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SCREENING FOR ENDOPHYTIC ACTINOBACTERIA TO ENHANCE GROWTH AND SALINITY TOLERANCE OF TOMATO PLANTS IN THE UNITED ARAB

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جامعة الإمارات العربية المتحدة
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Amira Hamdy Mohamed Mohamed Hassan

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Molecular Biology and Biotechnology

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Cover: Effect of endophytic actinobacterial isolate possessing ACCD activity on salinity tolerance of tomato seedlings under greenhouse conditions. From left to right: Tomato seedlings inoculated with the non-ACCD-producing E1-3EC before and after salinity stress and the ACCD-producing Z3-40 before and after salinity stress.

(Photo: By Amira Hamdy Mohamed)

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Declaration of Original Work

I, Amira Hamdy Mohamed Mohamed Hassan, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Screening for Endophytic Actinobacteria to Enhance Growth and Salinity Tolerance of Tomato Plants in the United Arab Emirates*", hereby, solemnly declare that this is the original research work done by me under the supervision of Prof. Synan F. AbuQamar, in the College of Science at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Salinity is one of the most decisive environmental factors limiting the productivity of crop plants, mainly in arid and semi-arid regions. An eco-friendly technology can be used to boost crop production in saline areas by using plant growth-promoting bacteria. Endophytic actinobacteria that produce the enzyme 1-Aminocyclopropane-1-Carboxylic acid (ACC) Deaminase (ACCD) can modulate the levels of Ethylene (ET) in plants to reduce the effect of abiotic stresses including high salt stress. The main objectives of this study were to: (1) Evaluate endophytic actinobacterial isolates from healthy tomato plants cultivated in the UAE that are capable of producing ACCD *in vitro*; (2) test the isolated endophytic actinobacteria possessing ACCD for their potential of producing Plant Growth Regulators (PGRs) *in vitro*; and (3) test the ability of tomato seedlings growing under high salt stress conditions using the most promising endophytic actinobacterial isolate producing ACCD in the greenhouse. To achieve this, twenty-five isolates of actinobacteria possessing the activity of ACCD were obtained from the roots of *Zygophyllum mandaveli* in Sweihan area, Abu Dhabi-UAE. *In vitro* screening demonstrated that two actinobacterial isolates produced ACCD, while tolerating up to 8% NaCl. In the greenhouse, the ACCD-producing isolate (referred to as Z3-40 isolate) significantly ($P<0.05$) enhanced growth of tomato seedlings in response to salt stress (120 mM NaCl). This was evident from the increase in the dry weight of roots by 2-fold and the length of roots and shoots by about 25%. These results were also associated with the reduced levels of the endogenous ACC by 3- and 3-fold in both root and shoot tissues, respectively, in plants inoculated with Z3-40 isolate compared to those of control or non-ACCD-producing isolate treatments. In conclusion, the production of ACCD by the endophytic actinobacterial isolate and its ability to enhance tomato growth under saline conditions mitigate the effect of salt stress through the reduction of endogenous ACC as well as ET levels in plant tissues. This investigation is expected to contribute to the development of sustainable agricultural strategies for utilizing saline water for the primary production in the United Arab Emirates, allowing local crop growers to use the high saline groundwater for irrigation.

Keywords: ACC deaminase, ethylene, endophytic actinobacteria, plant growth promoting actinobacteria, tomato

Title and Abstract (in Arabic)

الكشف عن الأكتينوبكتيريا المحيطة بالجذور والتي تعزز من نمو ومقاومة الملوحة لنبات الطماطم في الإمارات العربية المتحدة

الملخص

تعتبر الملوحة من أهم العوامل البيئية التي تحد من إنتاجية المحاصيل، وخاصة في المناطق القاحلة وشبه القاحلة. وعليه يمكن استخدام تقنية صديقة للبيئة لزيادة إنتاجية المحاصيل في المناطق المالحة وذلك باستخدام الأكتينوبكتيريا المعززة لنمو النبات. يمكن للأكتينوباكتيريا التي تعيش داخل جذور النبات والتي تنتج إنزيم 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD)، والذى بدوره يعمل على تقليل مستويات الإيثيلين داخل النبات للحد من تأثير الضغوطات الغير حيوية وأهمها الإجهاد الملحي. كانت الأهداف الرئيسية لهذه الدراسة هي: (1) تقييم عزلات الأكتينوبكتيريا من نباتات الطماطم الصحية المزروعة في دولة الإمارات القادرة على افراز انزيم ACCD في المختبر؛ (2) اختبار عزلة الأكتينوبكتيريا الداخلية المنتجة لانزيم ACCD لقدرتها على انتاج منظمات نمو النبات (PGRs) فى المختبر؛ (3) اختبار قدرة شتلات الطماطم على النمو في ظروف عالية من الإجهاد الملحي باستخدام عزلة الأكتينوباكتيريا الداخلية الواعدة التي تنتج انزيم ACCD فى تجارب البيوت البلاستيكية. لتحقيق هذا، تم عزل خمسة وعشرين عزلة من الأكتينوباكتيريا التي تعيش داخل جذور نبات *Zygophyllum mandaveli* من منطقة سويحان في امارة أبو ظبي في دولة الإمارات العربية المتحدة. لقد أثبتت الاختبارات المخبرية أن اثنين من عزلات الاكتينوبكتيريا قادرة على إنتاج ACCD، مع تحمل تركيزات ملحية عالية جدا قد تصل إلى 8 ٪ من كلوريد الصوديوم. أظهرت تجارب البيوت البلاستيكية، أن العزلة الواعدة المنتجة لانزيم ACCD (المشار إليها باسم العزلة Z3-40) عملت على تحسين نمو نباتات الطماطم بوجود تركيزات ملوحة وبنتيجة ذات دلالة إحصائية معنوية ($P<0.05$) كان هذا واضحًا في زيادة الأوزان الجافة مرتين، وزادت أطوال الجذور تقريبًا 25٪ وعززت أيضًا أطوال البراعم. كما أظهرت النتائج أن الاكتينوبكتيريا المنتجة لانزيم ACCD،عملت على تخفيض مستوى ACC إلى الثلث داخل الجذور، بالمقارنة مع المعاملات التي لم يتم اضافة الاكتينوبكتيريا أو تلك التي تم اضافة الاكتينوبكتيريا غير المنتجة لانزيم ACCD. إن إنتاج ACCD بواسطة الأكتينوباكتيريا الداخلية وقدرتها على تعزيز نمو الطماطم في ظل الظروف المالحة من خلال انخفاض مستويات النبات من الACC وما يترتب على ذلك من خفض مستويات الإيثيلين الداخلي في الأنسجة النباتية. ومن المتوقع أن تسهم هذه الدراسة في تطوير استراتيجيات لاستخدام المياه الجوفية عالية الملوحة لإنتاج الخضروات في دولة الإمارات العربية المتحدة، مما يسمح للمزارعين باستخدام هذه المياه للري.

مفاهيم البحث الرئيسية: ACC deaminase، هرمون الإيثلين، الأكتينوبكتيريا، هرمونات النمو النباتية، طماطم

Author Profile

Amira Hassan is currently a Research Assistant at the United Arab Emirates University, UAE. She has more than three years of research experience working to solve most important research challenges. Amira has participated in the Annual Conference of the Ecology of Soil Microorganisms 2022 Conference (Prague, Czech Republic); and SfAM's ECS Research Symposium Conference (Cardiff, Wales) in June 2022. She has also participated in the UAE GSRC 2022 Conference (Dubai, UAE). She has published 2 papers in a top 5%-ranked journals. Amira lives in Al Ain, UAE. She has received her bachelor's degree in Biotechnology from the Misr University for Science and Technology, Egypt.

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I owe thanks to a very special person, my husband, Dr. Mohamed Kamel for his continued and unfailing love, support and understanding during my pursuit of MSc degree that made the completion of thesis possible.

Dedication

To my beloved parents and family, dedicated to the memory of my mother, Hanaa Mohamed, who always believed in my ability to be successful in the academic arena. You are gone but your belief in me has made this journey possible

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List of Abbreviations

ABA	Abscisic Acid
ACCD	1-Aminocyclopropane-1-Carboxylate Deaminase
ACC	1-Aminocyclopropane-1-Carboxylic Acid
Cfu	Colony Forming Units
CWDEs	Cell-Wall Degrading Enzymes
DF	Dworkin and Foster's salts minimal agar medium
ECe	Electrical Conductivity
ET	Ethylene
HPLC	High-Performance Liquid Chromatography
IAA	Indole Acetic Acid
ISR	Induced Systemic Resistance
$MgSO_4$	Magnesium Sulfate
PGRs	Plant Growth Regulators
PGPB/R	Plant Growth-Promoting Bacteria/Rhizobacteria
Put	Putrescine
NaOCl	Sodium Hypochlorite
$(NH_4)_2SO_4$	Ammonium Sulfate
SNA	Starch Nitrate Agar

Chapter 1: Introduction

1.1 Overview

Salinity is a key limiting factor for agricultural yield globally. Due to global climate change, soil salinity has increased at an alarming rate in the United Arab Emirates (UAE). Nowadays, there is a significant focus on plant stress tolerance induced by plant-associated endophytic microorganisms. These microbes perform a crucial role in defending plants from diverse environmental stressors. Inoculating plants with bacteria that are capable of producing 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase (ACCD) can reduce Ethylene (ET) levels. Hence, endophytic bacterial that exhibit ACCD activity may be an alternative solution to help reduce endogenous ET contents *in planta* following a particular environmental stress *i.e.*, salinity. The novelty of this research was based on the use of endophytic actinobacteria that would help improve the performance of crops under salinity stress in the UAE or elsewhere.

1.2 Statement of the Problem

Agriculture suffers from global climate change causing vulnerability to food. Excess salt concentration in soil and water resources turns fertile fields to barren lands. Salinity is also associated with osmotic and ionic stresses in plants, which may inhibit plant growth. Osmotic stress occurs immediately upon exposure to salinity, whereas ionic stress arises after several days of exposure as a result of sodium (Na^+) and chloride (Cl^-) ions accumulation inside the cell. Osmotic stress affects the water balance inside the cell and reduces cell turgor pressure, cell elongation and cellular division rates. It has been reported that ionic stress modulates ion homeostasis inside the cell, resulting in changes in hormonal status, transpiration, photosynthesis, nutrient translocation and other metabolic processes. To remedy this, bacteria can be added to root to lower the levels of stress hormone ET in plant tissues. the utilization of plant growth-promoting microorganisms in agriculture can be of low-cost and eco-friendly technology to reinforce crop productivity in saline areas. Therefore, the hypothesis of this research is that the tomato plants have the ability to tolerate soil salinity in the UAE when inoculated with the endophytic actinobacterial isolate that produces ACCD.

1.3 Research Objectives

The overall objective of the research is to screen for endophytic actinobacterial isolates and assess their abilities to tolerate soil salinity in tomato plants in the UAE.

The specific aims are to:

(1) Evaluate endophytic actinobacterial isolates from healthy tomato plants cultivated in the UAE that are capable of producing ACCD *in vitro*.

(2) Test the isolated endophytic actinobacteria possessing ACCD for their potential of producing Plant Growth Regulators (PGRs) *in vitro*.

(3) Test the ability of tomato seedlings growing under high salt stress conditions using the most promising endophytic actinobacterial isolate producing ACCD in the greenhouse.

This research will also update the scientific information on different aspects of the tomato production chain from farm to consumers and would help improve the performance of crops under salinity stress in the UAE.

1.4 Relevant Literature

The beginning of the 21st century is distinguished by the worldwide depletion of water resources, environmental pollution, and the rising salinization of soil and water. Two risks to agricultural sustainability are an increase in the human population and a decrease in the agricultural area suitable for production (Shahbaz & Ashraf, 2013; Ullah et al., 2021). Different environmental stresses, such as strong winds, temperature extremes, salinization, droughts, and floods, have impacted agricultural crop production and cultivation. Salinization is one of the most damaging environmental stresses and usually causes a substantial reduction in cultivated land area, crop productivity, and crop quality (Yamaguchi & Blumwald, 2005; Shahbaz & Ashraf, 2013). Irrigation and soil management strategies have improved agricultural productivity on salinity-affected soils, but getting an extra benefit from these measures seems challenging (Zahir et al., 2008).

Soil deterioration is a key limiting factor in global agricultural production for all main crops (Bacilio et al., 2004). The overwhelming need to feed the world's rising

population while also preventing soil pollution, soil salinity, and desertification has made plant and soil productivity research critical. In such cases, appropriate biotechnology is required not only to enhance crop production but rather to promote soil health via interactions between plant roots and soil microorganisms (Lugtenberg et al., 2002).

1.4.1 Soil Salinity and its Impact on Plants

Soil salinity limits agricultural production and is responsible for more crop losses than any other cause. The threshold amount of salinity varies from plant to plant. The majority of cereal and legume plants are sensitive at 4 dS m^{-1} electrical conductivity (ECe; approximately 40 mM NaCl/0.2 MPa osmotic pressure), whereas vegetable plants are sensitive at 1–2.5 dS m^{-1} ECe (Etesami & Noori, 2019). Natural and artificial activities both contribute to soil salinity.

Natural processes such as weathering of rocks and minerals, more unpredictability in temperatures, changes in precipitation patterns, wind-borne salt from the seas, and the inclusion of saltwater or brackish water in coastal locations, are key sources of salinity. These activities discharge salts into the soil and groundwater, where they build over time. These mechanisms have a greater impact in arid and semi-arid locations. By the end of the twenty-first century, climate change is expected to raise global temperatures by 1.4–5.8°C and sea levels by 1.8–5.9 mm $year^{-1}$ (Teh & Koh, 2016).

As the temperature increases, more groundwater evaporates and salt deposits increase year after year. Furthermore, heavy rainfall hastens the leaching of salts from the soil into water sources (Goud et al., 2022). Human activities such as irregular irrigation with low-quality water, land clearance, insufficient drainage, and other bad agricultural practices contribute to secondary salinization. Irrigated areas are more susceptible to salinity than drylands. Excessive irrigation and poor drainage procedures raise the water table. Salts migrate from underground to the root zone or collect in the topsoil when the water table rises. Irrigation with inadequate water (salt-rich) causes salt deposition in topsoil. As a result, irrigated salinity has been identified as a severe issue since irrigated land delivers a significant quantity of food to the globe (Vaishnav et al., 2017).

Salt build-up worsens soil physical conditions while increasing alkalinity. During high salinity, cations in the soil, such as Ca^{2+} and Mg^{2+}, are replaced by Na^{+} ions, causing soil particle dispersion to rise (Rengasamy et al., 2022). Furthermore, salt promotes soil compaction while decreasing hydraulic conductivity and oxygen availability in the root zone. Plants suffer from nutritional deficits and salt toxicity as a result of these soil changes. The alkaline state has a significant impact on plant nutrient availability. Major nutrients are accessible at pH levels ranging from 6.5 to 7.5. Most cations, such as potassium (K), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn), become strongly attached to the soil when the pH rises over the optimum range, making them less accessible to plants and soil biota (Desai et al., 2022). Furthermore, salt has a detrimental impact on the nodulation process by lowering the nitrogenase enzyme activity of symbiotic bacteria, which results in a decrease in the nitrogen (N) content of the plants (Vaishnav et al., 2017). As a result, salinity not only affects crop productivity but also has a negative impact on soil microorganisms.

Under salt stress, agricultural crops have a range of reactions. Salinity not only reduces the agricultural productivity of most crops, but it also has an impact on soil physicochemical features and the area's ecological balance. Low agricultural production, low economic returns, and soil erosion are all consequences of salinity. Salinity impacts are caused by complex interactions among morphological, physiological, and biochemical processes such as seed germination, plant development, and water and nutrient intake (Akbarimoghaddam et al., 2011). Soil salinity causes ion toxicity, osmotic stress, nutritional deficiency (N, Ca, K, P, Fe and Zn), and oxidative stress in plants, limiting water absorption from the soil. Plant phosphorus (P) absorption is considerably reduced by soil salinity because phosphate ions precipitate with Ca ions (Bano & Fatima, 2009). Some elements, such as Na, Cl and boron (B), are particularly hazardous to plants. Excess Na in cell walls can quickly lead to osmotic stress and cell death.

Plants that are sensitive to certain elements may be harmed even at low salt concentrations if the soil has enough of the poisonous element. Because many salts are also plant nutrients, excessive salt levels in the soil may disrupt nutritional balance or interfere with nutrient absorption. Salinity has also an effect on photosynthesis, mostly

by decreasing leaf area, chlorophyll content, and stomatal conductance, and to a lesser extent by decreasing photosystem II effectiveness (Netondo et al., 2004).

1.5 Soils of the UAE: Orders and Groups

The UAE is located in an arid region with low-lying sandy deserts, extensive salts-flats in the coastal areas, gravelly plains covering wide areas in both the far west and east of the Emirate, and alluvial plains. These different landscape features strongly suggest the occurrence of soil diversity in the Emirate. The environment in the UAE is characterized by poor soil, low precipitation rate, extremely high temperatures, and a lack of natural waterways. All these features have a significant impact on the Emirati agricultural sector.

Soils in the UAE are formed from two orders called Aridisols and Entisols. These orders, in turn, are broken down into 6 suborders, 10 great groups, 41 sub-groups and 74 series. The 10 great groups are Aquisalid, Calcigypsids, Haplocalcids, Haplocambids, Haplogypsids, Haplosalids, Petrocalcids, Petrogypsids, Torriorthentssoils and Torripsamments soils (Figure 1). Aquisalid are saline or highly saline (salic horizon) soils found in coastal and inland salt flats (sabkhas). Despite the availability of groundwater within 1 meter of the soil surface, the high salt of these soils causes them to be physiologically dry. The majority of the textures are loamy or sandy. Because of the near-surface saline groundwater with a high salt concentration, aquisalids are deemed permanently unsuitable for irrigated agriculture (AD128), Aquisalids cover about 3% of the UAE soils (Emirates Soil Museum).

Haplosalids are extremely saline. Within one meter of the soil surface, haplosalids have a salt concentration (salic horizon), and are physiologically dry. The majority of the textures are loamy or sandy. This type of soil is deemed permanently unfavourable for irrigated agriculture (AD146), Haplosalids cover about 5.4% of the UAE (Emirates Soil Museum). Saline soils are the most common feature that can be recorded along the coastline of Emirates. The evaporation and continuous seawater intrusion, reaction between the sediment and the highly saline groundwater has produced these very strongly saline soils (Shahid et al., 2014).

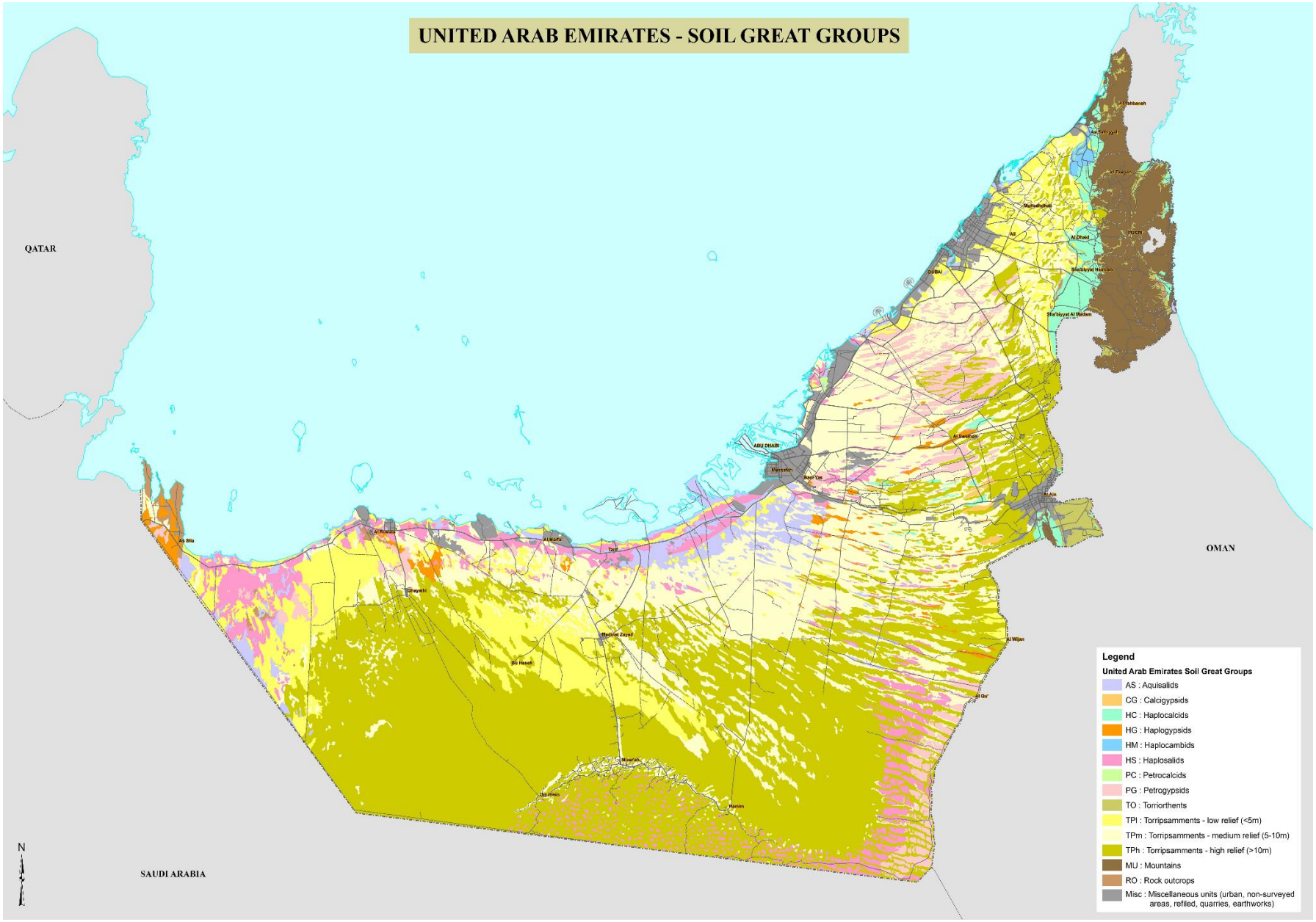

Figure 1: The UAE soil map showing the 10 great groups along with the sub-groups and series (Emirates Soil Museum)

1.6 The Role of ET in Plant Growth and Environmental Stresses

Plants are susceptible to a variety of environmental pressures, which may be classified as biotic or abiotic. Pathogenic fungi and bacteria are classified as biotic stresses, while abiotic challenges include salt, floods, drought, severe hot and cold temperatures, soil alkalinity, and heavy metal pollution (Glazebrook, 2005; Phour & Sindhu, 2022). Plants tend to develop response mechanisms, such as overproduction of the ET hormone, when exposed to one or more of the aforementioned conditions (Glick et al., 2007).

Ethylene is a phytohormone that is generated by all higher plants and mediates several phases of plant growth and development. It is involved in seed germination, root hair formation, lateral root elongation, and nodulation. Ethylene is essential for leaf withering, flower wilting, fruit ripening, and the creation of volatile chemicals linked to fruit fragrance (Arshad & Frankenberger, 2002). This phytohormone is only necessary in low concentrations, under any stress scenario; however, ET is generated in large amounts as part of the plant's response. This has a negative impact on plant development, manifesting as a decrease in root elongation and an acceleration of aging, senescence, and abscission (Arshad & Frankenberger, 2002; Glick et al., 2007). As a result, depending on its quantity in tissues and the physiological stage of the plant, ET may have a stimulatory or inhibitory impact on plants (Arshad & Frankenberger, 2002). As a consequence, any mechanism that decreases endogenous ET levels would have a negative impact on plant growth inhibition under stress circumstances (Glick et al., 2007).

1.6.1 In Planta ET Production

Ethylene is derived from its precursor, ACC, in plant tissues (Ma et al., 2003). The stress hormone ET is generated in two peaks in response to severe temperatures, soil pollution, mechanical wounding, and other biotic and abiotic stress conditions (Abeles et al., 1992; Rachappanavar et al., 2022). The initial peak occurs within a few hours after stimulus exposure and is lower in number and frequently difficult to detect. This peak functions as a stress signal, triggering the transcription of plant-protection genes such as Induced Systemic Resistance (ISR) (Glick et al., 2007). The second peak is greater and

begins a few days after the stress is introduced. This apex has growth inhibitory effects as well as visible plant damage.

1.7 Mechanisms of Salinity Tolerance in Plants

Salt stress may be caused by high soil salinity, which compromises numerous important cellular activities in plants. By interfering with a variety of physiological, biochemical, and metabolic processes (Pandey et al., 2015), many plant species; however, have developed ways to tolerate salt stress and may therefore flourish in saline soils (Zhao et al., 2020). Excess salt sensing causes plants to activate a signaling network and a comprehensive response, which includes the production of a variety of chemicals that help minimize the effects of excessive soil salinity and preserve cellular homeostasis (Zhu, 2016). The plant cell wall is a complex structure that performs several tasks during the plant's life cycle. Plant cell walls are vital for maintaining cell shape by resisting internal hydrostatic pressures and protecting cells in response to environmental stresses (Ezquer et al., 2020). Primary metabolites play a function in plant growth and development, while secondary metabolites are derived from primary metabolites, and both primary and secondary metabolites play important roles in plant adaptation to environmental stresses, including salt stress (Kumari et al., 2015; Ashraf et al., 2018). However, a lack of specific knowledge about the metabolic changes that occur in plants in response to environmental stresses hinders our understanding of how plants react to environmental stresses, particularly salinity stress.

To improve plant salinity stress tolerance, many techniques, including chemical priming and genetic engineering, have been used (Nguyen et al., 2018). Chemical priming may make plants more resistant to salinity stress. Natural metabolites and compounds and synthetic compounds have shown an outstanding chance to boost salt tolerance in many models and major agronomic crop plants without modifying their genomes. Furthermore, contemporary efforts have been made in plant genetic engineering techniques to improve plant tolerance to many kinds of abiotic stressors, including SS, based on changes in the expression levels of genes linked with osmoregulation, metabolic pathways, and metabolites (Nguyen et al., 2018). By activating multiple signaling pathways involved in stress detection, signal transduction,

osmotic control, and antioxidant enzyme synthesis, the genetic engineering technique has the potential to improve SS tolerance in crops (Ishaku et al., 2020).

In agronomy, crops treated with priming agents may activate many physiological and biochemical processes; hence, improving salt stress tolerance for crop stress management (Savvides et al., 2016). Furthermore, determining the activities of a specific gene or a group of genes, as well as the endogenous metabolites linked with them, would help in the investigation of the processes governing complex physiological, biochemical, and phenotypic features (Nguyen et al., 2018). By modifying the amounts of related transcripts, metabolite production, and enzyme levels for membrane lipid biosynthesis, metabolic gene modification and priming agents may boost plant stress tolerance (Nguyen et al., 2018).

Plants alter their physiological, biochemical, and molecular systems in response to salinity. Plants retain their water content under osmotic stress by undergoing morphological changes such as reduced cell elongation and division, growth inhibition of young leaves, shoot branches, and lateral root development, and stomatal closure (Wahab et al., 2022). These changes initially help the plants survive for a few days by conserving moisture in the soil and preventing further salt content increases. Furthermore, plants maintain a shoot-to-root ratio for survival under salt stress since a larger root accumulates more salts and will not be able to transfer to the vegetative growth (Moya et al., 1999). When compared to sensitive plants, salt-tolerant plants have a lower shoot: root ratio. Plant phenotypic changes are regulated by a variety of phytohormones, including auxin, Gibberellin (GA), Cytokinin (CK), Abscisic Acid (ABA), and ET (Wáskiewicz et al., 2016). The synthesis of phytohormones is interconnected; where the play a major role in integrated signaling pathways.

Auxins and CKs are engaged in a variety of growth processes such as cell division, differentiation, expansion, and so on. Auxins are primarily synthesized in mature and growing tissues, where they promote the production of lateral primordia (Dodd, 2003). ABA is often created during times of stress and activates adaptive responses for survival. During osmotic stress, ABA preserves the water potential within the cell by lowering transpiration activity through stomatal closure and even influences

photosynthetic rate (Pliego et al., 2011). By decreasing gibberellic acid concentration, ABA also suppresses leaf growth and shoot development. When present in high amounts, ET is a gaseous hormone that suppresses plant development. At concentrations below the threshold level, it, however, acts as a signaling molecule, regulating physiological responses like blooming and seed dormancy, among other things.

Salt ions are trapped within vacuoles at the cellular level, disrupting the cell's osmotic equilibrium. As a result, water leaks from the cytoplasm into the extracellular space, causing cell dehydration. Plants collect low molecular weight organic molecules in their cytoplasm that are compatible with metabolic activity, known as "compatible solutes" to sustain such an osmotic pressure. Proline, glycine betaine, trehalose, mannitol, and sucrose are the most prevalent solutes that accumulate under osmotic stress (Munns & Tester, 2008). These chemicals are accumulated in larger quantities in halophytes (above 40 mM) than in glycophytes (up to 10 mM) (Rhodes et al., 2002). Osmoprotectants for membranes, proteins, and enzymes are also provided by compatible solutes. They aid in the stabilization of the subcellular structure during dehydration and scavenge free radicals to protect plants from oxidative damage.

1.7.1 Metabolic Reprogramming in Response to Salt Stress

Environmental stresses, such as salinity, drought, and high temperatures may cause hyperaccumulation of a variety of metabolites in plants (Krasensky & Jonak, 2012). Plant tolerance to salinity stress is generally predicated on their ability to maintain a suitable level of primary and secondary metabolic processes as well as defensive responses (Singh et al., 2020).

Plants generate primary and secondary metabolites as excretory products throughout the evolution of SS, which exude from shoots, roots, and leaves at various phases of plant development (Singh et al., 2020). Because metabolites are by-products of different cellular activities, the plant metabolome is often regarded as the link between a plant's genotype and phenotype (Arbona et al., 2009). Thus, metabolomic analysis may assist in examining and uncovering major differences between SS-tolerant and SS-sensitive plants, as well as connecting the genotypic and phenotypic alterations that occur in plants reacting to salt stress (Llanes et al., 2018). Plant species and genotypes

targeted and non-targeted metabolomics have been utilized to investigate the metabolic reprogramming induced by salt stress. Targeted metabolomics is a tool for detecting, measuring, and interpreting particular or known chemicals in stressed plants (Van Meulebroek et al., 2016).

Non-targeted metabolomics, on the other hand, can offer a wide overview of the most abundant metabolites seen in plants under salt stress compared to unstressed control plants (Pandey et al., 2015). As a consequence, employing plant metabolomics to investigate changes in the levels of both primary and secondary metabolites is crucial for our knowledge of metabolic reprogramming in stressed plants (Pal et al., 2016).

1.7.2 Primary Metabolites and Their Response to Salt Stress

Primary metabolites are required for the normal function of plant cells and are directly involved in a variety of biochemical and physiological processes, such as photosynthesis and respiration, by providing energy and precursors for the biosynthesis of new macromolecules required for plant developmental processes (Apel & Hirt, 2004; Kumar et al., 2017). Primary metabolites that may act as osmolytes and osmoprotectants in plants exposed to abiotic stresses include sugars (mono-, di-, and tri-saccharides), polyols (such as sorbitol and mannitol), and amino acids such as proline (Shulaev et al., 2008; Gupta & Huang, 2014).

1.7.3 Carbohydrates: Compatible Solute Accumulation Under Salt Stress

Salt stress has a negative impact on carbohydrate metabolism in plants, and the build-up of sugars and polyols is important in osmotic adjustment, carbon (C) storage, and free radical scavenging (Ahanger et al., 2018; Tammam et al., 2022). To manage osmotic stress, maintain cell turgor pressure, and help in cell membrane stabilization, plants exposed to salt stress accumulate various soluble sugars such as sucrose, trehalose, and raffinose, as well as sugar alcohols such as sorbitol and mannitol (Slama et al., 2015; Ahanger et al., 2018). The most basic metabolic stress adaptation response found in plants is the production of suitable solutes or osmolytes. In a number of investigations, carbohydrates such as hexoses (fructose and glucose), disaccharides

(sucrose and trehalose), and oligosaccharides (raffinose and stachyose) have been identified as key osmolytes (Patton et al., 2007; Ahanger et al., 2018).

1.7.4 Amino Acid Production Under Salt Stress

Amino acids are critical metabolites in plants not just for protein synthesis and other vital cellular activities (Ahanger et al., 2018), but also as osmolytes to balance cellular osmotic potential and govern ion transport, as well as scavengers of reactive oxygen species formed in salt stressed-plants. Proline, for example, is generally recognized as an osmolyte that accumulates and shields plant cells from the effects of salt. Using four barley genotypes (CM72, Gairdner, XZ16, and XZ169), researchers revealed the relevance of variations in AA levels and the coordination of amino acid metabolism in plants under salt stress (Wu et al., 2013). Proline levels rose in all four genotypes in response to SS, while changes in the levels of other amino acids, such as alanine, aspartate, glutamate, threonine, and valine, were genotype-dependent (Wu et al., 2013). In comparable research of two genotypes, *Glycine max* (C08) and *Glycine soja* (W05), grown under salt stress conditions, alanine content was reduced in both genotypes; whereas serine and glycine rose exclusively in the W05 genotype (Lu et al., 2013). Furthermore, Cao et al. (2017) found that the quantities of eight amino acids and amines increased in all barley types when subjected to salt stress.

1.8 Model Plants Used in this Research

1.8.1 Tomato

In the present study, tomato (*Solanum lycopersicum*) was used as a model plant to investigate the growth promotion and salinity tolerance by actinobacteria isolated from the UAE soil. tomato is the most frequently planted and consumed vegetable in the world. Tomatoes are recognized as an essential food crop for all sectors of the economy, regardless of whether industrialized or poor nations. The large area under cultivation of approximately 4.2 million hectares produces 100 million tons per year. Tomato as a fleshy vegetable has diverse applications in food and fodder (Chandrasekaran et al., 2021). Vitamins C and E, β-carotene, lycopene, flavonoids, and lutein are all health-promoting components of tomato. The macronutrients [phosphorous (P), magnesium

(Mg), and calcium (Ca) and K] and trace elements (Cu, Fe, Mn and Zn) highlight the biocompatibility and sustainability of tomato (Odriozola-Serrano et al., 2009).

Tomato production and yield, despite variations in region-specificity, soil fertility, and productivity, are affected by stress complications such as drought, salt stress, temperature, and environmental complexities, ultimately affecting food security. Alleviation of abiotic stress can be addressed as a large-scale barrier in tomato production worldwide, demanding an effective alternate way in efficient plant stress abatement. Abiotic stress factors are on rise, emphasizing the need to halt the devastating predicaments of salt stress and soil contamination; thus, accounting for vulnerability in sustainable agriculture of over 10% of arable land, resulting in major yield loss of nearly 50% (Chandrasekaran et al., 2021), including tomato. Improvement of plant tolerance to abiotic stressess remains a key focus of agricultural research.

1.8.2 Zygophyllum mandaveli

Zygophyllaceae, that belongs to Caltrop family, is a plant with around 25 genera and 240 species growing in semi-desert and Mediterranean conditions (Shawky et al., 2019). Species of the genus *Zygophyllum* belong to a group of succulent plants that are drought- or salt-tolerant that survive in difficult, arid climates. Several researchers have included *Zygophyllum* as an important component of desert vegetation. The prevalence of this genus (and species) might be ascribed to both their remarkable endurance to environmental challenges and their unpalatability. *Zygophyllum* is a genus of 100 species found in desert and steppe environments from the Mediterranean to central Asia, South Africa and Australia. Most of plants of the genus *Zygophyllum* are small perennial herbs with fleshy leaves and flowers (Shawky et al., 2019).

1.9 Salinity Tolerance by Plant Growth-Promoting Bacteria

Salt-tolerant plants are classified as either salt-excluders or salt-includers. The former group of plants avoid salt in order to adapt to saline environments, while includers absorb and sequester salt. All biochemical strategies for dealing with salt stress include (1) selective ion accumulation or exclusion; (2) control of ion uptake by roots and transport into leaves; (3) ability to work more efficiently with ions at the cellular and

whole levels; (4) synthase of compatible solutes; (5) change of membrane structure; (6) induction of antioxidative enzymes; and (7) initiation of plant hormones (Khan & Rizvi, 1994; Parida & Das, 2005). Salt tolerance varies depending on the ionic content of the soil, and the developmental stage and the overall health of plants.

The application of beneficial microorganisms to increase salt tolerance in plants may be a feasible alternative approach to reclaim salinity-prone lands under cultivation (Munns & Tester, 2008). Microorganisms inhabiting within plants significantly contribute to plant growth promotion and salinity tolerance (Shrivastava & Kumar, 2015). These microbes enhance soil–water–plant relationships, manipulate phytohormonal signaling and trigger several other mechanisms that can be employed in an integrated fashion to reinforce salt stress tolerance in plants (Pandey et al., 2015).

Plant Growth-Promoting Bacteria (PGPB) are bacteria that are useful for plants to help them develop via direct or indirect methods (Glick, 1995; Glick et al., 2007). The term "PGPB" refers to two types of bacteria that are classified based on their habitat in the soil. Those that have symbiotic interactions with plants, such as nodule-forming bacteria and Plant Growth Prompting Rhizobacteria (PGPR), and those that live freely in the soil around the root region as rhizobacteria or inside plant tissues as endophytes (Frommel et al., 1991; Lucy et al., 2004). In general, salt-tolerant plants have lower shoot: root ratios than sensitive plants. In plants, these morphological changes are controlled by phytohormones, such as auxin, GA, CK, ABA and ET (Zhao et al., 2020).

1.9.1 The Enzyme ACCD

In the event of a stress occurrence, it would be ideal to encourage plant development under stress circumstances by allowing the first harmful peak to occur while preventing the commencement of the second damaging peak. This might be accomplished by using PGPB containing the enzyme ACCD. A few hours after being exposed to high amounts of ACC, PGPB synthesizes and releases ACCD as the end result of a complicated transcriptional regulatory system. The ACCD enzyme hydrolyzes ACC to produce ammonia (NH_3) and α-ketobutyrate, which bacteria may utilize as N and C sources (Glick et al., 2007). Thus, ACCD-producing bacteria may decrease ET

levels, and their use as a treatment on stressed plants would diminish the inhibitory effects of ET (Glick et al., 2007).

1.10 Endophytic PGPB

Plant-bacteria interactions have been researched for decades. However, a thorough knowledge of the processes used by PGPB has remained somewhat elusive, making it difficult to fully utilize these complex relationships to increase plant development in an applied environment. Bacteria may favourably affect plant development and health, and plants can "choose" their microbiome in order to have beneficial bacterial colonizers, including those existing inside plant tissues (Marasco et al., 2012; Rashid et al., 2012).

1.10.1 Mechanisms Used by Endophytic PGPB

Endophytic PGPB can colonize healthy plant tissues without causing disease symptoms on the host plants (Bacon & Hinton, 2006). Similar to PGPR, endophytic PGPB employ several mechanisms directly and indirectly affecting plant growth and development. Such mechanisms may include production of organic molecules (Compant, Duffy et al., 2005), NH_3 (Marques et al., 2010), solubilization of P (Verma et al., 2001), production of siderophores (Lodewyckx et al., 2002) and production of phytohormones. In addition, endophytic PGPB may promote plant growth as a consequence of expressing the enzyme ACCD which cleaves ACC; and thereby decreases ET levels in the host plant (Sessitsch et al., 2005).

1.10.1.1 Indirect Mechanisms

Endophytic PGPB may indirectly boost plant development by lowering or suppress the detrimental effects of pathogenic infections on plants. This could be attributed to the generation of antifungal metabolites and Cell-Wall Degrading Enzymes (CWDEs) (Glick et al., 2007; El-Tarabily et al., 2010). Furthermore, endophytic PGPB produce siderophores that function as Fe chelators, preventing pathogenic fungi from multiplication (Matthijs et al., 2007).

1.10.1.2 Direct Mechanisms

Plant Growth Regulators (PGRs) or phytohormones such as auxins, GAs, CKs and polyamines may be secreted by, endophytic PGPB and directly boost plant development (Nassar et al., 2003; Kuklinsky-Sobral et al., 2004). They can also produce enzymes that control endogenous ET levels (Glick, 1995). Furthermore, PGPB may promote plant development by improving the capacity of plant roots to absorb nutrients (Glick et al., 2007). They may produce siderophores that chelate ions and enhance their absorption via plant roots (Matthijs et al., 2007). PGPB may also solubilize P so that it can be readily absorbed via the plant roots (Rodriguez & Fraga, 1999) and fix atmospheric N_2 (Dobbelaere et al., 2003).

Surprisingly, the considerable impact of endophytic PGPB can play a crucial role when plants are stressed and not in their best circumstances. Some endophytic PGPB are more successful than others because they have one or more plant growth-promoting mechanisms (Figure 2), and they may use various mechanisms during their life cycles (Glick et al., 2007).

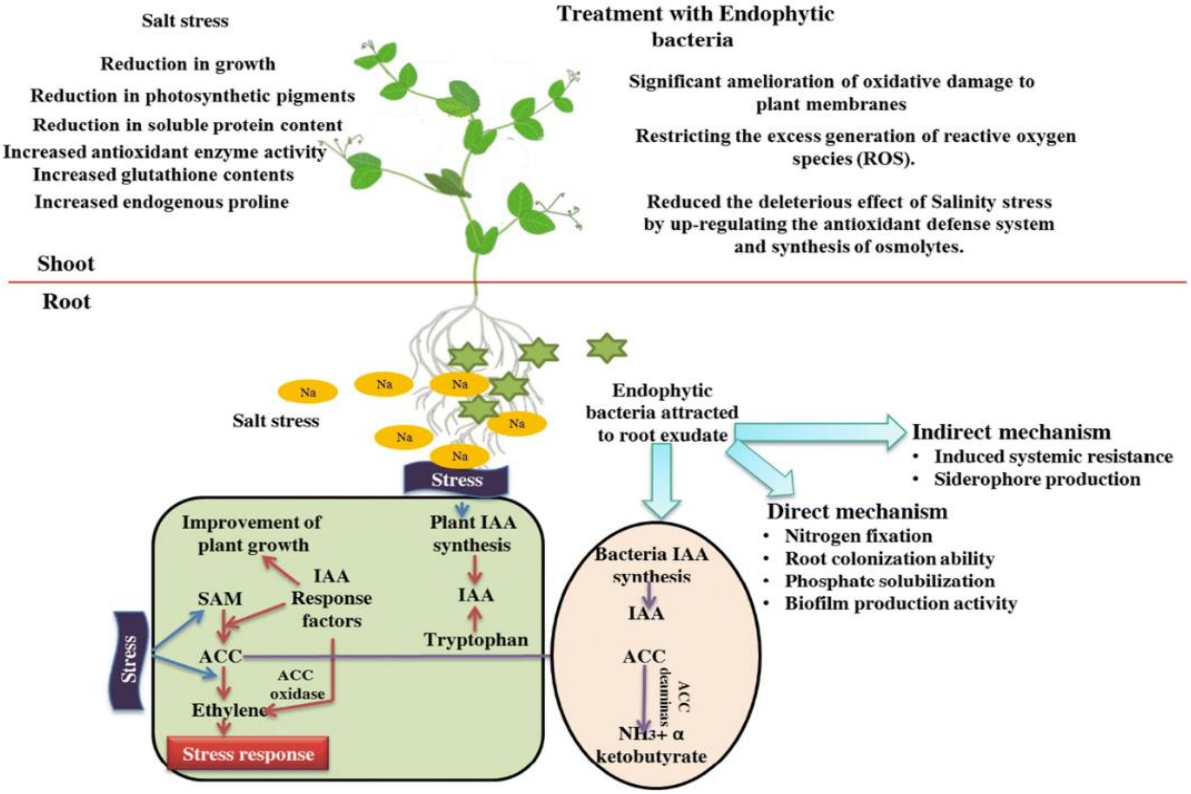

Figure 2: The effect of endophytic (actino) bacteria on plants under salinity stress conditions (adopted from Sofy et al., 2021)

Endophytic PGPB can colonize healthy plant tissues without causing disease symptoms on the host plants (Shulaev et al., 2008). Bacterial endophytes can provide several benefits to the host plant, particularly growth promotion and pathogen protection. Endophytes PGPB can communicate and interact with the plant more efficiently than rhizosphere-competent PGPB (Ali et al., 2012).

The main entrance sites for the endophytic bacterial population into plant tissues include primary roots and related lateral roots, as well as tissue lesions (Srensen & Sessitsch, 2015). Bacteria penetrate the root first, then colonize the stem, and finally colonize the leaf through the transpiration stream. As a result, the population densities of endophytic bacteria differ from tissue to tissue. Endophytes may also penetrate plants through stomata on leaves and young stems, lenticels in stems and root periderm, and germinating radicals and seeds (Compant et al., 2010). Endophytic colonization may alternatively be classified as obligatory, facultative, or passive.

1.11 Nature and Occurrence of Endophytic Bacteria

Endophytic bacteria are classified according to their significance in ecosystems and plant physiology. These bacteria populate in all plant compartments, most notably intercellularly and intracellularly of the inner plant tissues. Earlier, Lodewyckx et al. (2002) characterized isolation procedures and discovered 81 bacterial species that develop endophytic relationships with plants. Endophytic bacteria and plant associations encompass a wide range of bacterial taxa as well as host plants. Early research on the makeup of endophytic communities indicated that various plant hosts had a comparable population of bacterial endophytes (Mundt & Hinkle, 1976). *Bacillus* and *Pseudomonas* species have been discovered as commonly occurring bacteria in agricultural crops (Souza et al., 2013). The presence of various endophytic organisms is mostly determined by biotic and abiotic environmental variables in plants. A single host plant species may include numerous genera and species of endophytes, but the scope of the endophytic population may be determined by the tissue type of the plant or the season of isolation (Rosenblueth & Martinez-Romero, 2006). An extensive study of bacterial endophyte communities demonstrated that they colonize the entire plant, of which the roots often contain a higher number of species.

Previously, a majority of work has focused on identifying unculturable endophytes utilizing innovative metagenomic analytic methodologies in order to gain a clear picture of the variety of endophytic microbes (Akinsanya et al., 2015). Direct amplification of microbial DNA from plant tissue samples, along with the use of contemporary bioinformatics techniques, enables the investigation of bacterial community composition and phylogenetic structure inside plant organs or tissues (Sessitsch et al., 2012).

Tsurumaru et al. (2015) have demonstrated that endophytic colonization on the tap root of the sugar beet (*Beta vulgari* L.) is mainlky associated with alphaproteobacteria, followed by actinobacteria and betaproteobacteria. According to Maropola et al. (2015), the bacterial pathogens, *Agrobacterium*, *Erwinia*, *Herbaspirillum*, *Microbacterium*, *Pseudomonas*, *Sphingobacterium*, and *Stenotrophomonas* species dominated the sorghum root and stem microbiome.

1.11.1 Colonization of Plants by Endophytic Bacteria

Endophytic bacteria may easily have a direct positive influence on the plant host by living inside plant tissues, allowing them to be in intimate touch with the plant host in exchange for a steady source of nutrients. The process of colonization often begins at the roots and needs endophytic bacteria to recognize certain molecules in root exudates (de Weert et al., 2002; Rosenblueth & Martinez-Romero, 2006). These root exudates are produced by plants in order for them to interact with helpful microbes for their own ecological benefit (Compant, Reiter et al., 2005). Furthermore, endophytic bacteria have been reported to colonize the interior of the plant in a series of events similar to rhizosphere colonization by rhizobacteria (Hallmann et al., 1997). Endophytic colonization, on the other hand, is the result of a combination of environmental and genetic variables that enable bacteria to infiltrate the plant endosphere (Compant et al., 2010). Although endophytic bacteria often penetrate plants via the root zone, aerial plant components such as stems, leaves, flowers, and cotyledons may also be employed (Zinniel et al., 2002). Endophytic bacteria may now infect neighbouring plant tissues systemically once within the roots.

Bacterial endophytes are known to colonize plant roots after establishing themselves in the rhizosphere and rhizoplane, with subpopulations ranging from 10^5-10^7 Colony Forming Units (cfu) g^{-1} Fresh Weight (FW) (Hallmann, 2001). Upon bacterial adherence on the root surface, type IV pili-driven twitching motility can have access to the root entry points through lateral root emergence and wounds (Figure 3). Nonetheless, each endophytic bacteria has its own specific colonization pattern and preferred colonization sites (Zachow et al., 2010).

The penetration process into the host might be either passive or active. Passive penetration may occur via cracks at root emerging regions, root tips, or those caused by harmful organisms (Hardoim et al., 2008). Active penetration, on the other hand, is accomplished by competent endophytic bacteria using specialized attachment and growth machinery. Endophytic colonization and bacterial movement within host plants may be affected by the presence of lipopolysaccharides, flagella, pili, twitching motility, and quorum sensing (Duijff et al., 1997; Dörr et al., 1998; Böhm et al., 2007; Suárez-Moreno et al., 2010). Furthermore, it is known that the production of CWDEs, primarily pectinases and cellulases, is involved in bacterial penetration and dissemination inside the plant (Compant, Reiter et al., 2005). Although not verified experimentally, it has been claimed that endophytic bacteria generate lower quantities of CWDEs than phytopathogens; and hence, avoid activating plant defense mechanisms.

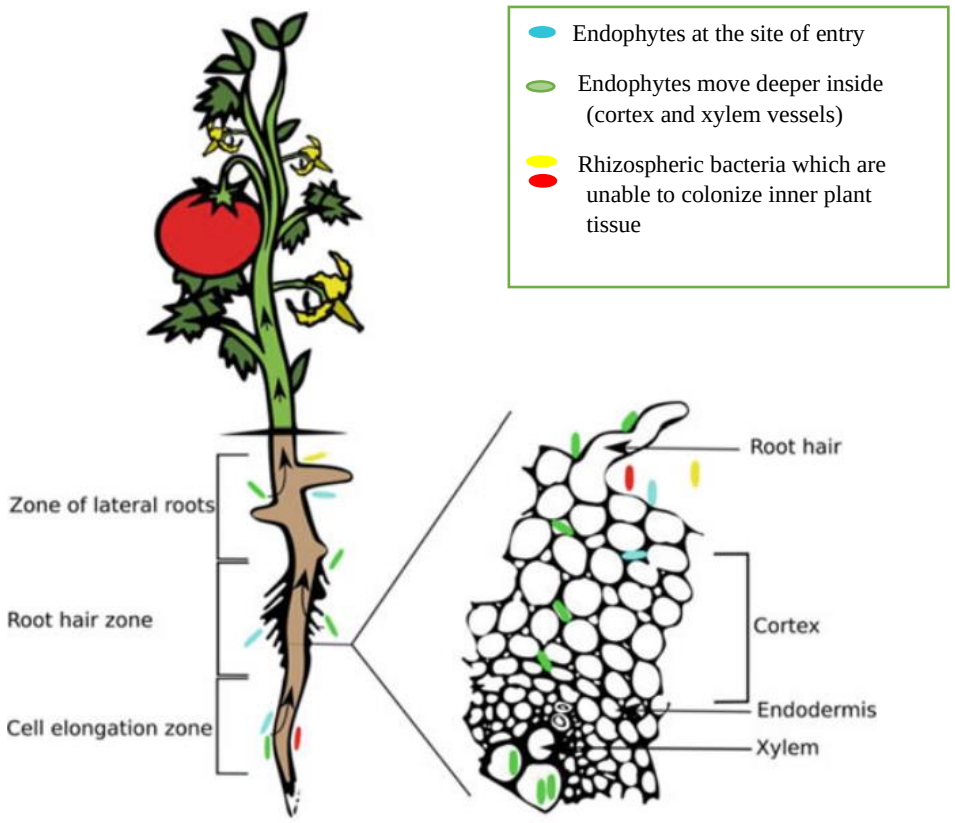

Figure 3: Endophytic (actino) bacterial colonization in plants (adopted from Mercado-Blanco & Lugtenberg, 2014)

Endophytic bacteria evade detection as pathogens by maintaining low cell densities ranging from 2-6 $\log_{10}$ cfu g^{-1} FW in comparison to pathogenic bacteria ranging from 7-10 $\log_{10}$ cfu g^{-1} FW (Zinniel et al., 2002). As a result, the presence of endophytic bacteria is governed by chance factors and bacterial genetic determinants that allow bacterial-plant interaction, resulting in active endophytic colonization (Hardoim et al., 2008). The plant host is also important in the selection of an endophytic partner, with the emission of specific root exudates and a selective plant defense response being important factors in the selection of certain endophytes (Rosenblueth & Martinez-Romero, 2006).

Endophytic bacteria may spread systemically after entering the roots and colonizing aboveground tissues. Under natural circumstances, they may produce stem and leaf population densities of 10^3–10^4 cfu g^{-1} FW (Compant et al., 2010). It is unclear if bacterial colonization of upper plant tissues has the same favorable impact on the plant host as root colonization. Due to the physiological requirements required to inhabit plant niches, only a few bacteria can colonize the aerial vegetative regions of their host plants

(Hallmann, 2001). Bacterial endophytes have been discovered in all of the plant species investigated. In the natural environment, an endophyte-free plant is therefore an uncommon exception (Partida-Martinez & Heil, 2011). In fact, a plant that lacks the accompanying beneficial bacteria is less equipped to cope with phytopathogens and is more vulnerable to stress situations (Timmusk et al., 2011). The sort of endophytic diversity present in a plant may be influenced by a number of variables, which are addressed in Figure 4.

1.11.2 Factors Affecting Endophytic Bacterial

Apart from the bacterial ability to colonize plants as endophytes, the host plant and environmental conditions can have a significant impact on the endophytic diversity of a certain plant. The kind of endophytic bacteria harbored by a host plant is determined by its age, genotype, geographical region, and also the tissue being studied (Hallmann & Berg, 2006). Furthermore, host plant growth stages can influence the endophytic life style, with nutrient-rich plant stages exhibiting higher bacterial diversity (Shi et al., 2014). Not only that, but environmental circumstances can impact a plant species' endophytic invaders. Penuelas et al. (2012) discovered that variations in climate had a substantial impact on the quantity and composition of endophytic bacteria inside leaf tissues. The approach employed to examine these bacteria is another major element influencing the detectable endophytic diversity of a plant. The type, concentration, and even duration of treatment time for a sterilizing agent used to recover bacteria can affect the spectrum of bacteria recovered from a plant (Hallmann et al., 1997; Hallmann & Berg, 2006).

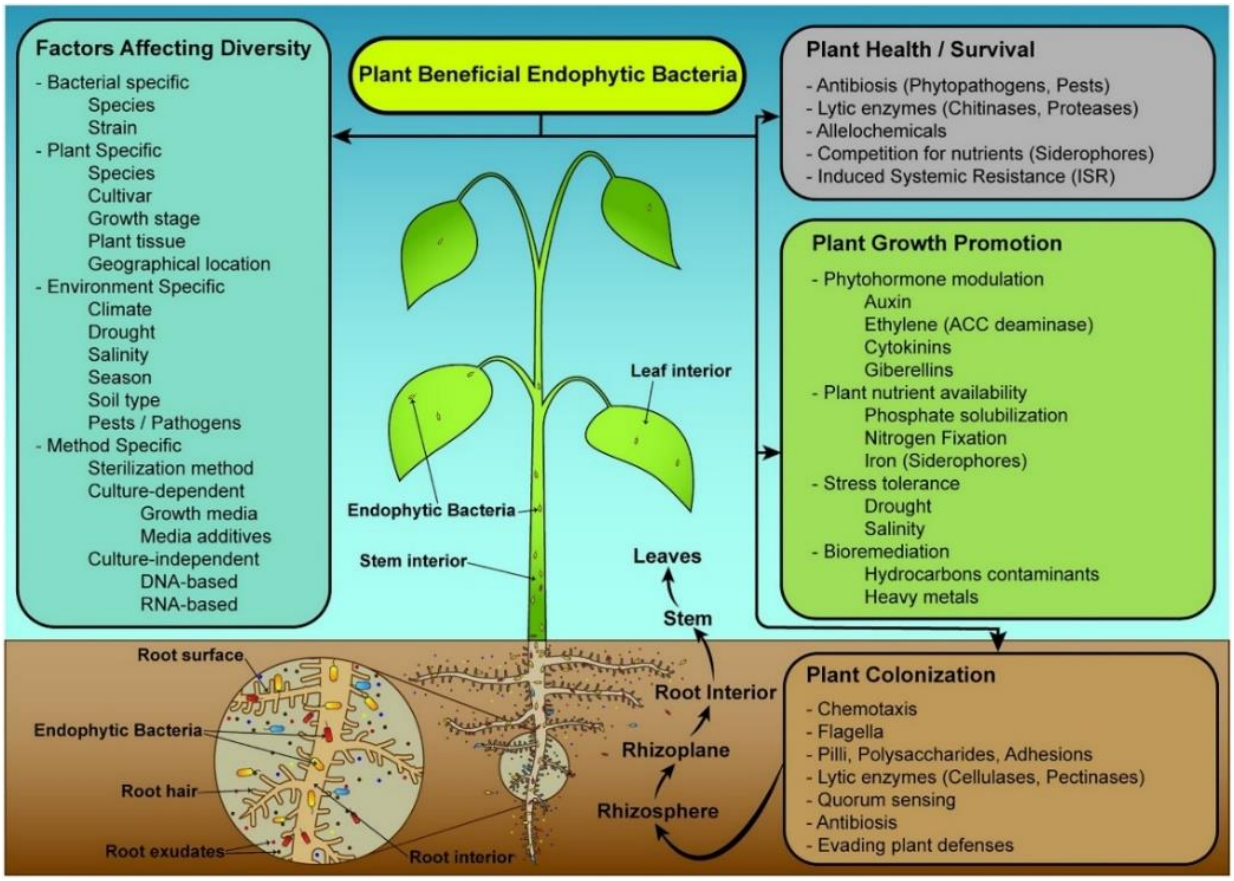

Figure 4: Mechanisms of plant growth promotion, colonization, and factors affecting the diversity of endophytic (actino) bacteria in host plants (adopted from Afzal et al., 2019)

Endophytic variety varies greatly across plant species growing in the same soil. Germida et al. (1998) discovered that canola and wheat plants growing in the same area had highly distinct endophyte bacterial communities. In addition, various cultivars of the same plant species cultivated in the same soil might have diverse endophytic bacterial residents. For example, Granér et al. (2003) discovered four different cultivars of *Brassica napus* with different endophytic bacterial occupants. Moreover, the type of soil utilized to grow a plant can influence its endophytic community. Rashid et al. (2012) reported several kinds of endophytic bacteria by cultivating a tomato cultivar in 15 different agricultural soils.

The preference imposed by the plant host in response to soil and stress conditions can also result in differences in endophytic communities. Siciliano et al. (2001) revealed that plants recruited endophytic bacteria with the requisite contaminant-degrading genes while growing in petroleum hydrocarbon polluted soil. In addition, wilt resistant oilseed

rape possessed a larger percentage of endophytic bacteria hostile to the wilt-causing *Verticillium longisporum* than susceptible cultivars (Granér et al., 2003). The presence of phytopathogens in plants has been identified as a critical component in the remodeling of endophytic bacterial populations. In their study, Bogas et al. (2015) observed that the reconstructed endophytic communities of asymptomatic and symptomatic *Paullinia cupana* plants challenged by *Colletotrichum* spp.

1.12 Actinobacteria: Classification and Identification

Actinobacteria are Gram-positive bacteria, and the genomes have a high G+C content in their DNA. They exhibit a variety of morphological differentiation, from simple cocci or rod to highly complex fragmenting hyphal (Xie & Pathom-Aree, 2021). Actinobacteria are widely distributed in both aquatic and terrestrial ecosystems (Mathew et al., 2020; El-Tarabily, Ramadan et al., 2021). The SA and NSA are the two primary groups of actinobacteria. The former accounts for more than 90% of the identified actinobacteria; whereas the latter is a tiny group accounting for only 5% of the actinobacteria recovered from isolation media (Alexander, 1977; El-Tarabily & Sivasithamparam, 2006). These reports identified *Streptomyces* 95.3%, *Micromonospora* 1.4%, *Nocardia* 1.98%, *Thermomonospora* 0.22%, *Actinoplanes* 0.2%, *Thermoactinomyces* 0.14%, *Streptosporangium* 0.10%, *Actinomadura* 0.1%, and *Microbispora* 0.18% of the times. Furthermore, Lechevalier & Lechevalier (1967) showed that NSA accounted for <0.2% of the 5000 actinobacterial soil isolates studied.

The identification of actinobacteria necessitates a thorough examination of their cultural, physiological, ecological, and morphological characteristics (Goodfellow & Cross, 1984; Goodfellow, 1989). Actinobacteria may also be distinguished by the kind of peptidoglycan, phospholipid, fatty acid pattern, wall chemotype, and whole-cell sugar pattern (Goodfellow & Cross, 1984). The use of *16S RNA* and DNA/DNA-hybridization analyses made it possible in the identification of actinobacteria to the species level (Maidak et al., 1999). In general, actinobacteria are responsible for producing over 1/2 of the bioactive secondary metabolites, antibiotics, anticancer agents, and enzymes identified in microbial sources (Hemashenpagam, 2011).

Actinobacteria produce 45% of the thousands of physiologically active chemicals found in microorganisms (Gebreyohannes et al., 2013). Actinobacteria have the most morphological differentiation among prokaryotic bacteria, owing to the production of diverse structures such as hyphae, mycelia, and a wide variety of spore types.

1.12.1 Endophytic Actinobacterial Diversity in Arid Ecosystems

Actinobacteria are mostly free-living microorganisms found in a variety of settings. Soil is the most important reservoir for actinobacteria and also represents the zone of the most active interaction between actinobacteria and plant root systems. The endophytic trait has been described mostly in the Actinobacteria class (Singh & Dubey, 2018). With the advancement of molecular identification tools, other endophytic candidates have been revealed as the following: *Thermoleophilia* class, e.g., *Solirubrobacter phytolaccae* (Wei et al., 2014) and *Patulibacter* (Ferrando et al., 2012); and *Rubrobacteria* class e.g., *Rubrobacteria* genus (Girija et al., 2018) and *Coriobacteria* class (Ren et al., 2018).

The ability of actinobacteria to survive not only in mesophilic conditions but also in thermophilic conditions reaching 60°C is an encouraging trait for their use as inocula (Edwards, 1993), and are considered aridity–winners (Marasco et al., 2021). Acidophilic actinobacteria are important in the inoculation of plants growing in acidic soil (Bull, 2011). Many halotolerant actinobacteria have been identified from saline environments and have been shown to be effective crop protection agents on plants under stress (Siddikee et al., 2010; Zhou et al., 2017; Qin et al., 2018). Some actinobacteria with extremophile nature might be a helpful tool for recovering damaged regions under harsh environmental circumstances; thus, boosting crop yield under a variety of stress situations, including high temperatures, pH, salt, and drought (Qin et al., 2011).

1.12.2 Actinobacteria as Plant Growth Promoting Bacteria

Actinobacteria are capable of promoting plant growth directly through a variety of mechanisms such as production of auxins such as Indole Acetic Acid (IAA), phytohormone, ACCD, induction of ISR, P solubilization, siderophore production, N fixation, antifungal activity, volatile organic compound production and promoting beneficial plant-microbe symbioses (Bhattacharyya & Jha, 2012; Kumar & Singh, 2020).

PGP actinobacteria could be used as biofertilizers by providing macro- and micro-nutrients such as biological N fixation (Vessey, 2003) and utilization of insoluble P (Chang & Yang, 2009), as biostimulants or phytostimulants by improving nutrient use and efficiency through the production of phytohormones (Lugtenberg et al., 2002), and as biocontrol agents against phytopathogens using antibiotics or siderophores (Vessey, 2003).

1.12.2.1 P Solubilization

Phosphorous is one of the most prevalent metallic elements in the earth's crust, and it may be found in both inorganic and biological forms in soils. It is used or absorbed by plants in its inorganic form, orthophosphate (H_2PO^{4-} and HPO_4^{2-}) (Hinsinger, 2001). The principal characteristics related with phosphorous nutrition include photosynthesis, energy transfer, signal transduction, nitrogen fixation in legumes, crop quality, and resistance to plant diseases (Khan et al., 2014). Microorganisms play an essential role in P solubilization by secreting organic acids, either by (I) decreasing the pH, (ii) chelating cations bound to P, or (iii) competing with P for adsorption sites on the soil. The decrease in medium pH shows that P-solubilizing bacteria secrete organic acids via a direct oxidation pathway on the cytoplasmic membrane (Zaidi et al., 2009). PGPR with P-solubilizing properties can give plants with soluble phosphate that can be easily absorbed through their root systems. The production of organic acids such as oxalic acid and citric acid is required for PGPBs to solubilize P. (Nimaichand et al., 2016). Through their hydroxyl groups, these acids chelate the cations attached to the phosphate, changing P into the soluble form (Santoyo et al., 2021).

1.12.3 Endophytic Actinobacteria Mitigating Saline Stress

Actinobacterial endophytes are well-acknowledged inoculants to promote plant growth and enhance their resistance toward various pathogens and environmental stresses (Mohamad et al., 2022). Endophytic actinobacterial have been investigated to stimulate plant growth through the activity of 1-aminocyclopropane-1- carboxylic acid (ACC) deaminase. This enzyme hydrolyzes ACC, which is the immediate biosynthetic precursor of the hormone ET in plant tissues, to ammonia and α-ketobutyrate. Inoculation of plants with ACC deaminase-producing PGPB lowers the levels of ACC,

reduces the harmful effects of ET synthesized as a consequence of stressful conditions as salinity (El-Tarabily et al., 2019).

ACC deaminase-producing PGPB have been tested to mitigate the inhibitory effects of salinity stresses on plant growth and development (Sarkar et al., 2018; Acuna et al., 2019). Hence, the introduction of ACC deaminase-producing PGPB may dramatically increase the productivity of plant crop.

Chapter 2: Methods

2.1 Root Sampling Procedure

From Sweihan area, Abu Dhabi, UAE (24°24'09 "N 55°05'42 "E), three root samples were collected from the highly salt tolerant plant, *Z. mandaveli*. According to Al-Alawi (2014), the groundwater in this area is considered as highly saline. Plant roots were washed with water, followed by surface-sterilization using 70% alcohol and then 1.05% Clorox (20% household bleach). Surface-sterilized roots were then washed 10 times with double deionized water and air-dried for 30 min in laminar air flow cabinet (El-Tarabily, Sham et al., 2021). Roots were immersed in a sterile phosphate buffered saline (PBS) solution with a pH of 7.0 for 10 min (Rennie et al., 1982). The roots were then surface-disinfested, as reported by Sardi et al. (1992). Each sample was sterile checked to ensure that the disinfestation processes were effective (Hallmann et al., 1997; Sturz et al., 1998).

2.2 Isolation and Preliminary Identification of Endophytic Actinobacteria from *Z. mandaveli* Roots

The roots were grinded as reported by Hallmann et al. (1997). The filtrate was serially diluted (10^{-2}, 10^{-3} and 10^{-4}) and aliquots of 0.2 ml were distributed with a sterile glass rod over the surface of Inorganic Salt Starch Agar (ISSA) (Küster, 1959) supplemented with 50 µg ml^{-1} of each nystatin and cycloheximide (Sigma-Aldrich Chemie GmbH, Germany). Three plates were dried in a laminar flow for 15 min before incubation at 28±2°C in dark for 7 days (El-Tarabily et al., 2019). All colonies were purified on oatmeal agar plates supplemented with 0.1% yeast extract (OMYEA; ISP medium 3) (Shirling & Gottlieb, 1966).

According to Cross (1989), actinobacteria were identified based on morphological and cultural traits based on the presence or absence of substrate mycelium, based on the observations of Prof. Khaled El-Tarabily (UAEU; Al Ain, UAE). Hyphae and spores of all isolates were kept in 20% glycerol at 70°C (Wellington & Williams, 1977).

2.3 Determination of NaCl Tolerance by Actinobacteria

Actinobacterial isolates were streaked in triplicate on ISSA medium supplemented with NaCl concentrations 40 g l^{-1} (4%) and 80 g l^{-1} (8%), and cultured at 28°C in the dark for 7 days (Williams et al., 1972). The cultures showing strong growth and sporulation on ISSA containing 4% NaCl (Sharma et al., 2016) were further used to determine their salinity tolerance to 8% NaCl. Isolates grew on 8% NaCl were only were only chosen for additional investigations.

2.4 Measurement of ACCD Synthesized by Actinobacteria

To screen for ACCD synthesis, 5-day-old actinobacterial isolates were streaked on the N-free Dworkin and Foster's salts minimum agar medium (DF) plates (Dworkin & Foster, 1958) supplemented with either ACC (3 mM; Sigma-Aldrich) or 2 g of $(NH_4)_2SO_4$ (control). The heat-labile ACC-filter sterilized (pore size 0.22 µm, Millipore Corporation, MA, USA) or $(NH4)_2SO_4$, the salt medium was autoclaved prior to adding. The plates were incubated in dark at 28°C for 7 days (El-Tarabily et al., 2019). Growth and sporulation on plates shows strong ACCD production. ACCD activity was quantified by monitoring the amount of α-ketobutyrate that was produced by ACC deamination following the derivatization of α-ketobutyrate with 2,4-dinitrophenylhydrazine (Sigma-Aldrich) as described by Honma and Shimomura (1978). Protein concentrations were measured using Bradford's method (1976).

2.5 Screening for P-solubilizing Actinobacteria

The aim of this experiment was to test phosphate-solubilizing ability by the Pikovskaya's agar medium (Pikovskaya, 1948) and tricalcium phosphate was substituted with insoluble rock phosphate as an indicator. P-solubilization was indicated by the absence of the blue color and the formation of a clear zone underneath the culture. The drop in pH and amount of released soluble P (Murphy & Riley, 1962) were used to assess the strains' ability to solubilize P.

2.6 Inoculate of Endophytic Isolates for Greenhouse Experiments

To produce inoculum for the greenhouse experiments, aliquots (4 ml) of 20% glycerol suspension of the isolates were inoculated into 250 ml of Starch Nitrate Broth

(SNB) (Küster, 1959) in 500-ml Erlenmeyer flasks and shaken on a rotary shaker at 250 rpm (El-Tarabily et al., 2019) for 5 days. In each sample, the concentration of actinobacteria was adjusted to 10^8 cfu ml^{-1}. A volume of 100 ml inoculum of each of the two actinobacterial isolate was applied to the seedlings as a soil drench. Due to the endophytic nature of the isolates, both were also injected in the surface-sterilized wounded crown area of the treated plants (Pleban et al., 1995; Downing & Thomson 2000). The scheme of the experiment can be found in Figure 5. Non-colonized SNB broth that had been autoclaved twice served as the control.

2.7 Evaluation of Actinobacterial Isolates to Different Salt Stress Regimes in The Greenhouse

The ability of the ACCD-producing isolate (Z3-40) inoculated in tomato plants (Castlemart II) (Agrimax Group S.L, USA) growing in different salt concentrations (0, 60 or 120 mM NaCl) was tested in the greenhouse. Each pot was watered with 100 mL of NaCl as a salt treatment twice per week.

The following treatments were carried out:

Control: Tomato seedlings treated with different salt treatments, without inoculation with any isolate.

E1-3EC: Tomato seedlings treated with different salt treatments and inoculated with E1-3EC (a -ACCD isolate).

Z3-40: Tomato seedlings treated with different salt treatments and inoculated with Z3-40 (a +ACCD isolate).

Three pots in which each containing two seedlings for each treatment were completely randomized. Treatments were carried out over a period of about 90 days (Figure 5). In Phase (I), actinobacterial isolate suspensions were administered once a week for four weeks in the first phase, in addition, to two times of injection in the crown region of tomato seedlings. This was followed by Phase (II), when seedlings were supplemented with different concentrations of salt treatments twice a week for two weeks. All control and inoculated seedlings were kept in a controlled greenhouse

environment at 27°C±3°C. All greenhouse trials were carried out in mid-February-May 2022.

Seedlings and fruits were collected by Week 12. Length and FW of shoots and roots were measured. Dry weight (DW) of shoots and roots were also recorded after 3 days of oven-drying at 70°C. Leaves and fruits were counted, and fruits were weighed out.

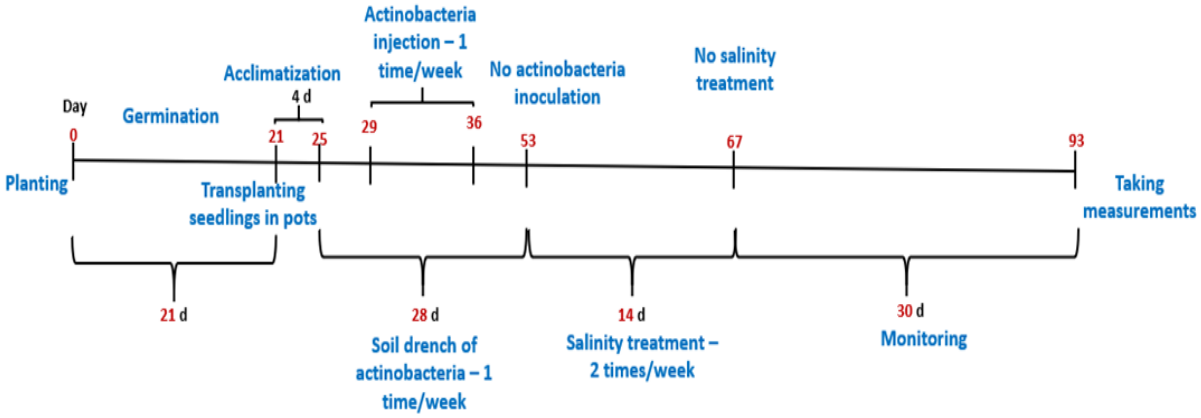

Figure 5: Schedule of the greenhouse experiment. The time (in days) of planting of seeds, transplanting and emergence of seedlings, application of actinobacterial isolates and salinity treatments; and harvesting of tomato seedlings for the greenhouse experiment

2.7.1 Measurement of Chlorophyll Contents from Leaves of Tomato Plants

The chlorophylls levels in the leaves of each treatment were measured according to Holden (1965). Briefly, 500 mg of fresh leaves were homogenized and kept in 50 mL flasks containing 25 mL of 80% acetone. These flasks were corked and kept in dark for 24 h; followed by centrifugation at 5000 *x g* for 15 min. The volume of the supernatant was made up to 40 mL with 80% acetone. The optical densities at wavelength of 663 (*chl a*) and 645 nm (*chl b*) (Holden, 1965) were measured using Shimadzu UV-2101/3101 PC scanning spectrophotometer (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). Chlorophyll content was expressed as mg g^{-1} FW.

2.7.2 Quantification of ACC From Root and Shoot Tissues of Tomato Plants

According to the method used by Lizada and Yang (1979), ACC was extracted from tissues of the terminal portions of the root and shoot tissues at the end of the greenhouse experiment. ACC derivatization was done by adding phenylisothiocyanate (Sigma-Aldrich) (Lanneluc-Sanson et al., 1986) using reverse-phase high-performance liquid chromatography (HPLC; Spectra Lab Scientific Inc., ON, Canada). This was carried out through the injection of 10 µL of the resulting phenylthiocarbamyl-ACC samples in dissolved cetonitrile onto a 10-µm reverse phase column (Waters Associates µ Bondapak C18, 4 mm x 30 cm) in a Waters Associates liquid chromatograph equipped with a differential UV detector set at 254-nm (Lanneluc-Sanson et al., 1986). A total of eight independent duplicate samples were examined.

2.8 Statistical Analysis

One-way ANOVA using SAS Software version 9 (SAS Institute Inc., NC, United States) was used to analyze the results obtained in this study. Mean values of treatments were compared using Fisher's protected Least Significant Difference (LSD) test at P=0.05 levels. Greenhouse experiments were repeated three times.

Chapter 3: Results

3.1 Actinobacteria Isolation

There were 25 different endophytic actinobacteria isolated from the ground root of *Z. mandaveli* on SNA media (Figure 6). No contamination in the sterility test was found, suggesting that the surface-sterilization procedures were appropriate. To determine the morphological characteristics, the isolated actinobacteria were cultured on OMYEA.

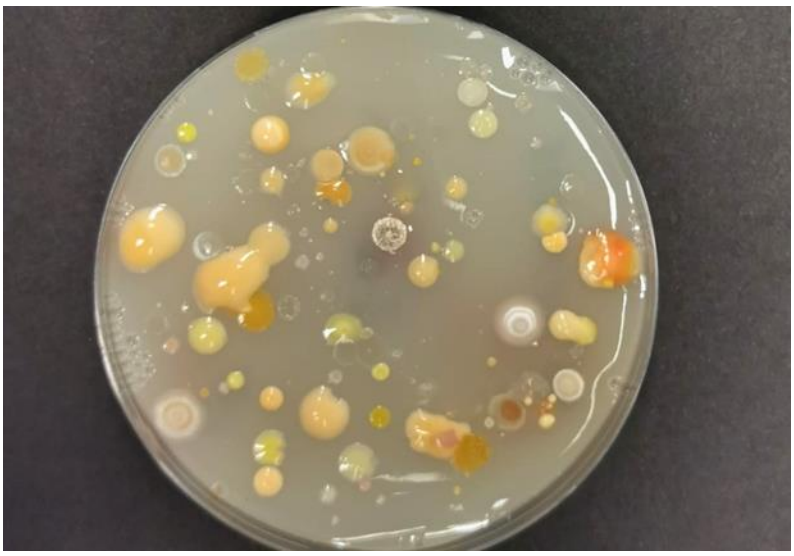

Figure 6: Actinobacteria colonies isolated from roots of the halophyte plant, *Zygophyllum mandaveli* on SNA media

3.2 Evaluation of Endophytic Actinobacterial Isolates on NaCl

All isolated actinobacteria were tested for their ability to tolerate up to 8% NaCl on SNA media (Figure 7). On SNA amended with 4% NaCl, 10 isolates were tolerant to this concentration. Two isolates, on the other hand, grew on media containing 8% NaCl. Both Z3-40 and E1-3EC were able to tolerate the highest level of salinity concentration of 8% NaCl (Figure 7). The rest of isolates were not tolerant to NaCl; and therefore, they were not used in the subsequent experiments.

Control E1-3EC Z3-40

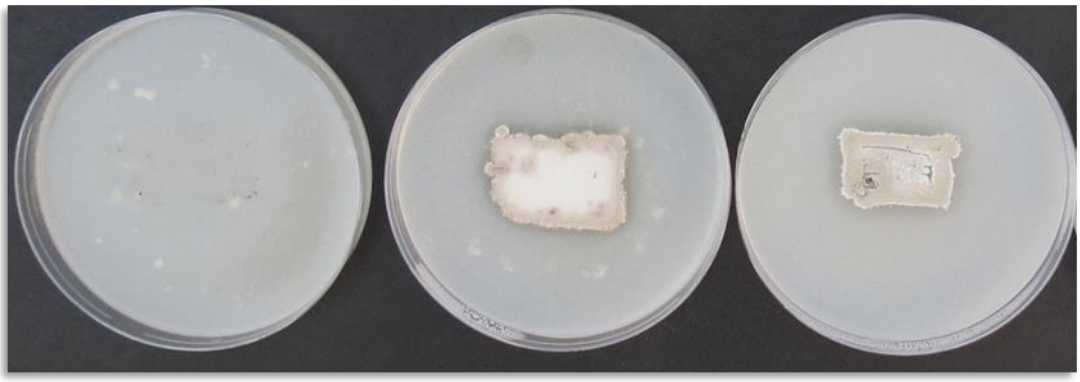

Figure 7: Salinity tolerance of actinobacterial isolates. Two isolates (Z3-40 and E1-3EC) exhibited growth on SNA media supplemented with 80 g l^{-1} of NaCl

3.3 Determination of P-solubilization in Actinobacterial Isolates

The salt-tolerant the two actinobacterial isolates that grew on media containing 8% NaCl were also cultured to determine the ability to solubilize P. Therefore, Z3-40 and E1-3EC isolates were tested on Pikovskaya's medium (Pikovskaya, 1948). A halo zone was developed around the isolates; thus, showing the ability of these isolates to solubilize P (Figure 8).

Control E1-3EC Z3-40

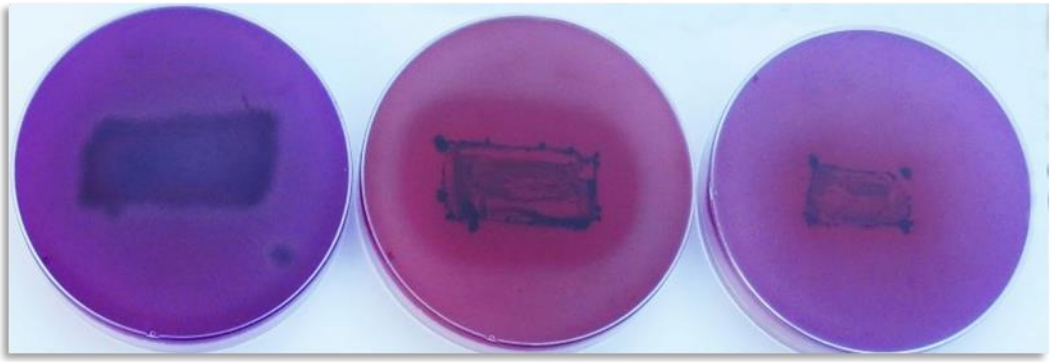

Figure 8: P-solubilization of E1-3EC and Z3-40 isolates on Pikovskaya's media. The halo zones refer to P-solubilization

3.4 Qualitative Determination of ACCD-Producing Actinobacteria

To check if Z3-40 and E1-3EC can synthesize the enzyme ACCD, assays on DF-ACC or DF-$(NH4)_2SO_4$ (control) were used (Figure 9). Isolate Z3-40 successfully showed strong growth and sporulation on DF-ACC media, suggesting high activity of ACCD in Isolate Z3-40. On the other hand, the actinobacterial isolate E1-3EC failed to grow on DF-ACC agar, suggesting that this isolate did not have ACCD activity and consequently was included as a control for the ACCD-producing isolate, Z3-40, in the greenhouse study.

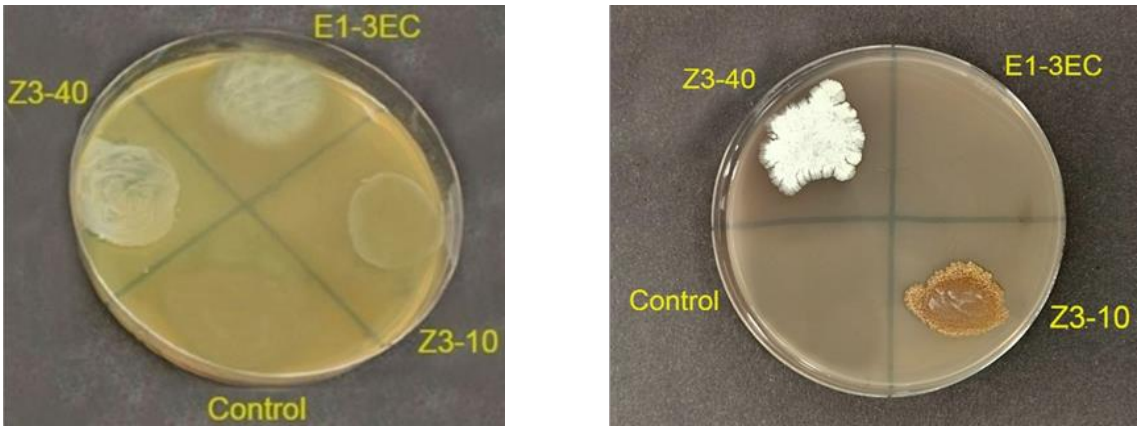

Figure 9: *In vitro* Determination of ACCD production. Actinobacterial isolates, Z3-40, Z3-10 and E1-3EC, grew and sporulated on the DF-$(NH4)_2SO_4$ control media (left); whereas the isolates Z3-40 and Z3-10, but not E1-3EC, grew well and sporulated on ACC-DF medium (right)

3.5 Evaluation of Salinity Tolerance of Tomato Seedlings Amended with Actinobacterial Isolates in The Greenhouse

3.5.1 Effect of Actinobacteria and Salinity on Shoot Tissues of Tomato Plants

Tomato seedlings were inoculated with the actinobacterial isolate Z3-40 (the ACCD-producer; +ACCD) to determine its's effect on alleviating salinity stress under greenhouse conditions. Isolate E1-3EC was, on the other hand, was used as the non-producing isolate of ACCD (-ACCD). Seedlings were treated with 60 and 120 mM NaCl post inoculation with the actinobacterial isolates. Tomato seedlings, serving as control,

were treated with 0 mM NaCl to determine if there were any growth promotion activities that was associated with these isolates (without salt stress). Tomato seedlings without any inoculation were also considered as controls for their corresponding Z3-40 and E1-3EC isolates treated with NaCl.

Prior to salinity treatment, all shoot lengths were similar (Figure 10A). There was negative impact when salt concentration increased from 60 to 120 mM in tomato seedlings in control and those inoculated with E1-3EC. This was evident with the significant ($P<0.05$) decrease in shoot lengths. However, this observation was not determined when Z3-40 was isolate was used. There was no significant ($P>0.05$) difference among plants inoculated with ACCD-producing isolate under any salt stress or no stress conditions (Figure 10A). Under salinity, plants treated with Z3-40 showed healthy appearance, similar to control plants or inoculated with E1-3EC without salt stress (Figure 10B).

The inoculation of Z3-40 isolates on tomato seedlings also increased FW (Figure 11A) and DW (Figure 11B) of shoots in response to 60 mM and 120 mM of NaCl, compared to those that were not inoculated (control) or inoculated with the E1-3EC isolate at the same concentration. This suggests that the actinobacterial Z3-40 isolate has the ability to relieve shoot tissues from stress and mitigate the effect of high salt stress on shoot tissues in tomato seedlings.

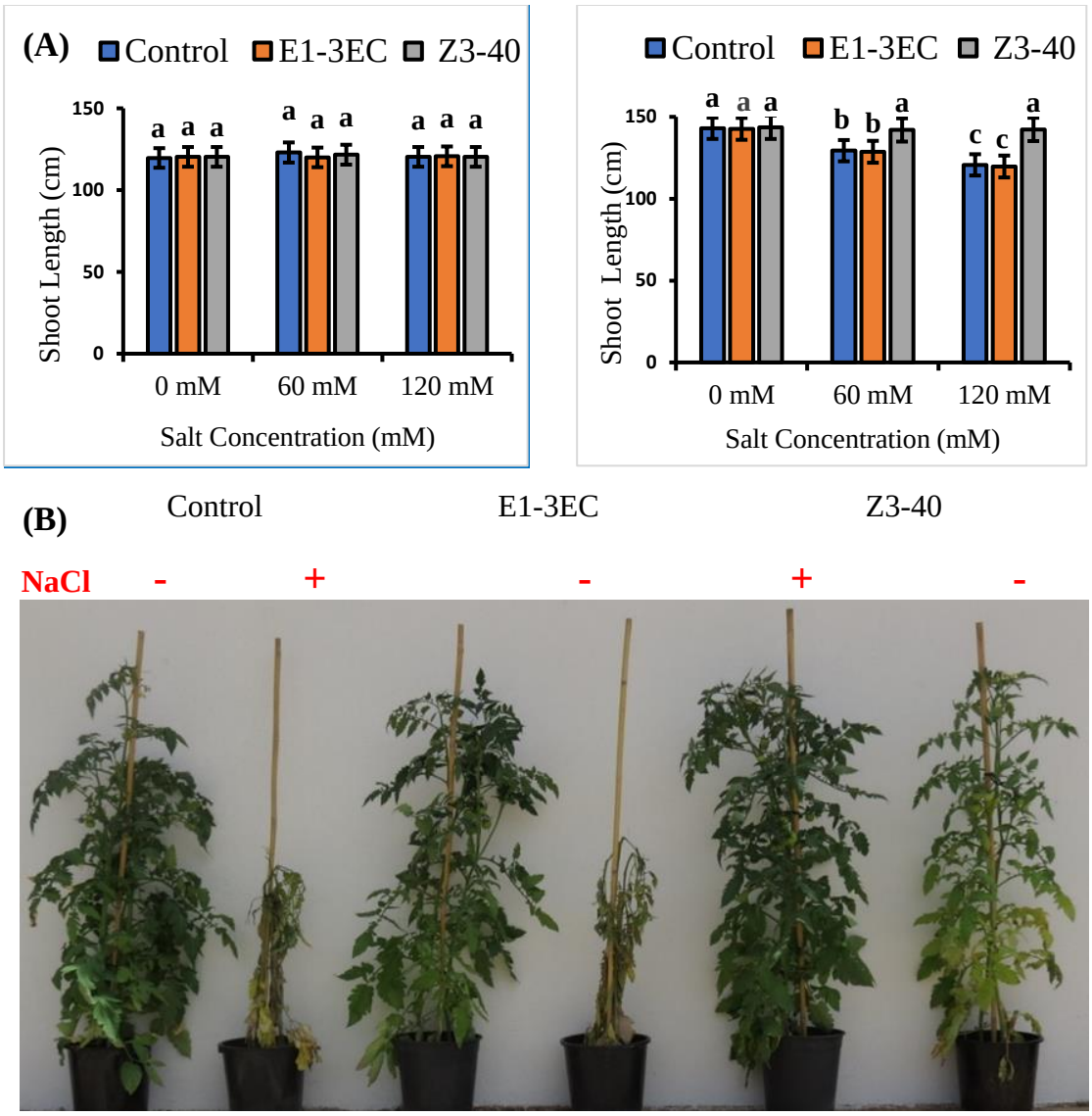

Figure 10: Effect of endophytic actinobacterial isolates possessing ACCD activity on salinity tolerance of tomato seedlings on shoot length of tomato seedlings under greenhouse conditions.

The effect of the non-ACCD-producing E1-3EC and ACCD-producing Z3-40 isolates on (A) shoot length before (left) and after (right) the application of salt stress treatments (60 and 120 mM NaCl); and (B) general appearance of the shoot of tomato seedlings inoculated with E1-3EC and Z3-40 isolates with and without salt stress treatments under greenhouse conditions. The means of 3 replicates ± SE for each treatment were used for statistical analysis. Values with the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test. ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase; NaCl, 120 mM NaCl; -/+, not applied/applied

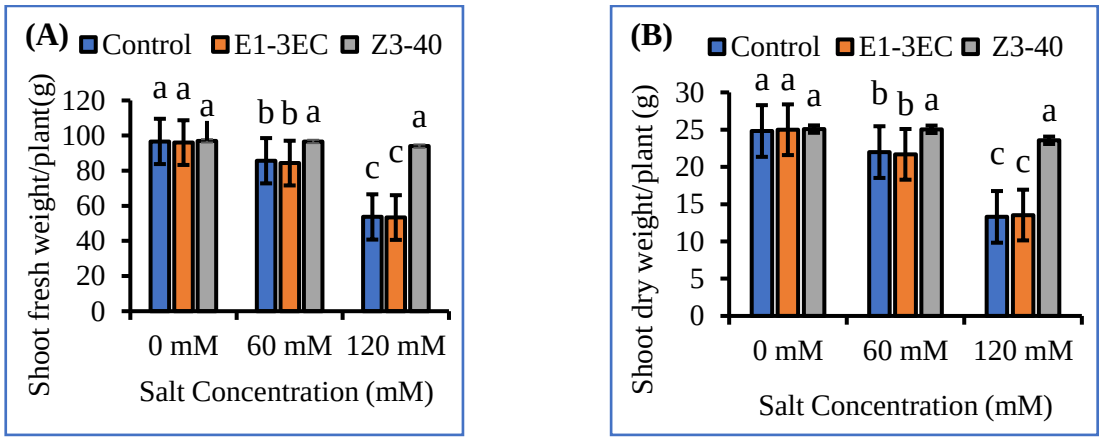

Figure 11: Effect of endophytic actinobacterial isolates possessing ACCD activity on salinity tolerance of tomato seedlings on shoot weight of tomato seedlings under greenhouse conditions.

The effect of the non-ACCD-producing E1-3EC and ACCD-producing Z3-40 isolates on shoot (A) fresh weight and (B) dry weight of tomato seedlings after the application of salt stress treatments (60 and 120 mM NaCl) under greenhouse conditions. The means of 3 replicates ± SE for each treatment were used for statistical analysis. Values with the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test. ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase

3.5.2 Effect of Actinobacteria and Salinity on Root Tissues of Tomato Plants

When root growth was assessed, similar trend was also found as observed with the shoot growth of tomato plants treated or not with NaCl (Figure 12A). Quantitively, FW and DW of roots were weighed out. Not surprisingly, none of the seedlings showed significant (P>0.05) difference in FW when any of the isolates or control was supplied at 0 mM NaCl (Figure 12B). At 60 mM NaCl, a significant (P<0.05) decrease occurred in root FW within the single isolate treatment compared to that at 0 mM NaCl. However, we did not find any significance (P>0.05) between the control seedlings and those inoculated with E1-3EC isolate but significant increase was in root FW of inoculated-seedlings of tomato with Z3-40 isolate at 60 mM NaCl. Similarly, the same trend of significance was observed in the 120 mM NaCl treated-seedlings, except that there was slight significant difference between control and E1-3EC (Figure 12B). In response to different salt concentrations of 60 and 120 mM NaCl, there was significant (P<0.05) difference in DW of roots among all seedlings of different isolate treatments (Figure 12C). Thus, it is worth mentioning that this significance is slightly minor and marginal (Figure 12C) and can be attributed to the low number of replicates used in the greenhouse experiments. Overall, the data indicate that the actinobacterial isolate Z3-40

enhances growth of tomato seedlings under salinity stress conditions, but not normal conditions.

3.5.3 Effect of Actinobacteria and Salinity on Tomato Fruits

By the end of the greenhouse experiment, the number of fruits harvested from non-inoculated- or inoculated seedlings with E1-3EC were not different at all levels of NaCl levels applied (Figure 13 A). However, the number of fruits per seedling inoculated with Z3-40 showed significant difference ($P<0.05$) at all tested salinity concentrations; and even reached to 2.5-fold increase in their number compared to those in control and inoculated seedlings with E1-3EC in response to 120 mM NaCl. The fresh weight of fruits was also measured in all treatments under the different salinity regimes. Under normal conditions, no significant ($P>0.05$) difference was found in all seedlings (Figure 13B). Clearly, there was significant ($P<0.05$) increase in the fruit fresh weights in the seedlings of the Z3-40 treatments compared to the other two treatments under salinity stress conditions at 60 and 120 mM NaCl.

3.5.4 Effect of Actinobacteria and Salinity on Leaves and Chlorophyll Contents in Tomato Plants

At 0 mM NaCl, one could not find any significant difference among control and the treatments of the two isolates (Figure 14A). With the application of 60 mM NaCl. The number of leaves was found to be significantly ($P<0.05$) higher in seedlings inoculated with Z3-40; however, no significant ($P>0.05$) difference was observed when tomato plants inoculated with E1-3EC compared to the control seedlings. This increase in the counts of leaves was doubled in inoculated-seedlings with the ACCD-producing isolate (Figure 14A). Although there was no effect of Z3-40 isolate on the chlorophyll contents of leaves of tomato seedlings, it was clearly illustrated that this isolate showed negative effect on the total chlorophyll content in seedlings exposed to salinity stress. In fact, chlorophyll content dramatically reduced in plants treated with Z3-40 in response to 60 and 120 mM NaCl treatments, compared to the control or the E1-3EC isolate treatments (Figure 14B). Although the reason of this reduction should be looked at carefully, there might be other plant growth regulators that may play a role in this isolate *i.e.,* Z3-40.

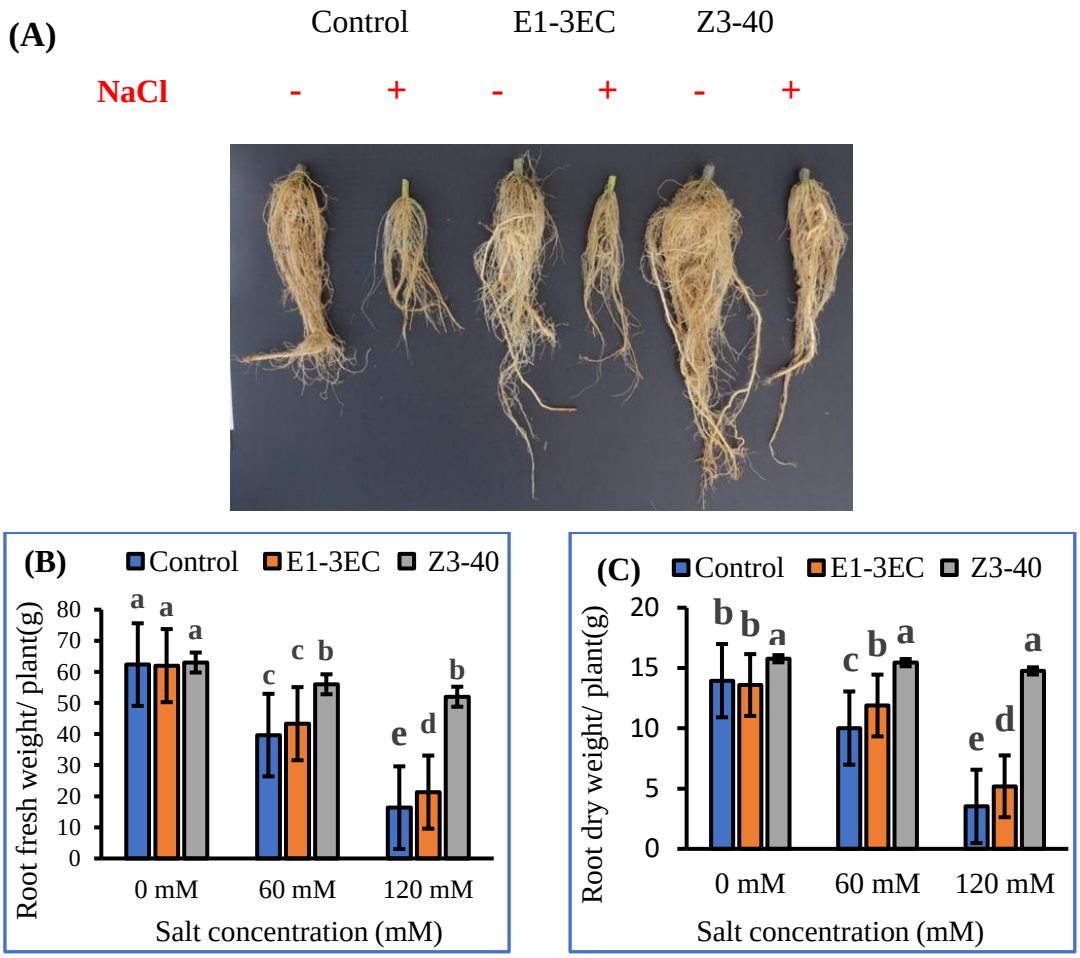

Figure 12: Effect of endophytic actinobacterial isolates possessing ACCD activity on salinity tolerance of tomato seedlings on root length, fresh and dry weight of tomato seedlings under greenhouse conditions.

The effect of the non-producing E1-3EC and ACCD-producing Z3-40 isolates on root (A) morphology; (B) fresh and (C) dry weight of tomato seedlings after the application of salt stress treatments (60 and 120 mM NaCl) under greenhouse conditions. The means of 3 replicates ± SE for each treatment were used for statistical analysis. Values with the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test. ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase; NaCl, 120 mM NaCl; -/+, not applied/applied

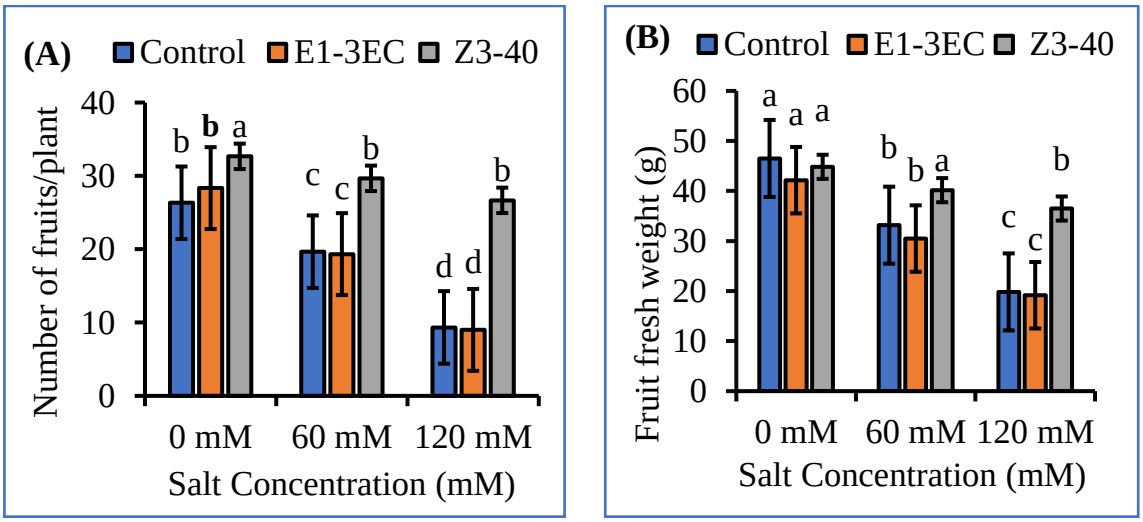

Figure 13: Effect of endophytic actinobacterial isolates possessing ACCD activity on salinity tolerance of tomato seedlings on number of fruits per plant and fruit fresh weight of tomato seedlings under greenhouse conditions.

The effect of the non-producing E1-3EC and ACCD-producing Z3-40 (isolates on the (A) number of fruits per plant and (B) fruit fresh weight of tomato seedlings after the application of salt stress treatments (60 and 120 mM NaCl) under greenhouse conditions. The means of 3 replicates ± SE for each treatment were used for statistical analysis. Values with the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test. ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase

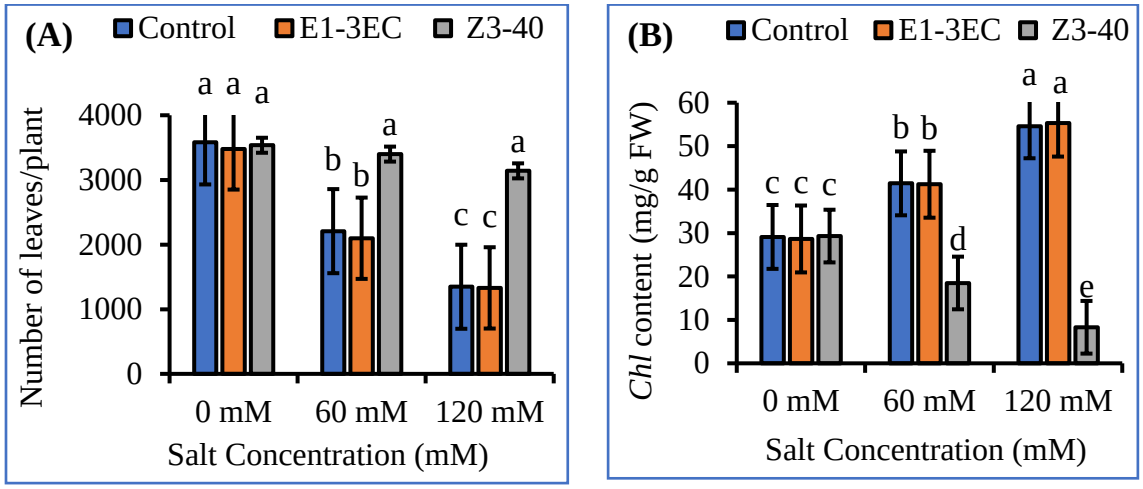

Figure 14: Effect of endophytic actinobacterial isolates possessing ACCD activity on salinity tolerance of tomato seedlings on number of leaves per plant and total *Chl* content of tomato seedlings under greenhouse conditions.

The effect of the non-ACCD-producing E1-3EC and ACCD-producing Z3-40 isolates on the (A) number of leaves per plant and (B) total *Chl* content of tomato seedlings after the application of salt stress treatments (60 and 120 mM NaCl) under greenhouse conditions. The means of 3 replicates ± SE for each treatment were used for statistical analysis. Values with the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test. ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase

3.5.5 Effect Actinobacteria and Salinity on ACC contents in Tomato Plants

To determine the efficiency of the ACCD-producing isolate in the mitigation of the effect of salt stress, tomato seedlings were inoculated with the actinobacterial isolates. There was no change in the endogenous levels of ACC in all tomato seedlings inoculated or not with any of the tested isolates (Figure 15). However, Z3-40 isolate reduced the levels of ACC in the shoot (Figure 15A) and root (Figure 15B) tissues of tomato seedlings treated with 60 mM NaCl. The endogenous ACC levels decreased by 4- and 2.8-fold in the shoots and roots of tomato seedlings treated with Z3-40 isolate at 60 and 120 mM NaCl, respectively, compared to the control and E1-3EC isolate (Figure 15). In general, ACC in roots were higher than in shoots in all treatments.

Together, these findings suggest that the alleviation of the effect of salinity stress by the ACCD-producing Z3-40 isolate is most probably associated with the low levels of the endogenous ACC. Thus, this will lead to the enhanced levels of all agronomic attributes in tomato plants under salt stress conditions that were treated with Z3-40 isolate.

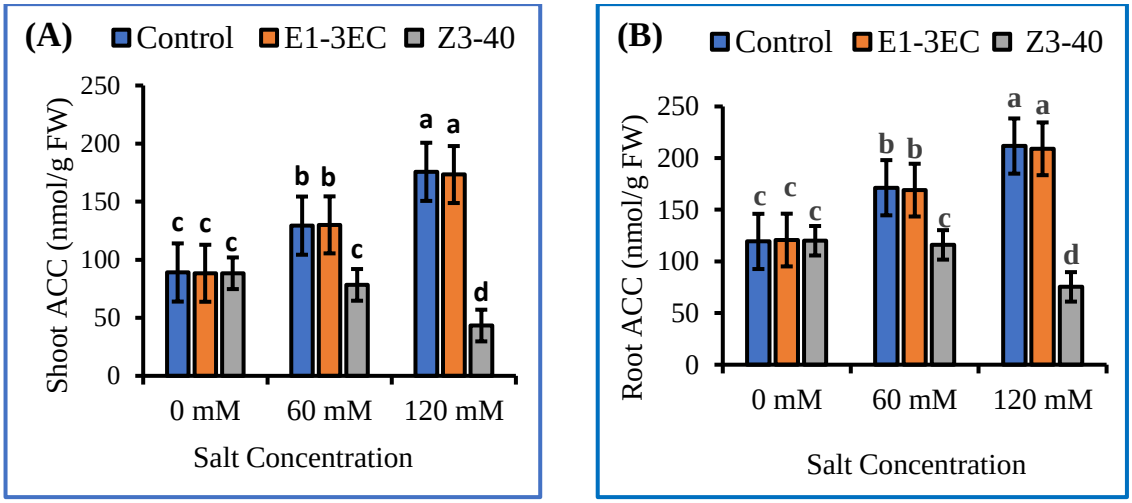

Figure 15: Effect of endophytic actinobacterial isolates possessing ACCD activity on salinity tolerance of tomato seedlings on measurements of the ACC content in the shoots and the roots of tomato seedlings under greenhouse conditions.

The effect of the non-ACCD-producing E1-3EC and ACCD-producing Z3-40 isolates on ACC contents in (A) shoots and (B) roots of the tomato seedlings after the application of salt stress treatments (60 and 120 mM NaCl) under greenhouse conditions. The means of 3 replicates ± SE for each treatment were used for statistical analysis. Values with the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test. ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase

Chapter 4: Discussion

Nowadays, agriculture sector is being hit by severe climate change circumstances, and the resulting salt intrusion has decreased agricultural fields, leading in food insecurity and unsustainability for the world's ever-increasing population, including the UAE (Ansari et al., 2019). Current techniques of irrigation, conventional breeding, and genetic engineering of salt-tolerant transgenic plants are extremely technical and labour-intensive, making them difficult to apply in practice (Singh et al., 2015; Niu et al., 2018). To combat salinity and improve crop yields in salinity-prone agricultural areas, the use of endophytic PGPB in the form of bioinoculants or biofertilizers has evolved as an element of climate-smart agriculture practice under challenging climate conditions (Sharma et al., 2016; Ansari & Ahmad, 2018).

When inoculated with plants, several endophytes have been shown to cope with salt stress and induce tolerance during plant growth and development under saline environments (Egamberdieva et al., 2019; Kearl et al., 2019). In this study, twenty-five salt-tolerant endophytes were isolated from the roots of the flowering plant, *Z. mandaveli* that is naturally found in the UAE. One of the significant findings of this study is the *in vitro* screening of P-solubilizing with the expectation that isolate Z3-40 can stimulate plant growth in saline fields in the form of biofertilizer. Similar results have been obtained in a previous study by Sultana et al. (2020).

The main objective of the current study was to determine if the inoculation with the ACCD-producing actinobacterial isolate can alleviate the effects of high salt stress in plant tissues. Thus, leading to reduced levels of ACC as well as the stress hormone ET which has resulted in plant growth promotion under salinity stress conditions. The enzyme ACCD cleaves ACC, the immediate precursor of ET in plants, to form NH_3 and α-ketobutyrate; and thereby modulate and lower the level of the phytohormone ET in developing or stressed-plants (El-Tarabily & Youssef, 2011). When the ACCD-producing endophytic PGPB are associated to a plant, they act as a sink for ACC ensuring that plant ET levels do not elevate to the point where root growth is impaired (Glick, 1995; Saravanakumar & Samiyappan, 2007). Direct consequences of this interaction significantly increased plant root and shoot length, an increase in biomass,

and protection of plants from inhibitory effects of ET synthesized as a direct consequence of a variety of biotic and abiotic stresses (Glick et al., 2007). The results of the current study are in agreement with those of Glick et al. (2007) in which actinobacteria were capable of producing ACCD, modulating and lowering the levels of the phytohormone ET in stressed plants. In addition, plant growth promotion efficiency can be achieved through the deployment of novel actinobacteria that can increase plant growth and its productivity during environmental stresses caused by the high levels of the stress hormone ET.

This is comparable to or even greater than previous reported endophytic PGPB that helped in the development in a variety of crop plants (Sharma et al., 2016). In general, low P levels in saline-prone cultivable areas force farmers to use excessive conventional P-containing fertilizers to boost agricultural production. This often causes possible surface water contamination, eutrophication and soil fertility loss. P-solubilizing microorganisms can boost crop development and production by mineralizing insoluble soil phosphate and releasing soluble P to plants. Thus, inoculating crops with P-solubilizing microorganisms, which can perform the same function in saline environments, is a viable technique for increasing global food supply while posing no environmental risk (Alori et al., 2017).

The actinobacterial isolate (Z3-40), used in the present investigation, was capable to grow and to produce heavy sporulation on DF medium amended with ACC as a sole source of N. Thus, this could be attributed to the stimulation of the enzyme ACCD; and subsequently convert ACC to α-ketobutyrate and NH_3 (Kende, 1993; Glick, 2007). Several bacteria, including the genera *Pseudomonas, Enterobacter, Bacillus*, and *Streptomyces* spp., have been reported to produce ACCD *in vitro*, reduce the levels of ACC inside plant roots/shoots, and increase root biomass and promote plant development and yield (Glick, 2007; Glick, 2014; El-Tarabily, Sham et al., 2021).

Here, the ability of actinobacterial isolate to promote the growth of tomato in saline soils through the production of ACCD was evaluated under controlled greenhouse conditions. Thus, the application of the actinobacterial isolate (Z3-40) increased and promoted plant growth in saline soils compared to control treatments. Similar findings

have previously been reported when the ACCD-producing bacteria were found to promote plant growth under salinity stress conditions (Sharma et al., 2016), which supported the findings of the current study.

One of the criteria for the selection of the actinobacterial isolate (Z3-40) was based on its ability to solubilize P. Under greenhouse conditions, the ability of the most promising isolate Z3-40 that belongs to the species *Streptomyces* to promote growth of tomato plants in saline soil was assessed. As a result, salt-tolerant endophytes with growth promotion capabilities can be of a promising resource for saline soil-based agriculture (Etesami & Beattie, 2018).

The use of filamentous actinobacteria rather than ordinary bacteria in the field of increasing plant growth under physiological stress circumstances and releasing the enzymatic activity of ACCD is quite novel in this work for more than one reason: (i) There is a significant lack of research on this form of bacteria, which is recognized for its extreme tolerance to high salinity (Qin et al., 2018; Olanrewaju & Babalola, 2019); and (ii) the use of this type of actinobacteria that are abundant in the UAE (El-Tarabily, 2008) will be more suitable for the local environment in the UAE, which has not been fully utilized so far.

Salt-tolerant endophytic PGPB can support the yield and overall growth of crops under salinity stress conditions (Sultana et al., 2020). We identified certain possible endophytes from the roots of plants growing in saline soil that had substantial plant growth promoting properties such as P-solubilization and ACCD production. As endophytes, they were already acclimatized to the plant system under stress, where they developed a symbiotic association with plants, a phenomenon that has been well-studied previously (Nautiyal et al., 2013; Abdel-Rahman et al., 2017; Singh & Jha, 2017).

Results from research world-wide with endophytic bacteria are significant and exciting. The study of endophytic bacteria is a challenging field of research, from a fundamental as well as an applied aspect. Descriptions of naturally-occurring and genetically-modified beneficial endophytic bacteria clearly point out the potential use of bacterial endophytes for enhancing plant growth and development (Hallmann et al., 1997). Most research in the past have concentrated on the plant growth promoting

bacteria from the rhizosphere, rhizoplane and other plant surfaces. The rhizosphere region has been the primary source of potential beneficial plant-associated bacteria due to the high diversity and population densities of the rhizosphere bacteria. There have been problems in the use of rhizosphere bacteria as plant-growth promoting bacteria. These problems include availability of nutrients, and space competition between the introduced microorganisms and the natural microflora, poor rhizosphere competence, as well as their incapacity to cope with extreme environmental conditions such as ultraviolet radiation, rainfall, moisture and temperature fluctuations (Kobayashi & Palumbo, 2000).

The endophytic bacteria have great attributes that make them relatively superior to all others. They are less exposed to the inhospitable environments of the soil and atmosphere, and in addition, are located in the tissues where relevant activities occur (Lodewyckx et al., 2002). Thus, endophytic bacteria could well be the next generation of rhizobacterial agents.

It is noteworthy to mention that similar pattern in growth and salinity tolerance has been found in tomato plants inoculated with the ACCD-producing actinobacterium isolated from the soil rhizosphere of Swehan area-UAE under the same greenhouse conditions (Elbadawi, 2022). Due to the nature of the endophytic ACCD-producing actinobacterial isolate (Z3-40) residing inside plant tissues, unexpectedly, the data in this project were in agreement with those of Elbadawi (2022) who used a soil-actinobacterial isolate. Endophytic bacteria have several advantages which make them more attractive beneficial bacteria than soil-actinobacteria. Endophytic actinobacteria can colonize and form associations within plant tissues, being protected from variable external environmental conditions and from competition for limited space and nutrients (Kobayashi & Palumbo, 2000). Thus, they are better able to use the protective environment of plant tissues than actinobacteria in plant growth promotion and abiotic stress tolerance.

Future research to enhance the capabilities of the endophytic actinobacterial isolate (Z3-40) will focus on increasing the dosage of inoculants, using younger plants and probably changing the inoculation methods (e.g., seed coating) of tomato. Thus, this

might have an impact on growth and salt tolerance between plants inoculated with the soil- and endophytic-isolates. In addition, identifying and characterizing the isolates at the molecular level *i.e.*, *16S rRNA* gene, is a top priority of this research.

Overall, the data generated in this study support the development of long-term biotechnological approaches of the use of endophytes in agricultural production enhancement under stressed situations along with sustainable and environmental-friendly use. Such an ACCD-producing isolate, in addition to the production of other plant growth hormones, can improve and increase the ability to withstand salinity and other environmental stress conditions; thus, contributing to the greening of the desert in the UAE.

Chapter 5: Conclusion

Soil salinity is a serious global concern due to its negative influence on agricultural production and sustainability. Salinity exists in all climates and can be caused by both natural and man-made factors. In general, saline soils develop in dry and semi-arid environments when rainfall is insufficient to supply crops with sufficient water requirements and/or drain mineral salts out of the root zone. This has led to suitable methods that can improve the conditions of these saline soils. As such, the current study obtained different native actinobacteria from UAE plants that produced the enzyme ACCD and exhibited strong root colonization abilities of tomato plant.

The application of salinity tolerant actinobacteria producing ACCD and solubilizing P significantly promoted tomato plant growth, including increased FW and DW, and increased length of root and shoot tissues compared with the control plants. In addition, it increased the photosynthetic chlorophyll pigment contents of in leaves. Moreover, the application of salinity tolerant actinobacterial isolate (Z3-40) significantly reduced the levels of ET content in the roots and shoots in plants compared with tomato plants grown in saline soils without the application of the actinobacterial isolate E1-3EC.

The use of ACCD-producing actinobacteria in the tomato plants improved the efficacy of soils under salinity stress. The presence of these actinobacteria in tomato roots helped them grow faster and produce large biomass. Under greenhouse conditions, the application of actinobacterial isolate (Z3-40) in tomato plants significantly reduced ET levels in saline soils. The reduction in the stress ET content, which is known to inhibit plant growth, was responsible for the improvement in tomato growth from soils under salinity stress.

Future research may include (1) examining modes of entry of endophytic bacteria into the host plant; (2) determining the population dynamics and activity of endophytic actinobacteria in their host plants; and (3) optimizing the practical applications to use endophytic bacteria for the improvement of plant stress tolerance. Accordingly, the salt-tolerant endophytic actinobacterial isolate identified in this study that possess multiple PGP properties can be used as a promising bioinoculant/biofertilizer to mitigate salinity stress conditions. The use of this “green” technology will have a wide range of positive

effects and can be a saver for saline-prone locations. In the future, these salt-tolerant endophytic PGPR enhancing crop yield in an economically viable way, can add extra value to the climate change preparedness strategy in the UAE.

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List of Publications

El-Tarabily, K. A., Ramadan, G. A., Elbadawi, A. A., Hassan, A. H., Tariq, S., Ghazal, E. W., Abo Gamar, M. I., and AbuQamar, S. F. (2021). The Marine endophytic polyamine-producing *Streptomyces mutabilis* UAE1 isolated from extreme niches in the Arabian gulf promotes the performance of mangrove (*Avicennia marina)* seedlings under greenhouse conditions. *Frontiers in Marine Science*, 8:710200. https://doi.org/10.3389/fmars.2021.710200.

El-Tarabily, K. A., Sham, A., Elbadawi, A. A., Hassan, A. H., Alhosani, B. K. K., El-Esawi, M. A., AlKhajeh, A. S., and AbuQamar, S. F. (2021). A Consortium of rhizosphere-competent actinobacteria exhibiting multiple plant growth-promoting traits improves the growth of *Avicennia marina* in the United Arab Emirates. *Frontiers in Marine Science*, 8:715123. https://doi.org/10.3389/fmars.2021.715123.

Amer, A. M., Abd El Maksoud, A. I., Abdeen, M. A., Hamdy, A., Mabrok, H. A., Amer, M. A., and El-Sanousi, A. A. (2018). Potency Of titanium dioxide nanoparticles on skin wound healing in rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 9(6): 909-923. https://www.researchgate.net/publication/332846543 Potency of titanium dioxide nanoparticles on skin wound healing in rats.

Appendix

List of The Media Used in This Research

1. Inorganic Starch Nitrate Agar (SNA) (Küster, 1959).
2. Oatmeal Yeast Extract Agar (OMYEA) (Küster, 1959).
3. Dworkin and Foster's Salts Minimal Agar Medium (DF) (Dworkin & Foster, 1958).
4. Pikovskaya's Agar Medium (Pikovskaya, 1948).

Composition of Media:

1- Inorganic salt-starch agar (Starch Nitrate Agar) (SNA) (Küster, 1959)

Soluble starch	10 g
Potassium nitrate	2 g
Di-potassium hydrogen phosphate	1 g
Magnesium sulfate	0.5 g
Sodium chloride	0.5 g
Calcium carbonate	3 g
Ferrous sulfate	0.01 g
*Trace salt solution	1 mL
Cycloheximide (Sigma)	50 μg mL^{-1}
Nystatin (Sigma)	50 μg mL^{-1}
Distilled water	1 L
Agar	20 g

*Trace salt solution (Pridham et al., 1956) composed of: 0.1 mg liter-1 of each of the following salts: ferrous sulfate, magnesium chloride, copper sulfate and zinc sulfate.

2- Oat-Meal Yeast Extract Agar (OMYEA) (Küster, 1959)

Twenty grams of oat-meal were steamed in 1 liter of distilled water for 20 min. The steamed oats were filtered through cheese cloth, and distilled water was added to continue the filtrate to 1 liter. Yeast extract (1 g) (Sigma) and agar (Sigma) (20 g) were added, and the final medium pH was adjusted to 7.2.

3- Dworkin and Foster's salts minimal agar medium (DF) (Dworkin & Foster, 1958)

Di- hydrogen potassium phosphate	4.0 g
Di-sodium hydrogen phosphate	6.0 g
Magnesium sulfate	0.2 g
Ferrous sulfate	1.0 g
Boric acid	10 μg
Manganese sulfate	10 μg
Zinc sulfate	70 μg
Copper sulfate	50 μg
Molybdenum oxide	10 μg
Glucose	2.0 g
Gluconic acid	2.0 g
Citric acid	2.0 g
Agar	20 g
Distilled water	1 L

4- Pikovskaya's Agar Medium (Pikovskaya, 1948):

Glucose	10.0 g
Calcium Phosphate $Ca_3(PO4)_2$	5.0 g
Ammonium sulfate $(NH4)_2SO_4$	0.5 g
Sodium Chloride	0.2 g
Magnesium sulfate	0.1 g
Potassium chloride	0.2 g
Yeast Extract	0.5 g
Manganese sulfate	0.002 g
Iron sulfate	0.002 g
Distilled water	1000 mL
0.5% Bromophenol Blue	5 mL
Agar	20.0 g

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The ability of native bacteria isolated from United Arab Emirates UAE soils with ACC deaminase activity to promote the growth of tomato plants in soils under salinity stress was evaluated under greenhouse conditions in an effort to gain the benefits provided by bacterial enzyme (1-aminocyclopropane-1-carboxylic acid) deaminase in salinity tolerant from the environment. The findings indicated that plant growth-promoting actinobacteria carrying ACCD might be useful as a bacterial inoculum for improving plant development, particularly in saltine soils.

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