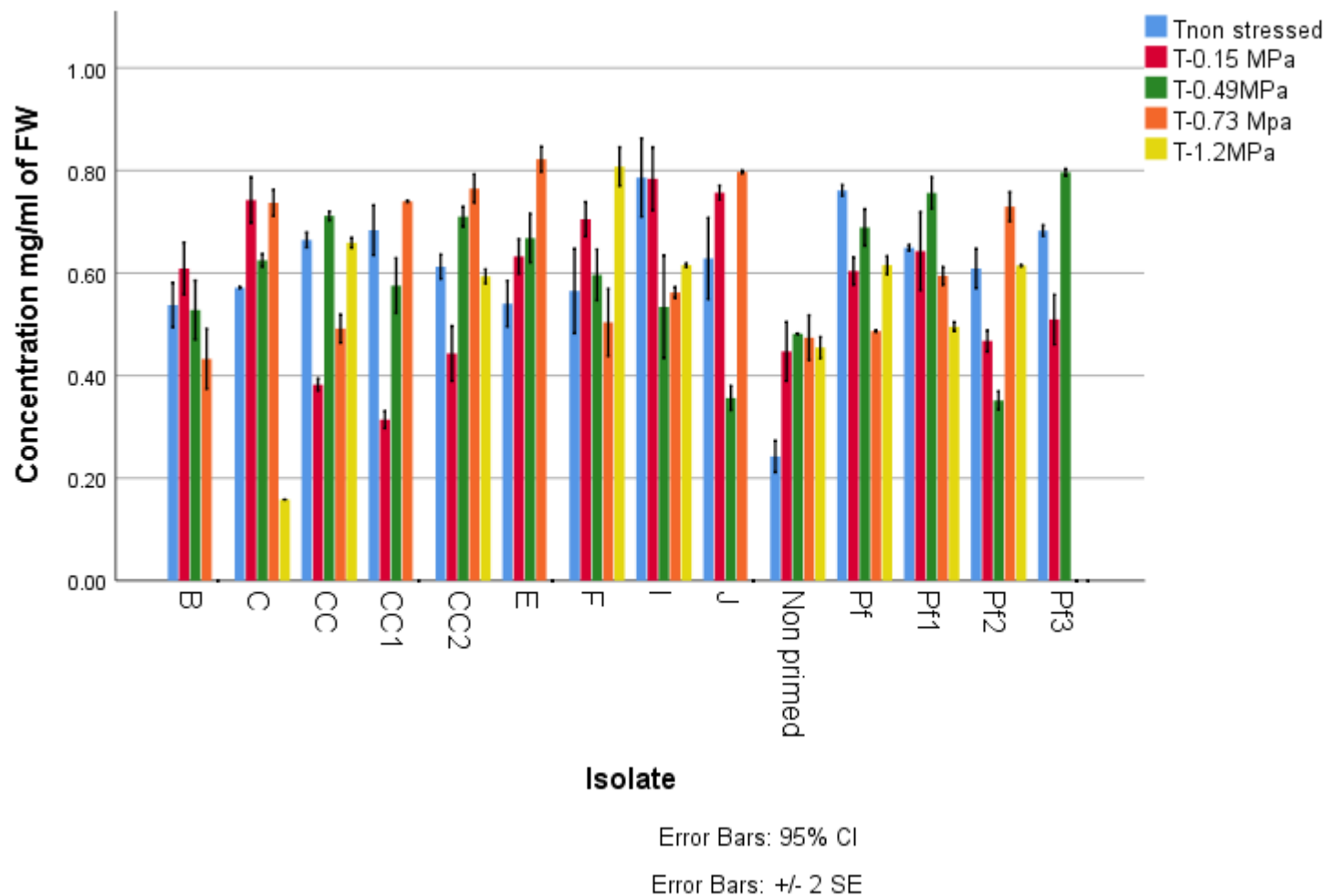


Figure (31): Total soluble protein concentration (mean $\pm$ SE) in leaves of maize seeds in mg/ml of FW.

Assessment of the best isolates that support maize growth and productivity:

On maize, the isolate demonstrating the best plant promotion abilities under no water stress was found to be CC as shown in table (6). Under low water stress of -0.15 MPa, the best performing isolates were found to be E and PF. In water stress of -0.49 MPa, many isolates showed high plant promotion abilities isolates (E, PF, PF3, J, C). Under moderate water stress (-0.73 MPa), isolate I demonstrated the best plant promotion abilities. In the highest water stress of -1.2 MPa, the best performing isolates were PF followed by CC and PF2.

It is also important to compare the isolates for the desired plant growth promoting potential as the bacteria have demonstrated in the green house to support maize growth. An isolate may demonstrate the best morphological parameter improvements but provide moderate enhancements of photosynthetic pigment concentrations or total soluble proteins, or the opposite. Such as isolate I, who demonstrated the highest concentration of total soluble proteins but not the highest morphological parameters or photosynthetic pigment. This is dependent on the isolate's unique potential in alleviating water stress which can be in different forms such as phytohormone production or osmolyte accumulation. Therefore, the inoculation of two or more isolate at once may result in high results for the combined tested parameters (morphological, photosynthetic and biochemical). The plants response to the effects of PGPRs can also cause variation in the combined parameters, different plants may respond better and therefore demonstrate better improvements in all parameters.

Table (19): Ranking of the best maize primed isolates (showing significance) compared to the non-primed maize in the parameters tested (Mean $\pm$ SE).

	Leaf Length (cm)	Leaf area (m ²)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Chl a (mg/g of FW)	Chl b (mg/g of FW)	Tot Chl (mg/g of FW)	Car (mg/g of FW)	TSP (mg/ml of FW)
T non stressed	J (65.0 $\pm$ 0.57)	CC (182 $\pm$ 2.00)	CC (60.67 $\pm$ 0.67)	CC (3.25 $\pm$ 0.25 g)	CC2 (0.496 $\pm$ 0.004)	CC (28.5 $\pm$ 1.32)	CC (5.39 $\pm$ 0.21)	CC (0.50 $\pm$ 0.005)	I (32.06 $\pm$ 1.09)	I (30.07 $\pm$ 1.37)	I (26.5 $\pm$ 1.21)	CC1(0.057 $\pm$ 0.000)	I (0.78 $\pm$ 0.038)
	PF1 (65 $\pm$ 0.00)	PF1 (162 $\pm$ 0.00)	PF2 (59.00 $\pm$ 1.15)	F (3.07 $\pm$ 0.32 g)	CC (0.357 $\pm$ 0.003)	I (26.00 $\pm$ 1.15)	CC2(4.06 $\pm$ 0.12)	PF (0.45 $\pm$ 0.011)	CC1 (28.56 $\pm$ 0.22)	PF3 (29.17 $\pm$ 1.61)	PF3 (25.72 $\pm$ 1.42)	F (0.056 $\pm$ 0.001)	PF (0.76 $\pm$ 0.005)
	PF (63 $\pm$ 0.88)	PF (158.33 $\pm$ 2.2)	F (55.67 $\pm$ 0.66)	CC2 (2.30 $\pm$ 0.20 g)	PF (0.312 $\pm$ 0.005)	PF1 (25.88 $\pm$ 1.25)	PF (3.60 $\pm$ 0.28)	PF1 (0.39 $\pm$ 0.009)	J (26.14 $\pm$ 1.12)	CC1 (25.46 $\pm$ 0.40)	CC1 (22.45 $\pm$ 0.36)	I (0.055 $\pm$ 0.001)	CC1 (0.68 $\pm$ 0.02)
Non primed	57.67 $\pm$ 0.33	115.33 $\pm$ 0.66	57.67 $\pm$ 0.33	0.95 $\pm$ 0.12	0.067 $\pm$ 0.004	15.033 $\pm$ 1.00	0.98 $\pm$ 0.04	0.059 $\pm$ 0.001	13.53 $\pm$ 0.42	12.74 $\pm$ 0.27	11.23 $\pm$ 0.24	0.029 $\pm$ 0.000	0.24 $\pm$ 0.015
T-0.15 MPa	CC2(54.33 $\pm$ 0.667)	CC2 (108.66 $\pm$ 1.33)	CC2(54.33 $\pm$ 0.66)	E (1.87 $\pm$ 0.15)	CC (0.352 $\pm$ 0.004)	E (20.53 $\pm$ 1.18)	E (1.73 $\pm$ 0.07)	E (0.14 $\pm$ 0.003)	PF (28.78 $\pm$ 0.78)	PF (30.14 $\pm$ 1.41)	B (22.38 $\pm$ 1.47)	B (0.072 $\pm$ 0.001)	I (0.78 $\pm$ 0.030)
	PF1(53.67 $\pm$ 0.88)	PF1 (107.33 $\pm$ 1.76)	C (48.00 $\pm$ 0.000)	CC (1.87 $\pm$ 0.18 g)	CC1 (0.143 $\pm$ 0.002)	F (19.0 $\pm$ 0.30)	CC1(1.40 $\pm$ 0.13)	CC2 (0.12 $\pm$ 0.006)	CC2 (24.91 $\pm$ 1.32)	B (25.38 $\pm$ 1.67)		PF (0.0723 $\pm$ 0.001)	J (0.75 $\pm$ 0.006)
	E (49.67 $\pm$ 0.88)	E (99.33 $\pm$ 1.76)		CC2 (1.79 $\pm$ 0.21)	CC2 (0.138 $\pm$ 0.004)	CC2 (18.76 $\pm$ 0.48)		F (0.11 $\pm$ 0.012)		PF2 (23.12 $\pm$ 0.82)		C (0.069 $\pm$ 0.000)	C (0.62 $\pm$ 0.006)
Non primed	33.33 $\pm$ 0.33	33.33 $\pm$ 0.57	33.33 $\pm$ 0.33	0.71 $\pm$ 0.03	0.05 $\pm$ 0.001	14.43 $\pm$ 0.98	0.63 $\pm$ 0.08	0.041 $\pm$ 0.002	10.64 $\pm$ 0.22	10.46 $\pm$ 0.20	9.22 $\pm$ 0.18	0.021 $\pm$ 0.000	0.447 $\pm$ 0.02

	Leaf Length (cm)	Leaf area (m ²)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Chl a (mg/g of FW)	Chl b (mg/g of FW)	Tot Chl (mg/g of FW)	Car (mg/g of FW)	TSP (mg/ml of FW)
T-0.49 MPa	E (51.67±0.88)	E (103.33 ± 1.67)	CC2 (50.00±1.15)	J (1.43 ± 0.11)	CC (0.165 ±0.002)	E (18.03 ± 0.56)	CC2(1.23 ±0.12)	J (0.11±0.012)			C (20.10 ±0.61)	PF (0.085±0.000)	PF3 (0.796±0.003)
	I (47.67±0.33)	CC2 (100 ± 2.30)		CC2 (1.35 ± 0.12 g)	J (0.125 ±0.002)			CC2(0.07 7±0.001)			PF1 (19.23 ± 0.44)	PF1(0.08 5±0.000)	PF1 (0.756±0.015)
	CC1 (47±0.57)	I (95.33 ± 0.66)			CC2 (0.105 ±0.011)						I (18.59 ± 0.25)	CC2(0.07 5±0.000)	CC (0.71±0.004)
Non primed	39.0 ± 0.57	58.50 ± 1.50	39 ± 0.60	0.80 ± 0.07	0.051 ± 0.002	12.33 ± 1.10	0.723±0.10	0.0417±0.001	22.2 ± 0.52	20.08 ± 1.53	17.70±1.35	0.040±0.001	0.48±0.000
T-0.73 MPa	I (49.33±1.20)	I (74.00 ± 1.80)	E (30.00±0.60)	CC (0.93 ± 0.12)	CC (0.09 ± 0.002)	B (15.34 ± 0.93)	I (0.86±0.13)		B (28.11 ± 0.24)	CC2 (28.52 ± 0.32)	CC2 (25.15 ± 0.28)	CC1(0.08 7±0.000)	E (0.822±0.012)
	F (38.33±0.66)	CC2 (67.33 ± 0.67)	I (49.33±1.20)			I (14.93 ± 0.56)				B (26.74 ± 0.43)	B (23.58 ± 0.38)	CC2(0.08 27±0.000)	J (0.797±0.001)
	CC (36 ± 0.57)	F (57.50 ± 1.00)	CC1(23.3 3±0.88)							CC1 (26.33 ± 0.21)	CC1 (23.22 ± 0.19)	CC (0.076±0.000)	CC2 (0.593±0.006)
Non primed	29.76 ± 0.34	31.66 ± 2.08	29.67 ± 0.32	0.57 ± 0.20	0.04 ± 0.004	11.34 ± 0.55	0.44 ± 0.01	0.022±0.002	24 ± 0.74	20.9 ± 1.89	18.43 ±1.66	0.050±0.003	0.047±0.002
T-1.2 MPa	CC2 (36.67 ± 0.67)	CC (50.50 ± 0.50)	CC (33.67 ± 0.34)		F (0.095 ± 0.016)			F (0.10±0.007)	PF (34.35 ± 0.43)	PF (37.07 ± 0.78)	PF (32.65 ± 0.69)	PF (0.081±0.000)	F (0.807±0.018)
					PF (0.091 ± 0.004)				CC (30.33 ± 0.28)	CC2 (34.39 ± 0.52)	CC2 (30.33±0.46)	PF2 (0.080±0.000)	CC (0.659±0.004)
									PF2 (27.81 ± 0.32)	PF2 (30.04 ± 0.58)	PF2 (26.49 ± 0.52)	C (0.068±0.000)	PF (0.61±0.008)
Non primed	31.67 ± 0.33	31.67 ± 2.08	31.67±0.33	0.67 ± 0.00	0.049 ± 0.001	12.2 ± 0.15	0.43 ± 0.00	0.0347±0.001	21.66 ± 0.11	20.93±3.70	18.46 ± 3.26	0.0421±0.005	0.454±0.001

It is important to remember that results in-vitro can differ greatly from the in vivo greenhouse results. And while the isolated bacteria demonstrate promising results in in vitro assays, the same isn't always true when these bacteria are faced with the changing conditions of greenhouse experimentation (Jeyanthi et al., 2018).

Arid and semi-arid regions experience joint stress of drought and heat, which leads to reduced plant stomatal conductance, photosynthetic activity, and CO₂ fixation (Ahmad et al., 2022). This occurs due to impairments in Rubisco enzyme activity and chlorophyll synthesis reductions (Ahmad et al., 2022). As a result, ROS accumulate causing protein denaturation and decreased plant biomass and growth (Ahmad et al., 2022). Increased production of Osmo regulators due to PGPRs improves the plants tolerance to water stress through the reduction of toxic molecules that negatively affect plant growth (Ahmad et al., 2022; Dikobe et al., 2021).

Overall, many of the harmful effects of drought such as root and shoot growth reduction, stomatal closure, oxidative damage, and turgor pressure reductions can be alleviated by PGPRs due to their ability to secrete phytohormones, antioxidants and osmoregulators, which enhance plant growth parameters as shown in the summary table (20) (Ashry et al., 2022; Ahluwalia et al., 2021).

Table (20): Methods implemented by PGPRs to reduce water stress effect on plants growth.

Plant response to water stress	Mitigation effects by PGPRs	References
Reduction in Shoot growth	Plant phytohormones synthesis and ethylene mitigation.	Ahluwalia et al., 2021
Reduction in root growth and impaired nutrient uptake from water	Plant phytohormone synthesis which endorses root length, growth, surface area and the formation of lateral roots. Additionally, phytohormones allow the roots to absorb water and nutrients more efficiently from the soil also enhance water uptake.	Mataranyika et al., 2022; Ahluwalia et al., 2021; Jochum et al., 2019; Lin et al., 2019; Calvo et al. 2017.
Closure of stomata	Plant phytohormone synthesis particularly cytokines and ethylene reduction.	Ahluwalia et al., 2021, Zia et al., 2021
Oxidative damage	Increase in antioxidant concentration and activity, as well as osmolyte production.	Ahluwalia et al., 2021; Zia et al., 2021; Lin et al., 2020
Reduction in turgor pressure and water potential	Osmoregulation through solute accumulation and osmo-protectant molecule production such as soluble proteins.	Ahluwalia et al., 2021; Zia et al., 2021; Lin et al., 2020

PGPRs are unique to each microclimate produced by a plants root deposits and exudates. Therefore, this study provides essential data for future research. The present results indicate the success of these PGPRs in alleviating drought stress. However, to successfully manage the detrimental effects of water stress on crops, a holistic approach must be followed (Ojuederie & Babalola, 2023). Additional studies are needed to understand the interaction between plants and microbes genetically, in order to mitigate drought stress.

Recommendations

1. The co-inoculation of two or multiple isolates can result in a higher drought tolerance in crops. Such as the inoculation using isolates PF, CC and PF2 which demonstrated the best growth parameters in this study.
2. Since PGPRs are unique to each microclimate created by plants deposits and exudates, further investigations can experiment with different crops which may have a greater compatibility and benefit from these PGPRs.
3. Isolates obtained in this investigation have demonstrated salinity tolerance abilities, although not tested on maize in our study, this ability can be explored and exploited further to assist crop growth in salt- affected soils.
4. Different PGPR application methods to plants can be compared to obtain the best results.
5. Native plants which reside in Bahrain tolerate water stress, high temperature and salinity regularly, accordingly, they represent a reservoir of potential PGPR strains that can assist crop growth in

these conditions. The management and protection of these native plants is therefore essential.

6. Identification of the most promising PGPR isolates using molecular markers can results in better effective comparisons according to maize growth.

Summary and conclusion

Drought is an environmental stress significantly affecting crop yield and productivity. PGPRs are largely recommended to alleviate the effects of stresses in crops, especially drought due to their plant growth promotion abilities. Because PGPR composition and diversity is affected by the surrounding plant exudates and environment, those isolated from arid or harsh environments seem to be better adapted to water stress. In addition to tolerating increasing osmotic pressures (-0.15, -0.49, -0.73, -1.2 MPa) the isolates examined demonstrated phosphate solubilization, ammonia production and salinity tolerance abilities. In this study, isolates obtained from the arid country of Bahrain allowed maize to better tolerate water stress at different osmotic pressures. Priming with these isolates under different osmotic pressures produced improvements in maize germination index and mean germination time compared to the non-primed seeds under water stress and without. Morphological parameters such as leaf length, leaf area, shoot and root length, shoot and root fresh and dry weight, as well as photosynthetic pigments concentration such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids and the total soluble protein concentration also improved significantly compared to the non-primed in both non-stressed and water stressed conditions. Therefore, the investigated isolates (especially isolates PF, CC and PF2) can be used further for research pertaining to maize and drought stress alleviation

especially in arid environments in order to create an efficient and suitable inoculum.

References

1. Abbas, J. A. (2006). Economic halophytes of Bahrain. In Sabkha Ecosystems: Volume II: West and Central Asia (pp. 113-120). Dordrecht: Springer Netherlands.
2. Abbasi Khalaki, M., Ghorbani, A., & Dadjou, F. (2019). Influence of nano-priming on *Festuca ovina* seed germination and early seedling traits under drought stress, in laboratory condition. *Ecopersia*, 7(3), 133-139.
3. Ahmad, H. M., Fiaz, S., Hafeez, S., Zahra, S., Shah, A. N., Gul, B., ... & Wang, X. (2022). Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: a review. *Frontiers in Plant Science*, 13, 875774.
4. Ahmad, I., Zaib, S., Alves, P. C. M. S., Luthe, D. S., Bano, A., & Shakeel, S. N. (2019). Molecular and physiological analysis of drought stress responses in *Zea mays* treated with plant growth promoting rhizobacteria. *Biol Plant*, 63, 536-547.
5. Alexander, A., Singh, V. K., & Mishra, A. (2020). Halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 induces salt tolerance by modulating physiology and biochemical activities of *Arachis hypogaea*. *Frontiers in Microbiology*, 11, 568289.
6. Ali, M., Gul, A., Hasan, H., Alipour, H., Abbasi, A. A., Abbas, S., ... & Taimoor, Z. (2020). LEA proteins and drought stress in wheat. In *Climate change and food security with emphasis on wheat* (pp. 193-205). Academic Press.
7. Antonucci, G., Croci, M., Miras-Moreno, B., Fracasso, A., & Amaducci, S. (2021). Integration of gas exchange with metabolomics: high-throughput phenotyping methods for screening

biostimulant-elicited beneficial responses to short-term water deficit. *Frontiers in Plant Science*, 12, 678925.

8. Ahluwalia, O., Singh, P. C., & Bhatia, R. (2021). A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resources, Environment and Sustainability*, 5, 100032.
9. Aranda, I., Cadahía, E., & Fernández de Simón, B. (2021). Specific leaf metabolic changes that underlie adjustment of osmotic pressure in response to drought by four *Quercus* species. *Tree physiology*, 41(5), 728-743.
10. Ashry, N. M., Alaidaroos, B. A., Mohamed, S. A., Badr, O. A., El-Saadony, M. T., & Esmael, A. (2022). Utilization of drought-tolerant bacterial strains isolated from harsh soils as a plant growth-promoting rhizobacteria (PGPR). *Saudi Journal of Biological Sciences*, 29(3), 1760-1769.
11. Astorga-Eló, M., Gonzalez, S., Acuña, J. J., Sadowsky, M. J., & Jorquera, M. A. (2021). Rhizobacteria from ‘flowering desert’ events contribute to the mitigation of water scarcity stress during tomato seedling germination and growth. *Scientific reports*, 11(1), 13745.
12. Baltazar, M., Correia, S., Guinan, K. J., Sujeeth, N., Bragança, R., & Gonçalves, B. (2021). Recent advances in the molecular effects of biostimulants in plants: An overview. *Biomolecules*, 11(8), 1096.
13. Bashir, S. S., Hussain, A., Hussain, S. J., Wani, O. A., Zahid Nabi, S., Dar, N. A., ... & Mansoor, S. (2021). Plant drought stress tolerance: Understanding its physiological, biochemical and molecular mechanisms. *Biotechnology & Biotechnological Equipment*, 35(1), 1912-1925.
14. Bazrgar, G., Kalat, S. M. N., Khorasani, S. K., Ghasemi, M., & Kelidari, A. (2023). Effect of deficit irrigation on physiological, biochemical, and yield characteristics in three baby corn cultivars (*Zea mays L.*). *Heliyon*, 9(4).

15. BiBi, A., Bibi, S., Al-Ghouti, M. A., & Abu-Dieyeh, M. H. (2023). Isolation and evaluation of Qatari soil rhizobacteria for antagonistic potential against phytopathogens and growth promotion in tomato plants. *Scientific Reports*, 13(1), 22050.
16. Bogati, K., & Walczak, M. (2022). The impact of drought stress on soil microbial community, enzyme activities and plants. *Agronomy*, 12(1), 189.
17. Bueno, C. B., Dos Santos, R. M., de Souza Buzo, F., de Andrade da Silva, M. S. R., & Rigobelo, E. C. (2022). Effects of chemical fertilization and microbial inoculum on *Bacillus subtilis* colonization in soybean and maize plants. *Frontiers in Microbiology*, 13, 901157.
18. Calvo, P., Watts, D. B., Kloepper, J. W., & Torbert, H. A. (2017). Effect of microbial-based inoculants on nutrient concentrations and early root morphology of corn (*Zea mays*). *Journal of Plant Nutrition and Soil Science*, 180(1), 56-70.
19. Dikobe, T. B., Mashile, B., Sinthumule, R. R., & Ruzvidzo, O. (2021). Distinct morpho-physiological responses of maize to salinity stress. *American Journal of Plant Sciences*, 12(6), 946-959.
20. Etemadi, N., Müller, M., Etemadi, M., Brandón, M. G., Ascher-Jenull, J., & Insam, H. (2020). Salt tolerance of *Cressa cretica* and its rhizosphere microbiota. *Biologia*, 75, 355-366.
21. Fahey, C., Koyama, A., Antunes, P. M., Dunfield, K., & Flory, S. L. (2020). Plant communities mediate the interactive effects of invasion and drought on soil microbial communities. *The ISME Journal*, 14(6), 1396-1409.
22. Flieger, J., Flieger, W., Baj, J., & Maciejewski, R. (2021). Antioxidants: Classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of nanoparticles. *Materials*, 14(15), 4135.

23. Franzoni, G., Cocetta, G., Prinsi, B., Ferrante, A., & Espen, L. (2022). Biostimulants on crops: Their impact under abiotic stress conditions. *Horticulturae*, 8(3), 189.
24. Gaete, A., Mandakovic, D., & González, M. (2020). Isolation and identification of soil bacteria from extreme environments of Chile and their plant beneficial characteristics. *Microorganisms*, 8(8), 1213.
25. Getahun, A., Muleta, D., Assefa, F., & Kiros, S. (2020). Plant growth-promoting rhizobacteria isolated from degraded habitat enhance drought tolerance of acacia (*Acacia abyssinica* Hochst. ex Benth.) seedlings. *International journal of microbiology*, 2020, 1-13.
26. Ghaffar, A., Hussain, N., Ajaj, R., Shahin, S. M., Bano, H., Javed, M., ... & Athar, H. U. R. (2023). Photosynthetic activity and metabolic profiling of bread wheat cultivars contrasting in drought tolerance. *Frontiers in Plant Science*, 14, 1123080.
27. Gull, A., Lone, A. A., & Wani, N. U. I. (2019). Biotic and abiotic stresses in plants. *Abiotic and biotic stress in plants*, 1-19.
28. Gupta, R., Kumari, A., Sharma, S., Alzahrani, O. M., Noureldeen, A., & Darwish, H. (2022). Identification, characterization and optimization of phosphate solubilizing rhizobacteria (PSRB) from rice rhizosphere. *Saudi Journal of Biological Sciences*, 29(1), 35-42.
29. Hartman, K., & Tringe, S. G. (2019). Interactions between plants and soil shaping the root microbiome under abiotic stress. *Biochemical Journal*, 476(19), 2705-2724.
30. Hernandez, R. E., Gallegos-Monterrosa, R., & Coulthurst, S. J. (2020). Type VI secretion system effector proteins: effective weapons for bacterial competitiveness. *Cellular microbiology*, 22(9), e13241.
31. Jeyanthi, V., & Kanimozhi, S. (2018). Plant growth-promoting rhizobacteria (PGPR)-prospective and mechanisms: a review. *J Pure Appl Microbiol*, 12(2), 733-749.

- 32.Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales, 138, 65-75.
- 33.Khan, N., Ali, S., Tariq, H., Latif, S., Yasmin, H., Mehmood, A., & Shahid, M. A. (2020a). Water conservation and plant survival strategies of rhizobacteria under drought stress. Agronomy, 10(11), 1683.
- 34.Khan, N., Bano, A., & Curá, J. A. (2020b). Role of beneficial microorganisms and salicylic acid in improving rainfed agriculture and future food safety. Microorganisms, 8(7), 1018.
- 35.Kour, D., Rana, K. L., Sheikh, I., Kumar, V., Yadav, A. N., Dhaliwal, H. S., & Saxena, A. K. (2020). Alleviation of drought stress and plant growth promotion by *Pseudomonas libanensis* EU-LWNA-33, a drought-adaptive phosphorus-solubilizing bacterium. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 90, 785-795.
- 36.Karnwal, A. (2017). Isolation and identification of plant growth promoting rhizobacteria from maize (*Zea mays L.*) rhizosphere and their plant growth promoting effect on rice (*Oryza sativa L.*). Journal of Plant Protection Research, 57(2).
- 37.Karnwal, A. (2020a). Effect of halotolerant plant growth-promoting rhizobacteria from *Bougainvillea glabra* on wheat and maize seedlings under NaCl stress. BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology, 101(4), 349-359.
- 38.Karnwal, A. (2020b). Effect of salt stress-tolerant bacterial endophytes from *Bougainvillea glabra* on the growth of *Triticum aestivum* L. var. HD 2687 and *Zea mays* var. PSCL-4642. BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology, 101(2), 89-99.

39. Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K., ... & Rakshit, S. (2020). Genetically modified crops: current status and future prospects. *Planta*, 251(4), 91.
40. Kumar, A., Singh, S., Mukherjee, A., Rastogi, R. P., & Verma, J. P. (2021). Salt-tolerant plant growth-promoting *Bacillus pumilus* strain JPVS11 to enhance plant growth attributes of rice and improve soil health under salinity stress. *Microbiological Research*, 242, 126616.
41. Lin, Y., Watts, D. B., Kloepper, J. W., Feng, Y., & Torbert, H. A. (2020). Influence of plant growth-promoting rhizobacteria on corn growth under drought stress. *Communications in soil science and plant analysis*, 51(2), 250-264.
42. Liu, J., Chen, N., Gao, B., Miao, M., Zhao, Y., Liu, S., ... & Ma, Y. (2024). Overexpression of the late embryonic genesis abundant protein MGL3 improves the drought tolerance of maize (*Zea mays* L.). *Crop Science*.
43. Mataranyika, P. N., Chimwamurombe, P. M., Venturi, V., & Uzabakiriho, J. D. (2022). Bacterial bioinoculants adapted for sustainable plant health and soil fertility enhancement in Namibia. *Frontiers in Sustainable Food Systems*, 6, 1002797.
44. Mazumdar, D., Saha, S. P., & Ghosh, S. (2020). Isolation, screening and application of a potent PGPR for enhancing growth of Chickpea as affected by nitrogen level. *International Journal of Vegetable Science*, 26(4), 333-350.
45. Mishra, S. K., Khan, M. H., Misra, S., Dixit, V. K., Gupta, S., Tiwari, S., ... & Chauhan, P. S. (2020). Drought tolerant *Ochrobactrum* sp. inoculation performs multiple roles in maintaining the homeostasis in *Zea mays* L. subjected to deficit water stress. *Plant Physiology and Biochemistry*, 150, 1-14.
46. Naylor, D., & Coleman-Derr, D. (2018). Drought stress and root-associated bacterial communities. *Frontiers in plant science*, 8, 303756.

47. Naz, N., Asghar, A., Basharat, S., Fatima, S., Hameed, M., Ahmad, M. S. A., ... & Ashraf, M. (2024). Phytoremediation through microstructural and functional alterations in alkali weed (*Cressa cretica* L.) in the hyperarid saline desert. *International Journal of Phytoremediation*, 26(6), 913-927.
48. Norton, J., Majid, S. A., Allan, D., Al Safran, M., Böer, B., & Richer, R. (2009). An illustrated checklist of the flora of Qatar (p. 67). Gosport: Browndown Publications.
49. Ojuederie, O. B., & Babalola, O. O. (2023). Growth enhancement and extenuation of drought stress in maize inoculated with multifaceted ACC deaminase producing rhizobacteria. *Frontiers in Sustainable Food Systems*, 6, 1076844.
50. Pepe, M., Crescente, M. F., & Varone, L. (2022). Effect of water stress on physiological and morphological leaf traits: A comparison among the three widely-spread invasive alien species *Ailanthus altissima*, *Phytolacca americana*, and *Robinia pseudoacacia*. *Plants*, 11(7), 899.
51. Rehman, M., Shahid, M., Mahmood, S., Ali, Q., & Azeem, M. (2023). Modulation of Growth and Antioxidative Defense Mechanism in Wheat (*Triticum aestivum* L.) Mediated by Plant-Beneficial Strain *Pseudomonas veronii* MR-15 under Drought Conditions. *Applied Sciences*, 14(1), 128.
52. Sadeq, M. A., Abido, M. S., Salih, A. A., & Alkhuzai, J. A. (2020). The effects of mesquite (*Prosopis juliflora*) on soils and plant communities in the deserted rangelands of Bahrain. *International Journal of Forestry Research*, 2020, 1-8.
53. Salimi-Sabour, E., Fattahi, M., Rezaei, K., Lotfali, E., & Khademian, (2022). *Prosopis farcta*: Potent Antifungal Activity Against Trichophyton mentagrophytes Strains; A Research Based on an Ethnobotanical Study. *Iranian Journal of Medical Microbiology*, 16(2), 127-133.
54. Santoyo, G., Urtis-Flores, C. A., Loeza-Lara, P. D., Orozco-Mosqueda,

- M. D. C., & Glick, B. R. (2021). Rhizosphere colonization determinants by plant growth-promoting rhizobacteria (PGPR). *Biology*, 10(6), 475.
- 55.Scagliola, M., Valentinuzzi, F., Mimmo, T., Cesco, S., Crecchio, C., & Pii, Y. (2021). Bioinoculants as promising complement of chemical fertilizers for a more sustainable agricultural practice. *Frontiers in Sustainable Food Systems*, 4, 622169.
- 56.Shirinbayan, S., Khosravi, H., & Malakouti, M. J. (2019). Alleviation of drought stress in maize (*Zea mays*) by inoculation with *Azotobacter* strains isolated from semi-arid regions. *Applied soil ecology*, 133, 138-145.
- 57.Soltani, J., Samavati, R., Jalili, B., Bagheri, H., & Hamzei, J. (2023). Halotolerant endophytic bacteria from desert-adapted halophyte plants alleviate salinity stress in germinating seeds of the common wheat *Triticum aestivum* L. *Cereal Research Communications*, 1-11.
- 58.Tripathi, N., & Sapra, A. (2020). Gram staining.
- 59.Ullah, S., & Bano, A. (2015). Isolation of plant-growth-promoting rhizobacteria from rhizospheric soil of halophytes and their impact on maize (*Zea mays* L.) under induced soil salinity. *Canadian Journal of Microbiology*, 61(4), 307-313.
- 60.Vaidya, S., Vanaja, M., Lakshmi, N. J., Sowmya, P., Anitha, Y., & Sathish, P. (2015). Variability in drought stress induced responses of groundnut (*Arachis hypogaea* L.) genotypes. *Biochem Physiol*, 4(149), 2.
- 61.Zhao, Y., Shi, R., Bian, X., Zhou, C., Zhao, Y., Zhang, S., ... & Zhang, T. (2019). Ammonia detection methods in photocatalytic and electrocatalytic experiments: how to improve the reliability of NH₃ production rates?. *Advanced Science*, 6(8), 1802109.
- 62.Zia, R., Nawaz, M. S., Siddique, M. J., Hakim, S., & Imran, A. (2021).

Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiological research*, 242, 126626.