POTASSIUM SOLUBILIZATION BY INDIGENOUS RHIZOBACTERIA ISOLATED FROM SALINE SOIL AND THEIR IMPACT ON EARLY GROWTH AND MACRONUTRIENT CONCENTRATION IN PADDY CROP (*Oryza sativa* L. Var. MRIA 1)

MUHAMMAD ASHFAQ

UNIVERSITI SAINS MALAYSIA

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by

MUHAMMAD ASHFAQ

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TABLE OF CONTENTS

CHAPTER 4 THE PLANT GROWTH-PROMOTING PROPERTIES OF POTASSIUM SOLUBILIZING RHIZOBACTERIA UNDER SALINITY STRESS………………………………...92

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ACC	1-AminoCyclopropane-1-Carboxylic acid
ACC-d	1-AminoCyclopropane-1-Carboxylic acid deaminase
ANOVA	Analysis Of Variance
ATCC	American Type Culture Collection
ATP	Adenosine Tri Phosphate
CAS	Chrome Azurol S
DAI	Days After Incubation
DF	Dworkin & Foster
DNA	Deoxyribonucleic Acid
EC	Electrical conductivity
EPS	Exopolysaccharides
HCN	Hydrogen Cyanide
HDTMA	Hexadecyl Trimethyl Ammonium Bromide
HKTs	High-affinity Potassium Transporters

IAA	Indole Acetic Acid
KSR	Potassium Solubilizing Rhizobacteria
KSB	Potassium Solubilizing Bacteria
MOP	Muriate of Potash
NBRIP	National Botanical Research Institute's Phosphate
NSCC	Non Selective Cation Channels
OD	Optical Density
PGPR	Plant Growth Promoting Rhizobacteria
PSB	Phosphate Solubilizing Bacteria
RNA	Ribonucleic Acid
rpm	Revolutions per minute
RWC	Relative Water Content
SE	Standard Error
SOP	Sulphate of Potash
TSB	Tryptic Soybean Broth

LIST OF SYMBOLS

%	per cent
=	equal
≈	Almost equal
µg/ml	Microgram per millilitre
µl	microliter
α	alpha
B	Boron
Ca	Calcium
Cl	Chlorin
cm	centimeter
dSm^{-1}	Deci Siemens per meter
ET	Ethylene
Fe	Iron
Fe^{+2}	Ferrous
Fe^{+3}	Ferric

g	gram
H	Hydrogen
ha	hectare
K	Potassium
KCl	Potassium Chloride
kg	kilogram
ml	millilitre
mM	millimolar
Mn	Manganese
N	Nitrogen
N	Normality
Na	Sodium
NaCl	Sodium Chloride
nm	nanometer
P	Phosphorus
pH	Potential of Hydrogen
Zn	Zinc

LIST OF APPENDICES

KETERLARUTAN KALIUM OLEH RHIZOBAKTERIA ASLI YANG DIPENCILKAN DARI TANAH MASIN DAN KESANNYA TERHADAP PERTUMBUHAN AWAL DAN KEPEKATAN NUTRIEN MAKRO DALAM TANAMAN PADI (*Oryza sativa* L. Var. MRIA 1)

ABSTRAK

Saliniti adalah faktor abiotik utama di dalam tanah yang mempengaruhi pertumbuhan tanaman dan mengurangkan kawasan penanaman secara berterusan. Dalam keadaan masin, natrium adalah kation yang paling penting. Kation kalium dan kation natrium bersaing satu sama lain untuk pengambilannya dalam tanaman, terutamanya melalui Pengangkut Kalium Afiniti Tinggi dan Saluran Kation Tak Memilih. Mikroorganisma memainkan peranan penting dalam melarutkan kalium terikat dan menjadikannya tersedia untuk tanaman. Dalam kajian ini, empat puluh dua rhizobakteria pelarut kalium telah dipencilkan dari sawah di kawasan pesisir Kuala Muda, Malaysia. Tiga belas strain daripadanya adalah positif untuk pelarutan fosforus, Asid Indola Asetik (IAA), siderofor, ammonia, pengeluaran eksopolisakarida (EPS), pengikatan nitrogen, dan deaminase 1-aminocyclopropane-1-carboxylate (ACC). Tiga belas strain ini tumbuh dengan jaya pada agar nutrien yang ditambah dengan NaCl hingga 8%; walau bagaimanapun, pertumbuhannya berkurang pada tahap kemasinan yang lebih tinggi. Lima bakteria pelarut kalium (KSB) (L1/4, L3/3, L3/4, L4/12, dan L5/1) telah dipilih untuk kajian lanjutan berdasarkan pelarutan kalium tertinggi 1(9.16, 18.33, 17.83, 17.25, dan 17.75µg/ml) di bawah tekanan 0, 3, 5 dan 7% NaCl. Strain ini dikenal pasti sebagai *Acinetobacter pittii* (L1/4), *Acinetobacter pittii* (L3/3), *Rhizobium pusense* L3/4), *Cupriavidus oxalaticus* L4/12) dan *Ochrobactrum ciceri* (L5/1). Keterlarutan kalium yang tertinggi telah dicatatkan pada suhu 30ºC, pH 7 dan sumber gula sebagai glukosa. Empat strain bakteria mampu menunjukkan sifat penggalak pertumbuhan tanaman (PGP); namun, sifat ini berkurangan dengan peningkatan tekanan kemasinan. Untuk mengkaji kesan strain KSB pada tanaman padi yang ditanam di dalam pelbagai tahap kemasinan, beberapa aspek fisiologi dan agronomi telah dikaji. Tanaman padi yang telah diinokulasi dengan strain KSB telah dibandingkan dengan tanaman yang tidak diinokulasi. Tanaman padi ditanam di ruang pertumbuhan dengan rawatan kemasinan 4, 8 dan 12 dSm^{-1} manakala tanaman padi ditanam di rumah tumbuhan dengan menggunakan 0, 1.32 dan 2.76 g NaCl kg^{-1} tanah. Dalam eksperimen bilik pertumbuhan, kemasinan telah mengurangkan percambahan benih (60 hingga 90%), kolonisasi akar (20.20 hingga 74.33x10^{5} CFU/100 mg), klorofil$_{\text{-tot}}$ (1.072 hingga 1.396 mg/g), kandungan air relatif (RWC), panjang pucuk dan Panjang akar (13.44 hingga 23.00 dan 3.88 hingga 7.11 cm) dan berat kering pucuk dan berat kering akar (7.77 hingga 11.72 dan 2.50 hingga 4.27 mg). Inokulasi strain KSB pada biji padi meningkatkan pertumbuhan tanaman secara signifikan. Peningkatan yang ketara pada percambahan biji benih (10%), panjang pucuk (23%), panjang akar (30%) telah direkodkan pada tanaman yang diinokulasi dengan *Acinetobacter pittii* dibandingkan dengan tanaman kawalan. Inokulasi pokok padi dengan *Cupriavidus oxalaticus* meningkatkan klorofil a dan b (19% dan 25%) berbanding dengan tanaman yang tidak diinokulasi. Inokulasi strain KSB pada benih padi dapat mengurangkan kesan bahaya kemasinan pada tanaman yang tumbuh di bawah tekanan kemasinan. *Acinetobacter pittii* menunjukkan kesan yang lebih ketara berbanding dengan strain lain. Strain ini meningkatkan kandungan air relatif (RWC) (88 hingga 91%), klorofil$_{\text{-tot}}$ (4.647 hingga 4.962 mg/g), karotenoid (1.3 hingga 2.5 mg/g), kepekatan kalium dalam pucuk (17.51 hingga 17.57 mg/g), kepekatan kalium dalam akar (7.22 hingga 8.84 mg/g), kepekatan fosforus dalam pucuk (5.182 hingga

6.155 mg/g) dan kepekatan fosforus dalam akar (2.877 hingga 3.497 mg/g). Inokulasi *Acinetobacter pittii* telah meningkatkan secara signifikan RWC (3,44 dan 3%), klorofil$_{-tot}$ (16 dan 12%), karotenoid (56 dan 22%), kepekatan kalium dalam pucuk (18 dan 34%), kepekatan kalium dalam akar (26 dan 19%), kepekatan fosforus dalam pucuk (17 dan 15%) dan kepekatan fosforus akar (19 dan 9%) berbanding dengan tanaman yang tidak diinokulasi dengan penambahan 1.32 g NaCl kg^{-1} tanah dan 2.76 g NaCl kg^{-1} tanah. Hasil kajian menunjukkan bahawa inokulasi bakteria pelarut kalium (KSB) dapat meningkatkan prestasi pertumbuhan tanaman padi di bawah tekanan kemasinan. Kesimpulannya, aplikasi bakteria pelarut kalium (KSB) berpotensi untuk digunakan sebagai cara yang ekonomi dan kos-berkesan untuk toleransi kemasinan dan mempromosi pertumbuhan tanaman padi.

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ABSTRACT

Salinity is a major abiotic factor in soils that severely affects plant growth and continuously reduces the cultivated area. Under saline conditions, sodium is the most important cation, which is very harmful to plants. The potassium and sodium cations compete for their uptake in plants, primarily through High-Affinity Potassium Transporters and Nonselective Cation Channels. Microorganisms play a vital role in solubilizing fixed potassium and making it available to plants. In the present study, forty-two potassium solubilizing rhizobacteria were isolated from paddy fields in the coastal area of Kuala Muda, Malaysia. Thirteen strains from the isolated rhizobacteria were positive for phosphorus solubilization, Indole Acetic Acid (IAA), siderophores, ammonia, Exopolysaccharides (EPS) production, nitrogen fixation, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. These thirteen strains grew successfully on nutrient agar amended with NaCl up to 8%; however, the growth was reduced at a higher salinity level. Five potassium solubilizing bacteria (KSB) (L1/4, L3/3, L3/4, L4/12, and L5/1) were selected for further studies based on the highest potassium solubilization (19.16, 18.33, 17.83, 17.25, and 17.75 µg/ml), respectively under 0, 3, 5 and 7% NaCl stress. These strains were identified as *Acinetobacter pittii* (L1/4), *Acinetobacter pittii* (L3/3), *Rhizobium pusense* (L3/4), *Cupriavidus oxalaticus* (L4/12) and *Ochrobactrum ciceri* (L5/1). The highest potassium solubilization was noted at temperature 30ºC, pH 7 and glucose as a carbon source. These four strains successfully showed the plant growth promoting (PGP) properties; however, these traits were reduced with increased salinity stress. Some physiological and agronomical aspects were investigated to study the impact of KSB strains on paddy plants grown under various salinity levels. The paddy plants inoculated with KSB strains were compared with non-inoculated plants. The paddy plants were grown in the growth chamber under salinity treatments of 4, 8 and 12 dSm$^{-1,}$ whereas paddy plants were grown in plant house using 0, 1.32 and 2.76 g NaCl kg^{-1} of soil. In growth room experiment, salinity reduced the seed germination (60 to 90%), root colonization (20.20 to 74.33×10^5 CFU/100 mg), chlorophyll-$_{tot}$ (1.072 to 1.396 mg/g), relative water content (RWC), shoot and root length (13.44 to 23.00 and 3.88 to 7.11 cm) and shoot and root dry weights (7.77 to 11.72 and 2.50 to 4.27 mg). Inoculation of paddy seeds with KSB strains significantly improved plant growth. The pronounced increase in seeds germination (10%), shoot length (23%), root length (30%) were recorded in plants inoculated with *Acinetobacter pittii* as compared to the uninoculated control. Inoculation of paddy plants with *Cupriavidus oxalaticus* had increased chlorophyll a and b (19% and 25%) compared to uninoculated plants. Inoculation of KSB strains on paddy seeds mitigated the harmful effects of salinity on plants grown under salinity stress. *Acinetobacter pittii* showed more pronounced effects as compared to other strains. The strain improved relative water content (RWC) (88 to 91%), chlorophyll-$_{tot}$ (4.647 to 4.962 mg/g), carotenoids (1.300 to 2.500 mg/g), shoot potassium concentration (17.51 to 17.57 mg/g), root potassium concentration (7.22 to 8.84 mg/g), shoot phosphorus concentration (5.182 to 6.155 mg/g) and root phosphorus concentration (2.877 to 3.497 mg/g). Inoculation of *Acinetobacter pittii* significantly increased RWC (3.44 and 3%), chlorophyll-$_{tot}$ (16 and 12%), carotenoids (56 and 22%), shoot potassium concentration (18 and 34%), root potassium concentration (26 and 19%), shoot phosphorus concentration (17 and 15%) and root phosphorus concentration (19 and 9%) as compared to the uninoculated plants grown in soil amended with 1.32 g NaCl kg^{-1} of soil and 2.76 g NaCl kg^{-1} of soil, respectively. The results showed that inoculation with KSB improved the growth performance of paddy crops under salinity stress. It is concluded that the application of KSB can be potentially used as economical and cost-effective means for salinity tolerance and growth promotion in paddy plants.

CHAPTER 1

INTRODUCTION

Soil salinity is a primary abiotic constraint to crop production, implementing detrimental consequences on millions of hectares of land (Munns, 2005; Munns & Tester, 2008; Shabala & Cuin, 2008). The annual losses in agricultural production due to salinity are more than US$ 27.3 billion (Flowers et al., 2010; Qadir et al., 2014). Out of 12781.3 million hectares of total world land, approximately 954.9 million hectares are salt-affected, which is 7.471% of whole land (Shokat & Großkinsky, 2019). There is a severe threat of salinity to agriculture in the world. The irrigated land, which is accountable for 1/3 of the world food security, produces salt-affected soil at least two times more than rain-fed land. The salinity in irrigated areas increases perception as critical trouble for crop productiveness (Munns, 2005; Munns & Tester, 2008). The salt-affected area is growing at 10% yearly because of many reasons like irrigation with saline water, low precipitation, excessive surface evaporation, poor cultural practices, and weathering of local rocks. It is expected that more than 50% of the arable region might be salt-affected up to 2050 (Jamil et al., 2011). It is assessed that a more than 50% increase in grain production of major crops like rice, wheat, and maize is compulsory to fulfil the food requirement of the estimated population by 2050 (Godfray et al., 2010). Soil salinity is identified by the availability of a high concentration of soluble salts in it, wherein NaCl is the most abundant and widely distributed soluble salt. In the root area, the saturation extract of saline soil has electrical conductivity (ECe) more than 4 dSm^{-1} or 40 mM NaCl or more (Abbas et al., 2019). Under such conditions of soil salinity, the yield of most crops reduces significantly.

Different techniques were employed to overcome the harmful effects of salinity, such as planting, regenerating, and maintaining native vegetation and good ground cover in recharge, transmission, and discharge zones, where possible. Using more groundwater in recharge areas by pumping water from bores and redirecting it to other storages, installing bores and interceptor drains in discharge areas, use of suitable quality water to irrigate adjacent fields, installing sub-surface drainage and maximizing cropping opportunities and minimizing fallow land also helps (Cuevas et al., 2019).

Application of mineral potassium fertilizer reduces the effects of salinity. It increases the concentration of K^+ in plants by decreasing the absorption of Na^+ in plants and ultimately increasing the crop's yield under saline conditions (Ashraf et al., 2012). Growth enhancement has been reported by the addition of K^+ fertilizers to salt-affected soils in pepper and cucumber (Kaya et al., 2003), potato (Elkhatib et al., 2004), rice (Bohra & Doerffling, 1993), tomato (Kaya et al., 2001), pearl millet (Heidari & Jamshidi, 2011) and barley (Endris & Mohammed, 2007). The extensive use of chemical fertilizers is problematic for many reasons such as high cost, timely availability, inadequate resources, high cost of production, etc. Moreover, the injudicious use of chemical fertilizers poses harmful effects on the environment (Ahmad et al., 2016). Therefore, it is imperative to evolve or use cost-effective and eco-friendly biotechnology for sustainable crop production under saline conditions.

Sodium cations are most prominent in saline soils. Sodium cations taken up by plants from salt-affected soil disturbs an adequate Na^+:K^+ ratio in the cell cytoplasm. At a low concentration of K^+ in the nutrient medium, Na^+ competes with K^+ for its transportation through high-affinity potassium transporters (HKTs) and nonselective cation channels (NSCCs). Relatively higher K^+ concentration in the root zone under saline conditions may compete more efficiently with Na^+. It can effectively reduce the uptake of sodium cations by plants. It will not only help to decrease Na^+ concentration but will also improve the K^+:Na^+ ratio in the cytoplasm, which is required for optimum enzymatic activities (Wakeel, 2013).

Microorganisms play a vital role in the natural potassium cycle. Some species of rhizobacteria are capable of solubilizing potassium in an accessible form in soils. Silicate solubilizing bacteria were found to dissolve potassium, silicon, and aluminium from insoluble minerals (Aleksandrov et al., 1967). Previous studies showed that most of the potassium in the soil exists in the form of silicate minerals. The potassium becomes available to the plants when the minerals are slowly weathered or solubilized (Bertsch et al., 1985). Rhizobacteria can solubilize rock K, mineral powder such as mica, illite, and orthoclase by producing organic acids (Friedrich & Soriano, 1991). The specific focus on K solubilizer for saline soil is essential because potassium is an essential nutrient that can compete with Na^+ during uptake by all plants in the salt-affected environment. It is a crucial element in many physiological and biochemical processes in plants. The isolation and screening of salt-resistant potassium solubilizing rhizobacteria would be very important to add the body of knowledge on existing potassium solubilizing rhizobacteria from saline soils in Malaysia.

Rhizobacteria associated with plant roots may possess various plant growth promoting properties that may help increase plant growth and yield of crops and are applicable in directly and indirectly alleviating the effect of abiotic stresses. Directly, PGPR facilitates plant nutrients uptake from surrounding environments by phosphorus solubilization, producing siderophores to sequester iron by nitrogen fixation (Etesami & Beattie, 2018). Furthermore, PGPR can modulate plant growth by providing phytohormones such as indole acetic acid (IAA) or reducing the ethylene production by the activity of the 1-Aminocyclopropane-1-carboxylate (ACC) deaminase enzyme and Exopolysaccharides production (Glick, 2014). The investigation of KSB strains for PGPR under salinity stress would help develop a multifunctional biofertilizer for agriculture in saline soils.

The relationship between rhizobacteria and plants has been extensively known; however, there is a lack of information regarding the effect of salt-resistant potassium solubilizing rhizobacteria having different plant growth promoting properties on the growth and nutrient uptake of most cultivated variety MRIA 1. This is of utmost importance, reflecting the perception of salinity mitigation in coastal salt-affected rice-growing areas, in which seawater intrusion has become an urgent threat in recent years due to global climate change.

1.1 Research objectives

By keeping in view, the above problems, the research is planned to achieve the following objectives.

1. To isolate, screen, and identify potassium solubilizing rhizobacteria from saline soil and the effect of abiotic conditions on potassium solubilization.
2. To assess the plant growth-promoting properties of potassium solubilizing rhizobacteria under salinity stress.
3. To assess the root colonization potential of potassium solubilizing rhizobacteria under *in vitro* conditions.
4. To determine the impact of potassium solubilizing rhizobacteria on early growth and macronutrient concentration in paddy crop under different saline conditions.

CHAPTER 2

LITERATURE REVIEW

Potassium solubilizing microorganisms (KSB) are significant contributors to improve soil productiveness. They are particularly useful in facilitating the release of potassium from its fixed form to plant accessible format, which promptly provides an optimistic impact on vegetative growth, maturity, and crop produce properties. Additionally, they offer a substantial boost to plant immunity against pests. KSB also improves potassium uptake in plants, which is valuable in countering the adverse effects of sodium (Na). Hence, it can be inferred that using salt-tolerant and potassium solubilizing microorganisms in saline soil can offer long-standing soil sustainability, better crop throughout, and environmental maintainability. This factor would consequently lead to better financial situations in a country. This chapter encompasses a comprehensive review of research works pertaining to our study.

2.1 Salinity

Salinity is the most significant and extreme ecological strain on plant life (Munns & Tester, 2008). An estimate influences almost 20% of all crop fields and more than 7% of the global soil surface (Shokat & Großkinsky, 2019). The global economy suffers a loss of US$ 27.3 billion per year due to less crop productivity (Qadir et al., 2014). Also, environmental changes increase the risks of further salinization in various regions; hence, a focused and specialized effort is needed to mitigate the undesirable effects of salinity on crop production and avoid agrarian land deterioration due to salt accumulation (Turral et al., 2011).

2.1.1 Soil salinization

The soil salinity is mainly triggered by ecological factors such as saltwater interference, marine salts deposited through wind and rainfall, and deterioration of rocks and minerals (Flowers et al., 2010). Several other factors, such as irrigating fields with salty waters, mineral fertilizers, and soil modifications, also increase soil salinity (Kotuby-Amacher et al., 2000). The build-up of water-soluble salts deposits identifies soil salinization in the soil, such as cations of sodium, calcium, magnesium, and anions of (Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^-).

Salinity is the quantity of the dissolved salts in water or soil. It is often calculated through electrical conductivity (E.C.), which shows the electrical current passing through a material. E.C. indicates the concentration and constituents of dissolved salts or electrolytes in soils. It is usually measured in deci-Siemens (dSm^{-1}) (Tanji, 2006). Soil salinization is categorized into classes by using its E.C. value. The non-saline type has E.C. value from 0-2, slightly saline soil has a value in the range 2-4, moderately saline has a value from 4-8, strongly saline has a value from 8-16, and very strongly saline soil has a value greater than 16 dSm^{-1} (Zaman et al., 2018).

2.1.2 Effects of Salinity on Plant Growth

Salinity causes nutrient imbalance by inducing a competition in nutrient uptake and mineral transport in the plant. This nutritional imbalance causes subsequent nutritional disorders, impacting plant development, vegetative growth, and crop produces (Grieve, 1999). Salinity lessens nitrogen uptake (Chen et al., 2010). Also, it reduces phosphate uptake by decreasing phosphate availability (Fageria et al., 2011). Additionally, it reduces the total uptake of potassium and its transportation by reducing the potassium in the shoot and root (Fageria et al., 2011; Ghoulam et al., 2002).

Plant growth is mainly affected by salinity because of decreased water uptake by plants from the soil through osmotic effects. The abundance of sodium (Na^+) and chlorine (Cl^-) affect ions balance and cause nutritional deficiencies due to the competition of sodium ions with others such as K^+, NO_3^-, and $H_2PO_4^-$, these ions are vital for plant nourishment (Tester & Davenport, 2003). Sodium (Na^+) toxicity is more severe than chlorine (Cl^-) because sodium induces cell swelling and disturbs protein synthesis and enzyme activation mechanisms, which results in less energy and other anatomical variations (Tester & Davenport, 2003). Further physiological and structural changes are also observed due to salinity, such as leaf staining, suppression in seed germination, sprout development, blossoming, and fruit-bearing (Rao et al., 2006; Sairam & Tyagi, 2004; Tester & Davenport, 2003). Few studies have examined the chronology of salinity and its effects on plants (Munns & Sharp, 1993). The authors have presented a growth response concept consisted of two phases. The first stage is named as osmotic phase, this is a short phase, and it affects plant growth mainly because of water stress and salt accumulation around the root. The second phase is ion-specific, initiated by a high concentration of salt in cell vacuoles of emerging leaves, which reduces the carbohydrates supply to growing cells, diminishing the growth of younger leaves. The second phase takes more time and is comparatively more prolonged than the first phase (Munns, 2002).

Plants are classified into three main categories based on having different techniques to tolerate salinity. The first category of plants manages salt stress by minimizing osmotic stress through decreasing leaf area and stomatal conductance. However, this mechanism is suitable only in the presence of abundant soil water. The second category of plants manages by avoiding the toxic sodium deposits in leaves through Na^+ exclusion by roots. The third category has the tissue tolerance mechanism, which compartmentalizing Na^+ and Cl^- ions at the cellular and intracellular levels to avoid high accumulation at the cytoplasmatic level. This activity transpires in the mesophyll cells of leaves, and over time, toxic sodium levels are developed (Munns & Tester, 2008).

2.1.3 Crop yields and salt stress

Several crops show declined yields even at salinity levels lesser than the defined threshold at EC= 4 dSm^{-1}. The resistance against the salinity of a few crops under salt-stressed environments is given in Table 2.1. Hence the growth rate of salt-sensitive plants is visibly affected with exposure to the saline environment, even for a limited time. More tangible effects such as yellow staining, death of older leaves, and diminished plant growth are observed with extended exposure to saline conditions. However, salt-tolerant plants may survive under moderate saline conditions, yet they may show decreased productivity in terms of flowering and yield (Munns, 2002). Paddy is considered a susceptible cereal crop because the salt tolerance threshold level of most paddy varieties is 3 dSm^{-1}, at which the growth and yield of paddy start decreasing (Hoang et al., 2016). However, the soil is not considered saline soil.

Table 2.1 Tolerance threshold values of some crops to saline conditions.

Sensitive (0-4 dSm^{-1})	**Moderately tolerance (4-6 dSm^{-1})**	**Tolerant (6-8 dSm^{-1})**	**Highly tolerance (8-12 dSm^{-1})**
Almond	Tomato	Sunflower	Olive
Clover	Corn	Wheat	Rye
Onion	Lettuce	Fig	Wheatgrass
Rice	Soybean	Pomegranate	Barley
Potato	-	-	-

2.2 Reclamation of Salt-affected soils

Either salinity can be reclaimed, or plants can be adapted to minimize the effects of salinity and increase crop growth in salt-affected soils (Maas, 1993). Different mechanisms can be used to reclaim salt-affected soils, such as reducing salt availability in soil, developing soil structure, and reducing soil pH (Yu et al., 2011). Soil salinity can be reduced by growing and harvesting the halophytes or removing the salts present in the soil away from the root zone of crops to be grown (Swallow &

O'Sullivan, 2019). While reclaiming soil, it is considered that it must be reducing soluble salts, sustain soil porosity, and offer a suitable environment for the movement of water in the soil and improve plant growth and yield (Qadir et al., 2000).

Different techniques can be used for the desalinization of salt-affected soils. Adequate drainage of soil is assumed as the main requirement for the success of every method (Qadir et al., 2000). Though, the success of every technique is subjected to various circumstances:

1. Nature and quantity of salts in the soil.
2. Accessibility of quality water.
3. Soil texture
4. Under-ground water quality and level.
5. Area required to be desalinized.
6. The nature of plants to be planted after desalinization.
7. Environmental conditions
8. Economic viability (Qadir et al., 2000).

The most prominent methods of soil reclamation are discussed briefly;

2.2.1 Flushing

This method is adapted for the soil where salts are accumulated on the soil surface in the crust. The soil surface is flushed out by passing water. A significantly less quantity of salts is removed in this method, which is ineffective (Abdel-Fattah, 2019; Qadir et al., 2000).

2.2.2 Salts Scrapping

The salts may be taken off through scrapping, which is gathered on the surface of the soil. This method is also not practically applicable (Abdel-Fattah, 2019; Qadir et al., 2000).

2.2.3 Leaching

Salts are pushed to move far away from the root zone of plants present in the upper 45-60cm soil surface (Keren & Miyamoto, 2011; Qadir et al., 2000). This technique of soil reclamation is applicable where good quality water and soil profiles, which facilitate the downward movement of salts, are available. The water essential for soil reclamation depends on various factors like soil texture, salt level, soil volume, and plants to be grown (Biswas & Biswas, 2014). It may be carried out in various ways, like continuous ponding, intermittent ponding, and sprinkling (Qadir et al., 2000). Reclamation of soil through leaching also has various disadvantages, like it requires plenty of water for a long time. In the circumstances where a surface seal creates on soil, intermittent ponding may prompt the development of fractures to permit water penetration (Abdel-Fattah, 2019). Sprinkler leaching of salts from the soil is a cost and energy-intensive technique.

2.2.4 Subsurface Drainage

In this technique of soil reclamation, salts are collected in collection ponds by removing out from the root zone through leaching. Soluble salts in salt-affected soils may be removed in deep open drains or perforated pipe buried in and carried to collection drains. This method is socially acceptable to farmers (Raju et al., 2016). This technique of soil reclamation also has its disadvantages. The agricultural land is utilized to form open deep ditches, and thus cultivated area becomes reduced. It also requires many bridges for the movement of machinery for fields approach. Frequent cleaning and maintenance of open drains are required for weed control and repairs. On the other hand, there is no reduction in agricultural land in case of buried pipes and also requires very limited maintenance cost. However, construction costs of pipes may be unaffordable for farmers because of high cost of materials, equipment and skilled labour engaged.

2.2.5 Biomimicry

Biomimicry is a very modern method introduced for the reclamation of saline soil. It tries to mimic the capillary action of vascular plants, which helps the plants collect the effloresced salts on the surface of salt-affected soils (Swallow & O’Sullivan, 2019). In this method, crystal inhibitors, like ferrocyanides, are used to avoid crystal development and improve evapotranspiration (Gupta et al., 2014), helping create dendritic crystals (Klaustermeier et al., 2017). It enables a capillary pump in the soil to permit the fast ascending transfer of soil water comprising the soluble salts (Sghaier & Prat, 2009). The salts become effloresced on the surface of soil because of evapotranspiration and can be mowed. This technique of soil reclamation is yet at a very initial stage. It needs to conduct field trials before recommending it as a valuable tool for reclamation of saline soil (Swallow & O'Sullivan, 2019).

2.2.6 Phyto-desalinization

In this technique of soil desalinization, salt-resistant plants (halophytes) are grown in salt-affected soils or applying plant parts, such as farmyard manure, straw mulch. Halophytes obtain Na^+ and Cl^- from saline soil and hoard these salts in the tissues of plants, which facilitates desalinizing the soil (Manousaki & Kalogerakis, 2011; Shelef et al., 2012; Walker et al., 2014). The plantation of halophytes in saline soils also stimulates the leaching of salts from root away from zone and reduction in soil (Rasouli et al., 2013; Walker et al., 2014). The halophytes plants used for soil desalinization can moderate the absorption Na^+ and Cl^- and maintain the acceptable concentration of K^+ and Mg^{2+} in the cell cytoplasm required for cellular functions

(Flowers & Colmer, 2015). The halophytes can be distributed into three main categories a) salts excluding, b) salts secreting, and c) salts accumulating (Walter, 1961). The application of organic material like farmyard manure and straw has resulted in the reclamation of saline soils. Mulching saline soils with straw resulted in increased leaching after rainfall (Cui et al., 2018). The mulching of saline soil with straw minimize the evaporation of water from the soil surface and loss of water through surface runoff and also enhances water intake and infiltration (Gholami et al., 2014; Leogrande & Vitti, 2019; Prosdocimi et al., 2016; Ram et al., 2013). The application of organic material to saline soils reduces the salinity as well as increases plant growth. Tejada et al. (2006) found that cotton compost and poultry manure application significantly increased soil physical, chemical, and biological properties. It is found that organic farming may enhance the desalinization of saline soils.

2.3 Plant growth promoting rhizobacteria in saline soil

The influence of PGPR on plant growth is acknowledged in several studies. The effects of PGPR application on plant development are manifold. They help fix nitrogen from the atmosphere; they convert the fixed P to available form and stimulate its transportation. They involve in iron chelation by siderophores and in the making of hydrolytic enzymes and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. They contribute to manufacturing organic molecules such as vitamins and amino acids, phytohormones, hydrogen cyanide, and antibiotics (Babalola, 2010).

2.3.1 Nitrogen fixation

Nitrogen fixation converts atmospheric nitrogen into ammonia by diazotrophic bacteria consumed by plants to manufacture nitrogenous biomolecules. Diazotrophic microbes are classified into three categories based on their nitrogen fixation mechanisms: symbiotic nitrogen fixers, rhizospheric nitrogen fixers, and endophytic fixers. The symbiotic nitrogen fixers use organic acids manufactured by plants to fix nitrogen; these fixers are found in plant nodules. Rhizospheric bacteria utilize carbon from exudates for nitrogen fixation, and endophytic nitrogen fixers use carbon found in plant tissues and access it from decomposing plant material (Sessitsch et al., 2012; Terakado-Tonooka et al., 2013). The production of ammonia (NH_3) is also another fundamental PGP trait of PGPR. The production of ammonia by PGPR provides nitrogen to the plants and increases plant growth and development (Marques et al., 2010). Some PGPR can produce hydrogen cyanide (HCN) and ammonia, which synergistically affect plant growth. The ammonia production by rhizobacteria directly supports plant growth by providing nitrogen, an essential nutrient required by the plants and synthesising chlorophyll, proteins, enzymes, DNA, and RNA. The application of nitrogen fertilizers improves plant growth, yield components, and significantly the value of the crops grown in salt-affected soils. Nitrogen increases the salinity resistance of plants as nitrogen plays nutritional and osmotic roles in saline soils (Chen et al., 2010). Ammonia production by rhizobacteria can help fulfil the plant's nitrogen requirements and minimize the root colonization of host plants by pathogens. A previous study conducted on *Avicennia marina* (Forsk) proved that salt stress significantly decreased the concentration of N and K in plant tissues (Naidoo, 1987). In salt-affected soil, applying nitrogen to the plants reduces the harmful effects of higher soluble salts on plant growth and nutrient uptake (Ahanger et al., 2019). Using nitrogen to mustard crops under saline conditions regulated ethylene and proline biosynthesis, photosynthesis, and plant development (Iqbal et al., 2015).

2.3.2 Phosphorus solubilization

Phosphorus is one of the essential macronutrients required for plant growth and is utilized to synthesize nucleic acids, Adenosine Triphosphate (ATP), and various enzymes. In soil, a significant portion of phosphorus exists in fixed forms such as $Ca_3(PO_4)_2$, $FePO_4$, $AlPO_4$, and organic phosphorus, unavailable for plants. The availability of P becomes reduced in high saline soil, and saline ions in soil inhibit P uptake by the plant through roots (Bano & Fatima, 2009; Karimzadeh et al., 2020; Rojas-Tapias et al., 2012). The availability of P to the plant becomes reduced in saline soils because of its fixation with calcium, aluminium, and iron. (Sashidhar & Podile, 2010). Patel et al. (2009) also reported that the nitrogen and phosphorus contents in seedlings of *Butea monosperma* were significantly reduced when they were grown under saline conditions. Costa & Medeiros (2018) reported that the nutrient content such as nitrogen, phosphorus, and potassium was significantly reduced in the watermelon plants when grown with saline water having 0.57, 1.36, 2.77, 3.86 and 4.91 dSm^{-1} electrical conductivities. Phosphorus usually exists in insoluble organic and inorganic forms at 0.04-0.12 %, while its solvable form exists at much lower levels, such as 0.1 % or less (Zou et al., 1992). Naturally occurring fixed phosphorus found in the upper layer of fertile soils usually is 20 to 80 % of total phosphorus, and almost 40 % is phytate fraction (Dalai, 1977). Converting insoluble phosphate to soluble and plant accessible form is another desirable feature of PGPR; the microbes capable of solubilizing phosphate are known as phosphate$^-$solubilizing bacteria (PSBs). The insoluble P can be solubilized by phosphate solubilizing rhizobacteria by manufacturing various organic acids. Thus, phosphorus becomes available for plants and gradually decrease soil pH. Phosphate solubilizing rhizobacteria can be used as valuable biofertilizers for plants to reduce the harmful effects of salts and recover the quantitative and qualitative characteristics of plant and soil efficiency. Karimzadeh et al. (2020) reported that the inoculation of salt-resistant phosphorus solubilizing rhizobacteria could significantly improve P uptake in wheat plants. Kadmiri et al.

(2018) found that applying salt-resistant phosphorus solubilizing and IAA producing rhizobacteria significantly enhanced wheat plant height shoot and root biomass under saline conditions. These rhizobacteria also enhanced total soluble contents and plant enzymatic activities.

2.3.3 Indole Acetic Acid (IAA) Production

Phytohormones or plant growth regulators are naturally occurring elements manufactured in small amounts by floras or microbes that can control several physiological mechanisms. They are responsible for initiating roots, promoting root structure, and branching roots. Plant growth regulator participates in a complex network of activities; they act as a chemical messenger among cells and control the chemical response (Kochar et al., 2013; Woodward & Bartel, 2005). IAA is one of the highly significant plants growth regulators which enhanced plant growth in maize crops grown in salt-affected soils (Kaya et al., 2010). IAA is a valuable plant growth regulator that mediates many plants like cell division, cell elongation, and root system development, encouraging plants to take up more nutrients and water from the rhizosphere (Egamberdieva, 2009). The author also reported that IAA producing rhizobacteria significantly improved the development of wheat plants by reducing the adverse effects of salt stress. IAA is also known to play a significant role in plant resistance against salinity stress. Alam et al. (2020) noted that salinity stress negatively influenced plant growth parameters and photosynthetic pigments in tomato plants. However, foliar application of IAA significantly improved plant growth and reduced the harmful effects of salinity stress.

Plant growth regulators are commonly applied to overcome the harmful effects of different biotic and abiotic stresses on plant growth. PGPR can produce plant growth regulators and even improve their amount, which positively influences plant growth.

They regulate the number of hormones in roots and shoots, control hormone levels, and adjust the long-range signalling process from root to shoot (Dodd, 2005). Some IAA producing salt-resistant PGPR under salt stress have been identified. The most prominent *Azotobacter, Arthrobacter, Zospirillum, Pseudomonas, Stenotrophomonas* and *Rahnella* (Abd-Allah et al., 2018; Egamberdieva et al., 2008, 2018; Piccoli et al., 2011). Various studies have verified that the application of IAA producing rhizobacteria enhanced plant growth and yield under salt-stressed conditions. Yao et al. (2010) found that the application of *Pseudomonas putida* in cotton crop modulated IAA production in plant tissues and significantly improved plant growth under saline environments. Goswami et al. (2014) isolated IAA producing rhizobacteria *Kocuria turfanensis* from halotolerant plant *Suaeda fruticosa* from a saline area. This strain was evaluated for the growth promotion of groundnut under salt-stressed conditions. Under saline conditions, the application of IAA producing rhizobacteria increased by 17% and 13% more plant length and fresh biomass, respectively. Similarly, Khan et al. (2019) isolated salt-resistant IAA producing PGPR from saline soil and evaluated for plant growth promotion of soybean seedlings under saline soils. They found that the application of IAA producing rhizobacteria significantly improved the plant growth parameters by alleviating the adverse effects of salinity on plants.

2.3.4 Siderophores production

Iron is known as an essential micronutrient, and it exists in several types of soils. Iron is an ample nutrient in the soil; however, it is generally a deficient micronutrient for plants because of its insolubility in certain forms (Fe^{+3}). The iron deficiency in soil may appear if ferrous (Fe^{+2}) ions are not regularly unconfined from soil minerals. Plants absorb (Fe^{+2}) form, which is required for chlorophyll contents synthesis and plays an essential function in several enzymatic activities that take part in the respiratory process of the plants. Manganese and copper are two oxidizing agents, which can change Fe^{+2} into insoluble Fe^{+3}. It has a dynamic role in numerous metabolic activities comprising the oxidative phosphorylation & photosynthesis process (Fardeau et al., 2011; Messenger & Barclay, 1983). The excess soluble salts in soil significantly reduce micronutrients, like Fe, to plants (Wallender & Tanji, 2011). The previous research proposed that salinity associates adversely with Fe availability to plants (Abbas et al., 2015; Q. Li et al., 2016; Purohit et al., 2016). Thus, iron deficiency to plants meaningfully decreases the quality and quantity of crop production. Zayed et al. (2011) found that the application of Fe to rice crops significantly promoted the plant growth and yield parameters under saline conditions. Siderophores are organic compounds with a low molecular weight, which are synthesized by rhizobacteria in iron-deficient environments. The critical role of such compounds is to chelate iron and make it accessible for plant and microbial cells. Amira et al. (2015) also reported that ZnO and Fe_2O_4 as nano fertilizers under saline conditions to rice crop significantly improved root length, shoot length, number of branches, number of leaves, fresh shoot and dry weights and fresh root and dry weights. They also reported that the application of ZnO and Fe_2O_4 also significantly decreased Na^+ and Cl^- uptake in rice plants. Siderophore producing rhizobacteria also play an essential role in the biological control of various phytopathogens. Siderophores produced by rhizobacteria chelate with iron firmly and make it inaccessible to pathogens, consequently hindering the growth of phytopathogens (Ahmed & Holmström, 2014; Beneduzi et al., 2012). The application of auxin and siderophores producing *streptomyces* significantly enhanced the growth of wheat plants in salt-stressed environments (Sadeghi et al., 2012). They also found improved germination percentage and uniformity, shoot length and dry weight under saline conditions. The application of bacterial inoculum improved N, P, Fe, and Mn in wheat plants grown under salt-stressed soils. Applying siderophores to produce endophytic rhizobacteria in rice crop significantly increased plant growth and biomass (Rungin et al., 2012).

2.3.5 ACC deaminase activity

Ethylene (E.T.) is a vaporous hormone that controls several essential phenomena in vegetation, such as the appearance of seedlings, leaf, and flower deterioration with age, tissue abscission, and fruit ripening. This hormone also improves the plant endurance against organic and environmental stresses such as harsh weather, infectious diseases, waterlogged soil, famine, heavy metals toxicity, extremely saline soil, and compaction of the rhizosphere (Dodd et al., 2010; Morgan & Drew, 1997). Because of some stress, a continuously high ethylene concentration restricts the root elongation process, leading to diminished root development, subsequently affecting overall plant health and growth (Babalola, 2010). ACC, a direct precursor of ethylene in plants, is cleaved into NH_3 and α-ketobutyrate in the presence of the ACC deaminase enzyme. ACC deaminase producing microbes can metabolize ACC, manufactured and discharged by plant tissues (Glick et al., 1998). Under stress conditions, ACC is made and secreted by seeds or roots, which permits ACC deaminase producing PGPR in the soil to degrade it, then ACC is used by microbes. Plant tissues generate more ACC to maintain equilibrium inside and outside the cells, reducing the quantity of ACC in plant cells for Ethylene synthesis.

The enzyme ACC-deaminase produced by various rhizobacteria catalyzes ACC deprivation, decreasing ethylene's harmful level in plants under stress. ACC deaminase producing bacteria can facilitate the plants to grow under stressful environmental circumstances, including salinity, flooding, drought, heavy metal contamination, and phytopathogens. Consequently, the application of ACC deaminase producing rhizobacteria can restrict the effect of ethylene on plant growth and permits the plant to improve plant growth under stress conditions. Gupta & Pandey (2019) reported that ACC deaminase producing PGPR isolated from saline soil significantly decreased ethylene stress in French beans subjected to salinity stress. Significant increases were observed in shoot length, fresh shoot weight, root length, fresh root weight, and total chlorophyll content of French bean plants grown under saline conditions. Similar findings were also reported in tomato plants where the application of ACC deaminase producing endophytes *Pseudomonas fluorescens* and *Pseudomonas migulae* significantly improved the salinity tolerance (Ali et al., 2014). The tomato plants inoculated with ACC deaminase producing bacteria produced significantly higher growth, yield parameters, and photosynthesis pigments. Various previous studies showed that the application of ACC deaminase producing rhizobacteria improved considerably salt tolerance and growth of plants (Li & Jiang, 2017; Misra et al., 2019; Misra & Chauhan, 2020; Ullah & Bano, 2015).

2.3.6 Exopolysaccharides production

Exopolysaccharides (EPS) are high molecular weight polymers that contain sugar residues and widely vary in structure and role. Many bacteria can produce and excrete exopolysaccharides. The influence of EPS producing PGPR on the combination of root adhering soils has been reported by Alami et al. (2000). The PGPR, which can produce ESP, has a vital role in plants grown in saline soils (Rossi & De Philippis, 2015). PGPR capable of EPS production can face salt stress through the preparation of rhizo-sheaths around plant roots. The EPS produced by PGPR attaches with Na^+ cations, reducing availability to the plants (Tewari & Arora, 2014; Timmusk et al., 2014). The application of *Bacillus subtilis* to *Arabidopsis thaliana* caused a significant reduction in the influx of Na^+ via downregulating the expression of HKT1/K^+ transporter (Zhang et al., 2008). The application of *Pseudomonas aeruginosa* to sunflower also reduced the harmful effects of salinity through EPS production and resulted in significant increased growth and yield (Tewari & Arora, 2014). Thus the bacteria capable of producing EPS have ameliorative effects against salinity to improve soil structure. Upadhyay et al. (2011) found that the inoculation of EPS producing rhizobacteria to wheat significantly enhanced salt tolerance by increasing the growth and development of plants. PGPR also considerably improved the K^+/Na^+ ratio by reducing the uptake of Na^+ under saline conditions. The application of *Azotobacter chroococcum* to faba beans under saline conditions significantly increased the N, P, and K uptake, proline content, K^+/Na^+ ratio, and RWC%; however, the uptake of Na^+ and Cl^- were reduced significantly (El-Ghany & Attia, 2020). In various studies, the application of EPS producing PGPR in reducing salinity stress in tomato (Isfahani et al., 2019), chickpea (Qurashi & Sabri, 2012), wheat (Ashraf et al., 1999), faba beans (Alaa, 2018)

2.4 Alleviating Na^+ stress by K^+ application

Higher availability of sodium in soil influences the mineral to the nutrient ratio in plant tissues, mainly its effects on calcium and potassium. Maintaining the optimum K^+:Na^+ ratio is significant in evaluating the salt resistance of salt-stressed plants as sodium deposits affect the potassium nutrition of plants. Selecting and breeding only those crops that can maintain higher potassium to sodium ratio under saline stress is a realistic strategy to cope with salinity and reduce economic losses (Santa & Epstein, 2001). Several studies have thoroughly examined and verified the harmful effects of salinity on vegetation growth, mainly due to ionic disparity (Cerdá et al., 1995; Lynch & Läuchli, 1985). Maintaining optimum potassium concentrations in saline soils is required for healthy crop growth and yield (Greenway & Munns, 1980). Potassium insufficiency at the cell level may induce oxidative stress due to salt, even though several studies provide enough knowledge about potassium and sodium homeostasis and offer supporting evidence for using potassium fertilizers to minimize sodium uptake by plants. However, few works have given conflicting evidence related to the potential effects and use of potassium fertilizers on crop growth in saline soil (Adhikari et al., 2020).

2.4.1 Foliar application of potassium source

Plants developing under salt stress can take up a small quantity of potassium through roots due to the competition among potassium and sodium cations hence suffering from potassium deficiency. However, this deficiency can be managed by supplying additional potassium to the plant through foliar spray. Kaya et al. (2001) applied KH_2PO_4 by the foliar method to a tomato crop in a salt-stressed environment; they managed to minimize the chlorophyll and cell membrane damage and lessen the biomass development triggered due to salt stress. Ahmad and Jabeen (2005) applied 250 ppm KNO_3 as foliar to bottle gourd in saline stressed environment and observed a reduction in toxic effects of ions and 77% improvement in fruit yield. Likewise, similar results on sunflower crop grown under saline stress with KOH foliar spray application had improved the potassium concentration in leaves and enhanced the growth and yield (Akram et al., 2009). It is identified that several sources of potassium have contributed to improving crop production by increased conductance in stomata, enhanced photosynthesis, and regulate efficient water usage under saline stress. Additionally, the potassium levels in roots and leaves were also increased. Similarly, an improvement in eggplant's vegetative growth and fruit yield is reported using K_2HPO_4 (Elwan, 2010). A significant reduction of sodium deposits in fruits is also observed by applying 10 mM K_2HPO_4. However, it was noted that KNO_3, KH_2PO_4, and K_2CO_3 are more efficient than KOH and KCl (Akram et al., 2009). Amjad et al. (2014) also confirmed that potassium improved plant growth and yield of the tomato crop, particularly in salt-resistant varieties. Fruit quality features such as dry matter percentage, titratable acidity, pH, and soluble solids were significantly enhanced with the foliar and soil application of K in salt-affected soils. Din et al. (2001) reported that the addition of potassium into the saline soil, as well as foliar application in rice crop, significantly increased tillers, straw yield, grain yield, K concentration in root and shoot, decreased sodium in root and shoot and also improved K^+/Na^+ ratio in paddy plants. Ashraf et al. (2013) stated that soil and foliar application of potassium in the shape of SOP and MOP counteracted the adverse effects of salinity on wheat crop under salinity stress conditions. Foliar application of KNO_3 and $Ca(NO_3)_2$ significantly improved shoot and dry root weights by minimizing the adverse effects of salinity in strawberry plants (Yildirim et al., 2009). An improved P, Fe, and Zn contents were observed in shoots and roots of plants due to foliar application of potassium under salt stress. However, on the other hand, foliar application of potassium on soybean plants did not mitigate the harmful impact of salinity on the early growth of plants (Adhikari et al., 2020).

2.4.2 Soil application of potassium source

Optimal vegetative growth and better yield demand a regular and stable source of essential nutrients (Sairam & Tyagi, 2004). More than enough potassium level in roots is incompatible for sodium uptake by plants through NSCCs and HKT1, and increased sodium levels in the roots cause competition among cations and thus disturb the zone nutrient balance. A significant improvement in crop growth has been observed in pepper and cucumber due to potassium fertiliser application in saline soils (Kaya et al., 2003). Similar effects are noted on potatoes (Elkhatib et al., 2004), rice crops (Bohra & Doerffling, 1993; Kibria et al., 2015; Munir et al., 2019) and wheat (Kausar et al., 2016; Singh & Sharma, 2001). Further, potassium application to pearl millet in saline stressed conditions had improved vegetative growth and crop by increasing potassium concentration in tissues and reducing sodium levels (Heidari & Jamshidi, 2011). Likewise, adding potassium in the root area of the barley crop in saline soil, with EC ≈ 13 dS m^{-1}, showed an improved vegetative growth (Endris & Mohammed, 2007). Wakeel (2013) reported that applying potassium in double quantities on a wheat crop has resulted in lower sodium levels and higher potassium levels in plant shoots. However, substantial growth in dry matter was reported at saline levels 4 and 8 dSm^{-1}, although no visible enhancement in vegetative progress was recorded at 12 and 16 dSm^{-1} salinity compared to control.

On the other hand, potassium fertilizer on corn grown in salt-stressed soil did not enhance the vegetative development and grain yield. The use of potassium did not remove the toxic effects of sodium, regardless of having better potassium to sodium ratio in plant tissues (Bar-Tal et al., 1991). The potassium application significantly improved growth characteristics, including nitrogen and antioxidant metabolism in two wheat cultivars (Ahanger & Agarwal, 2017). They also reported that the added potassium also improved the K^+:Na^+ ratio in plants.

2.4.3 Hydroponic studies

Although few works have demonstrated inconsistent results in improving the crops with additional potassium fertilizer in the saline environment, generally, potassium supplements tend to lessen sodium uptake in plants and strengthen potassium accessibility. Tzortzakis (2010) reported that potassium and calcium application to hydroponically grown endives alleviated the adverse effects of salinity. Similarly, when strawberry were planted under salt stress conditions, supplemented with potassium in the form of K_2SO_4, an improvement in the damaging effects caused by salt was observed in the affected leaf region (Khayyat et al., 2009). Hence it can be inferred that increased potassium concentration and reduced sodium and chlorine accumulations in plant tissues may increase crop yield in saline environments. Potassium tends to overcome the toxicity of sodium by decreasing its absorption by plants and providing additional calcium also improve vegetative growth (Kopittke, 2012). It is also supported by a study that demonstrated that adding potassium to *Jatropha curcas* seedlings significantly reduced sodium uptake in saline conditions (Rodrigues et al., 2012). The application of potassium, the xylem, has reduced the sodium levels in plant tissues by reducing sodium absorption. These results indicate the inhibition of sodium absorption at the root zone due to the high potassium levels in that area. Another study has been carried by Ashraf et al. (2012) on sugarcane under salinity stress. They evaluated the ameliorative role of potassium to overcome the adverse effects of sodium chloride (NaCl) in sugarcane genotypes under different stress levels (0, 100, 130, and 160 mM). They reported that the application of NaCl significantly reduced the potassium in plant tissues; however, the addition of potassium to sugarcane genotypes minimized the adverse effects of NaCl and improved crop growth and yield. Another work examined the use of potassium on two different species of broad bean to resist saline effects (Bulut et al., 2011). They applied potassium nitrate (KNO_3) and potassium acetate (CH_3COOK) as nutrient supplements. Nevertheless, they did not observe an elevated level of potassium in plant organs; neither any substantial improvement in plant development

2.5 Availability of potassium in soil

Typically, the soil contains a small amount of potassium in the soil solution. The upper 30 cm layer of soil has 5-25 kg/ha K in soil solution form (Fotyma et al., 2013). Most potassium deposits in soil are frequently maintained through negative ions on clay particles and organic matter. Clay lattice may hold K^+ in loosely or tightly bound form depending on its position on clay. The loosely bound potassium is radially accessible to plant, while the tightly bound potassium is slowly accessible to plants. Distinct soil properties have varying potential to bind $K^{+,}$ which depends on soil type, deposits of organic material, and other contents in the rhizosphere. Typically, sand has minimal deposits of readily available potassium, although clay itself comprises K^+.

2.5.1 Forms and availability of potassium

Potassium is one of the most abundantly found components in the lithosphere; it contributes approximately 2.4% by weight and is seventh among significantly found elements. However, only 1-2% of potassium is accessible for plant uptake (Meena et al., 2014). Potassium occurs in the soil in many distinct forms, such as soluble K^+ ions, loosely bound, and firmly fixed. Plants can readily take up soluble and loosely bound potassium, whereas firmly fixed potassium must be converted into the soluble form before the uptake. Depending upon the nature of the soil, up to 98% of total K in soil is not accessible to the plants. Natural components like feldspars and micas cover a substantial amount of potassium and other fundamental content of soil. Plants cannot use insoluble potassium until the fixed potassium is released due to environmental weathering over a long period. However, this process is time-consuming to fulfil the potassium requirements of plants.

2.5.2 Environmental factors affecting potassium solubilization

The solubilization of K^+ in the soil is influenced by several biotic and abiotic factors such as physicochemical properties of soil, aeration, and pH. The occurrence of mycorrhizal fungi, rhizosphere microbes, and root substances also contributes to solubilize K^+. These factors may also affect the solubilizing capacity of microbes.

Gahoonia et al. (1997) have proposed three theories to explain the mechanisms of mineral fermentation. Firstly, mineral fragmentation because of root activity boosts the mineral solubilization by bacteria because of more surface area; second is root secretions that contribute indirectly by supplying substrates to microbes for weathering metabolite synthesis; third, the microbes generate weathering agents, and phytohormones which trigger the root expansion, alter root exudation and improve nutrient absorption and mineral mobility. The concentration of potassium in different plant tissues may vary according to environmental conditions. The K solubilization from feldspar, illite and muscovite is higher in aerobic environments than in anaerobic environments (Badr, 2006), while a better growth and potassium solubilization were observed in *Bacillus edaphicus*, with illite than feldspar (Sheng & He, 2006).

2.6 Potassium solubilizing bacteria (KSB)

Various scientists have presented a wide diversity of potassium solubilizing rhizobacteria. Fei et al. (2008) isolated 23 potassium solubilizing rhizobacteria from rhizosphere soil, and all strains were characterized as able to produce IAA, and 43.5% could produce siderophores. Out of 23 strains, two AC2 and AFM2 strains were found the most efficient strains, identified as *Bacillus mucilaginosus* and *Agrobacterium tumefaciens.* Zarjani et al. (2013) isolated rhizobacteria from Iranian soils capable of K solubilization from different sources. Out of six efficient strains, five belonged to *B. megaterium,* one to *Arthrobacter* sp. Further, Sheng (2005) isolated potassium solubilizing strain *Bacillus edaphicus* from cotton rhizosphere soil, which could solubilize mineral potassium and significantly improve growth and nutrient concentration in cotton and rape crops. Liu et al. (2012) isolated KSB from the soil, which was identified as *Paenibacillus mucilaginosus*. Sangeeth and Suseela (2012) isolated KSB from black pepper soil and concluded *Paenibacillus glucanolyticus* as the most promising strain. Prajapati et al. (2013) isolated KSB, which was identified as *Enterobacter hormaechei*. Zhang & Kong (2014) isolated KSB from tobacco rhizosphere soil. Out of all 27 KSB strains were evaluated for K solubilization in liquid broth. Out of 27 strains, 17 belonged to *Klebsiella variicola*. Two belonged to *Enterobacter cloacae*, two belonged to *Enterobacter asburiae*, and the other six belonged to *Enterobacter aerogenes*, *Pantoea agglomerans, Agrobacterium tumefaciens, Microbacterium foliorum*, *Myroides odoratimimus, and Burkholderia cepacia,* respectively. Meena et al. (2015) isolated 12 potential KSB from different kharif crops. Out of 12 KSB, seven belonged to *Agrobacterium tumefaciens*, two belonged to each *Rhizobium pusense*, and *Flavobacterium anhuiense*, whereas one belonged to *Rhizobium rosettiformans*. Khani et al. (2019) also isolated K solubilizing rhizobacteria from the soil and identified *Enterobacter cloacae* and *Pseudomonas* sp. as the most efficient KSB.

2.7 Mechanisms of K solubilization

The mechanism of potassium solubilization refers to converting and mobilizing the insoluble and fixed K^+ to plant accessible form. This process is supplemented by the generation of several organic acids accompanied by acidolysis, complexolysis, and chemical reactions vital for the K^+ solubilization (Uroz et al., 2009). The organic and inorganic acids produced by rhizobacteria transform fixed potassium (mica, muscovite, biotite, and feldspar) to an accessible form of potassium, increasing the total amount of nutrients to the plant. Potassium solubilizing microbes produce several kinds of organic acids, while potassium solubilization. Zhang & Kong (2014) observed organic acids in bacterial culture. These mechanisms allow weathering on phlogopite through aluminium chelation and an acidic suspension of the crystal network (Abou-el-Seoud & Abdel-Megeed, 2012; Meena et al., 2014).

However, presently, the potassium solubilization mechanism by rhizospheric microbes is not fully understood. Xia et al. (2002) found that the pH of the soil, oxygen exposure, and microbial strains affected the solubilization of potassium from minerals. The efficacy of the potassium solubilizing through microbes varies with environmental conditions and with the presence of potassium$^-$containing minerals (Uroz et al., 2009). The *B. edaphicus* can solubilize more K^+ in broth, improving growth on illite than feldspar (Sheng & He, 2006). Native and active soil microbes can engage and mobilize tightly bound K^+ from raw minerals; for instance, silicate microbes effectively solubilise K^+, silica, and aluminium from insoluble minerals. The presence of hydrogen ions in the soil also influences the release of potassium from minerals. The amount of K^+ solubilization is increased in medium containing microbes from 84.8 to 127.9% compared to the medium without microbes (Sheng & He, 2006). In another study, an increase in the rate of K^+ solubilization is noted, 4.90 mg/L at pH 6.5 to 8.0, by using silicate solubilizing microbes (Badr, 2006). Likewise, it was noted that 4.29 mg/L with *B. mucilaginous*; in the medium treated with muscovite mica (Sugumaran & Janarthanam, 2007).

The potassium solubilizing mechanisms are mainly categorized into three processes first is accomplished by reducing the pH of the soil, second is performed by improving the chelation of potassium bonded cations, and third is acidolysis of neighbouring area microbes. The production of organic acids and protons by KSB is indicated by reducing soil pH (Zarjani et al., 2013; Uroz et al., 2009). While acidolysis either chelation of Si and Al ions related to potassium or readily solubilize the potassium due to the slow release of loosely bound potassium (Römheld & Kirkby, 2010). The organic acids generated by microbes promote the acidic environment in the surrounding soil, which subsequently contributes to liberating the bounded potassium ions.

The production of organic acids and lowering the pH is assumed as a major mechanism of potassium solubilization from mineral forms; however, it is noted that acidolysis only is not sufficient for potassium solubilizing because the reduction in pH does not correlate with the rate of potassium solubilization in few cases (Rosa-Magri et al., 2012; Zhang & Kong, 2014).

Furthermore, it is observed that when microbes convert potassium from fixed to a soluble form, they also release several organic acids. Hence it is theorized that the production of these organic acids also influences the enhancement in vegetative growth due to microbe potassium solubilization. A study examined the K^+ solubilization of illite and feldspar through rhizobacteria and noted the release of several organic acids (Sheng & He, 2006). They found 2-ketogluconic acid, oxalic acid, malic acid, tartaric acids, gluconic acid, succinic acid, and citric acid. Tartaric acid is the utmost abundantly found acid during the potassium solubilization process (Zarjani et al., 2013; Prajapati & Modi, 2012). Few more organic acids were found during solubilization processes, such as tartaric acid, acetic acid, fumaric acid, citric acid, succinic acid, lactic acid, malonic acid, propionic acid, glycolic acid and oxalic acid (Wu et al., 2005).

Natural potassium solubilization mechanisms can typically solubilize potassium minerals in lab conditions (Zarjani et al., 2013; Maurya et al., 2014) and agricultural and greenhouse environments (Basak & Biswas, 2012; Prajapati et al., 2013). Additionally, the deposits of microbes in the rhizosphere comprise a substantial amount of fixed potassium that can be made available for plant usage (Subhashini & Kumar, 2014; Zhang & Kong, 2014).

2.8 Effect of potassium solubilizing rhizobacteria on plant growth

Treating different plants and seeds with potassium solubilizing microbes typically demonstrated a substantial improvement in germination, seedling vitality, vegetative growth, crop yield and enhanced uptake of potassium by the plant in the greenhouse environment (Singh et al., 2010; Awasthi et al., 2011; Basak & Biswas, 2012; Ai-min et al., 2013; Zhang & Kong, 2014). Aleksandrov (1958) first experimented using minerals with silicate bacteria to increase wheat and maize crop vegetative growth and grain yield. Remarkably, the studies performed on several crops, such as fodder crops, wheat, maize, and Sudan grass, indicated a considerable reduction in synthetic or natural fertilizers by inoculating potassium solubilizing microorganisms (Xie, 1998). Few studies have suggested that the improvement in potassium nutrition of plants is may be due to the enhanced root growth and long root hairs caused by specific microbes (Sindhu et al., 2014; Zeng et al., 2012).

Inoculating the plants and crops with KSB strains has improved plant growth and crop yield (Basak & Biswas, 2012; Singh et al., 2010). Using efficient and relevant microorganisms with K^{-}solubilizing potential can substantially improve the Potassium Use Efficiency (PUE) in cultivatable areas. The use of KSB offers a sustainable nutrient supply to the vegetation. Furthermore, KSB effectively enhances plant growth by enhancing nitrogen fixation, P, and K solubilization (Maurya et al., 2014; Zhang & Kong, 2014). Singh et al. (2010) investigated the effectiveness of *Bacillus mucilaginous, Azotobacter chroococcum,* and *Rhizobium* sp. in mobilizing the potassium from mineral mica on wheat and maize crop in the phytotron growth chamber and hydroponic environment. They found that high potassium deposits were pronounced in wheat and maize crop having only raw mica for potassium source, indicating an elevated deposition of biological material and potassium nutrients, better absorption by plants, and enhanced chlorophyll and raw proteins in plant organs. Inoculating the *B. mucilaginous* strain of rhizobacteria provides better potassium mobilization than *Azotobacter chroococcum* and *Rhizobium* sp. A study was conducted on the wheat crop grown in potting soil with low available potassium to examine the potassium mobilization using the wild-type strain NBT of *B. edaphicus* (Sheng & He, 2006). Their study showed a substantial enhancement in root and shoot growth and a significant increase in NPK levels of plants compared to un-inoculated soil.

Furthermore, beneficial effects on plant health and growth are observed in several crops by treating with potassium solubilizing microbes such as cotton and rape (Sheng, 2005), sorghum (Badr, 2006), brinjal (Ramarethinam & Chandra, 2006), pepper and cucumber (Han et al., 2006), wheat (Sheng & He, 2006), sudangrass (Basak & Biswas, 2009; Basak & Biswas, 2010) and, tobacco (Zhang & Kong, 2014). Likewise, Zahra et al. (1984) recorded a considerable increase in organic matter. An approximately 17% more rice yield was observed by adding silicate solubilizing bacteria *B. cirulans* for potassium solubilization. According to Badr (2006), the inoculation of KSB on sorghum plants in three distinct soil types such as clay, sandy and calcareous; they found a 48%, 65%, and 58% higher dry matter, 71%, 110%, and 116% increase in phosphorus and 41%, 93%, and 79% increase in potassium uptake concerning soil type. Similarly, another study recorded a 25% increase in dry matter and 35.4% in the oil content of groundnut crops (Sugumaran & Janarthanam, 2007). They also noted increased available phosphorus and potassium 6.24 to 9.28 and 86.57 to 99.60 mg/kg, respectively, in untreated and treated soil with *B. mucilaginous*.

Archana et al. (2012) recorded the favourable impact of an efficient KSB *Bacillus* sp. on growth and grain yield of maize exhibited by a considerable improvement in plant growth, nutrient uptake and grain yield than the crop entirely dependent on synthetic fertilizer. Supanjani et al. (2006) isolated potassium and phosphorus minerals and treated them with potassium and phosphorus solubilizing microbes, enhancing phosphorus and potassium availability from 12% to 21% and 13% to 15%, respectively. Plants inoculated with K solubilizing rhizobacteria have approximately 16% and 35% more photosynthesis and leaf area than control, respectively. The process of treating the soil containing potassium and phosphorus minerals with potassium and phosphorus solubilizing bacteria may be a possible maintainable substitute for synthetic fertilizers. Moreover, Korea and China are already using KSB as biological fertilizers as substantial parts of agricultural land. In these countries, potassium in the soil for plants is deficient, which can cause significant crop reduction (Xie, 1998). Thus, using KSB as biofertilizers for crop development can significantly decrease the usage of chemical fertilizers and promote a more sustainable environment (Maurya et al., 2014; Prajapati et al., 2013; Zhang & Kong, 2014). Hence, it is essential to isolate and identify more and more microbial species that can efficiently solubilize minerals and expand the pool for potential biological fertilizers. These bacterial fertilizers would be a huge step towards an eco-friendly and sustainable agricultural environment (Liu et al., 2012). Tan et al. (2014) reported that the locally isolated PGPR with potassium solubilization capability demonstrated the substantial effects on early growth and vigour of rice seedlings under controlled conditions. Shultana et al. (2020) reported that inoculum phosphorus and potassium solubilizing strain UPMRE6 significantly improved the rate of photosynthesis, transpiration and stomatal conductance of three rice varieties under saline conditions.

2.9 Types of soils for paddy

The soils play a significant role in rice production. The soil texture affects available water capacity and organic matter to the plants grown. Generally, clay soil is richer in organic matter than sandy soil due to higher physical protection characteristics from clay (Six et al., 2000). Higher organic matter contents usually mean higher available water capacity. Hudson (1994) concluded that an increase in organic matter from 0.5 to 3% almost doubled the available water capacity of the soil. The reduction in soil organic matter and soil compaction can significantly reduce the crop yield (Powers et al., 2004). Moreover, the soil also affects root growth which is the main plant organ for water uptake. Generally, the more extensive the roots have a higher elongation potential and increase soil water and nutrient uptake. Soil texture significantly affects rice grain yield (Dou et al., 2016). They reported that 46% higher rice grain yield was recorded in clay soil than sandy loam soil. Similar results were also recorded by Ye et al. (2007). Tsubo et al. (2007) also reported similar response, rice grown in higher clay-content soils had greater grain yield and biomass accumulation than those grown in lower clay content soils.

2.10 Effect of salinity on paddy

Paddy (*Oryza sativa* L.) is the second most important crop after wheat, which provides food to half of the world's total population (Nakbanpote et al., 2014) and is grown in various parts of the world, especially in Asia, Latin America, and Africa. About 90% of total paddy grown in the world is cultivated in Asia, of which 30, 21, and 18% are produced in China, India and Pakistan, respectively, whereas the remaining 30% of paddy is grown in Thailand, Indonesia, Burma, and Japan (Calpe, 2006; Khush, 2005). Paddy has a higher yield potential; however, about 10 to 15% lower yield is obtained (Virmani et al., 1991). Many environmental factors, including biotic and abiotic factors, management practices, and nutrient deficiency, significantly affect paddy growth and yield. Abiotic stresses, particularly salinity, are the most important yield-reducing factors. Excessive salts present in soil affect the growth and yield components of paddy crops, including stand establishment adversely; panicles, tillers, and spikelets per plant; floret sterility; individual grain size; and even delayed heading (Grattan et al., 2002). In another study, eight salt tolerant paddy genotypes were subjected to 8.5 dSm^{-1} salinity and a significant decrease in yield per plant, chlorophyll concentrations, fertility percentage, and the number of productive tillers, panicle length and number of primary branches was observed (Ali et al., 2004). Nakbanpote et al. (2014) also reported that the salt stress ranging from 4-16 dSm^{-1} significantly reduced paddy seed germination and seeding growth. Similar findings were also concluded by Shrivastava & Kumar (2015) and Hoang et al. (2016). Similarly, Zhang et al. (2018) concluded that paddy plants grown under 3, 6, 9, and 12g/l NaCl were adversely affected.

2.10.1 Effect of PGPR on paddy

Molecular breeding and genetic engineering methods are being adopted to minimize the adverse effects of salinity and increase crop tolerance against biotic and abiotic stresses (Nautiyal, 2013). The development of transgenic plants to reduce the damaging effects of environmental stresses are allied to yield penalty, high cost, loss of plant gene functions, and other regularity issues (Singh et al., 2015). The use of plant growth-promoting rhizobacteria tolerant salinity, which can successfully colonize with the roots of the respective crop, may be an effective alternative to develop stress against salinity. Previous studies show that using salt-resistant PGPR in paddy could enhance germination, promote seedling growth, antioxidant enzymes, and osmolyte accumulation (Nautiyal, 2013; Paul & Lade, 2014; Rima et al., 2018; Sarkar et al., 2018). Zhang et al. (2018) isolated 162 rhizobacteria from the paddy rhizosphere, salt-tolerant up to 150 g/l NaCl. Most of these rhizobacteria applications significantly improved salt tolerance, growth, and rice yield under salt-stress conditions. Bal et al. (2013) also reported similar results. Ji et al., (2020) found that the application of rhizobacteria having different plant growth-promoting properties could improve the salt tolerance of paddy plants against salinity stress. The salt-tolerant PGPR may alleviate the salinity problems in crops grown in salt-affected areas (Shultana et al., 2020). They reported that the inoculation of locally isolated strains PGPR had substantial effects on relative water content, electrolyte leakage, and the Na/K ratio of the BRRI dhan67 rice variety under saline conditions. Similarly, Doni et al. (2014) reported that the inoculation of *Clostridium* and *Pseudomonas* to paddy seeds significantly enhanced rice seedling wet weight and biomass. Mohamad et al. (2019) reported that the application of PGPR having different plant growth promoting properties increased the rice yield by reducing the panicle grain shattering in the Malaysian climate. Similarly, Habib et al. (2016) reported that the locally isolated strains UPMR 7 and UPMR 17 significantly improved paddy plants' early growth compared to uninoculated plants. Othman et al. (2017) reported that the inoculation of *Acinetobacter* sp. improved plant growth and root in rice plants. The inoculation of PGPR may improve the water use efficiency (Omar et al., 2014). They reported that the inoculation of PGPR improve the water use efficiency in paddy plants under water limited environments. Ouyabe et al., (2020) concluded that under greenhouse conditions, most rice growth parameters were improved by inoculation with the strain S-7. It can be supposed that rhizobacteria having different plants growth promoting properties have pronounced effects on rice growth under both salinity stressed and normal conditions.

2.11 Conclusion

As discussed at the beginning of this chapter that the increase in salt-affected soils is increasing globally. It is expected that more than 50% of the arable region might be salt-affected up to 2050 (Jamil et al., 2011). Various techniques are being adapted to minimize the impact of salts on plant growth. The methods being utilized to reduce the adverse effects of salinity have their disadvantages, as discussed in section 2.2 of this chapter. Na^+ is the most critical cation in saline soils, which adversely affects plant growth. Na^+ cations compete with K^+ for their uptake in plants under saline soils, ultimately disturbing the optimum K^+: Na^+ ratio in plant cells. The application of potassic mineral fertilizer has proven beneficial in reducing the uptake of Na^+ in plants and improving the K^+: Na^+ ratio in plant cells. The application of chemical fertilizers is complex because of various causes like inadequate resources, timely availability, high cost, etc. Additionally, the extreme application of mineral fertilizers harms the environment (Ahmad et al., 2016). Hence, developing and using approximately cost-effective and eco-friendly technology for sustainable paddy crop production under saline conditions are imperative. The specific focus on K solubilizer having PGP properties for saline soil is necessary. Exploring the salt-resistant potassium solubilizing plant growth promoting rhizobacteria may provide great insight into the indigenous bacterial community for saline soils, overcoming the harmful effects of salinity and improving plant growth and yield of paddy plants grown under salt-affected areas to ensure food security.

CHAPTER 3

ISOLATION, SCREENING, AND IDENTIFICATION OF POTASSIUM SOLUBILIZING RHIZOBACTERIA FROM SALINE SOIL AND EFFECT OF ABIOTIC CONDITIONS ON POTASSIUM SOLUBILIZATION

3.1 Introduction

Since the beginning of agriculture, the need to improve crop yield is a problematic perspective that is the primary target to meet the food requirement of a growing population. Numerous abiotic factors such as temperature, salinity, soil pH, drought, agrochemicals, and heavy metal contamination affect agriculture produce (Ahmad, 2014). Among these factors, soil salinity is a significantly high-risk factor for agricultural production Shrivastava & Kumar (2015). Basic soil processes like respiration, decomposition of residues, soil biodiversity, nitrification, denitrification, and microbial activities are adversely influenced by high salts concentrations (Egamberdieva et al., 2019). A few farming practices disturb crop productivity in salt-affected soils, such as irrigating with low-quality water, and poor drainage also leads to depositing salts in the soil (Arora et al., 2018; Rengasamy, 2010). Increased evaporation from the soil surface also resulted in salts deposits in the upper layer of soil, causing secondary salinization (Xue & Akae, 2010).

Sodium cations are most prominent in saline soils. Sodium cations taken up by plants from salt-affected soil disturbs an adequate Na^+:K^+ ratio in the cell cytoplasm. K^+ deficiency can generally be detected under salinity stress. Potassium plays a mitigating role in various abiotic stresses such as drought, salinity, metal toxicity, high or chilling temperatures (Wang et al. 2013). The plants take up Na^+ through different transporters (Mueller-Roeber & Dreyer, 2007). Optimum K^+:Na^+ ratio can be well-maintained by limiting Na^+ absorption through Non-Selective Cation Channels (NSCCs) and High-affinity Potassium Transporters (HKTs) by root cells or plasma membrane. Furthermore, maintaining K^+ concentration at the cellular level above a certain threshold and keeping high potassium to sodium ratio in the cell cytoplasm is necessary for better plant productivity and salt tolerance (Wakeel, 2013). However, an enhanced K supply also resulted in higher K^+ accumulation in plant tissue, which causes a higher K^+/Na^+ ratio due to decreased Na^+ concentration (Assaha et al., 2017).

Potassium is one of the most abundant elements in the soil, around 2.5% of the lithosphere. Approximately 90 to 98% of total potassium found in soil is mineral-shaped, which is a tightly bound type of potassium and almost inaccessible for plants. Microbes play a dynamic role in the environmental potassium cycle by releasing the bound potassium in the interlayer spaces and minerals. Certain rhizobacteria species can solubilize potassium and make it available to plants (Ahmad et al. 2018). A substantial number of K solubilizing rhizobacteria is found in soil and rhizosphere. These KSB belong to different genera like *Bacillus, Paenibacillus, Acidothiobacillus, Pseudomonas* and *Rhizobium,* etc., which can solubilize different K minerals in soils like mica, orthoclase, muscovite, illite, feldspar, biotite (Etesami et al. 2017). The KSB has been reported in the rhizosphere and non-rhizosphere soil, submerged/paddy soil, and saline soil (Bakhshandeh et al. 2017; Bhattacharya et al. 2016). These KSB solubilize insoluble and fixed potassium through different mechanisms such as lowering soil pH by producing organic and inorganic acids and acidolysis (Ahmad et al., 2018; Meena et al., 2015).

The specific focus on K solubilizer for saline soil is essential because potassium is an essential nutrient that can compete with Na^+ during uptake by plants in the salt-affected environment. The present study was, therefore, intended to address the issue of potassium solubilization in saline soils. Thus, the work aims to isolate salt-tolerant potassium solubilizing bacteria (KSB) from the paddy rhizosphere growing under saline soil conditions.

3.2 Materials and Methods

This research was carried out at the Microbiological Lab. 208/A09, School of Biological Sciences, Universiti Sains Malaysia, for isolation, screening, and identification of KSB and to find out salt-resistant bacteria capable of K solubilization under saline conditions.

3.2.1 Collection of soil samples

The soil samples were collected from five different sites (L1, L2, L3, L4 and L5 with GPS locations 5.604831-100.342389, 5.610096-100.343107, 5.609866-100.343051, 5.620098-100.344107 and 5.61374-100.342281, respectively) of the rice-growing rhizosphere along the coastal area of Kuala Muda, Kedah State, Malaysia (Figure 3.1) on July 17, 2018. The sites for sampling were randomly selected after testing their salinity by Direct Soil EC Tester - HI98331. The rice fields having a salinity of more than 4 dSm^{-1} were chosen for the isolation of rhizobacteria. At the time of sampling, the rice crop was at the milky and dough stage. Two neighbouring rice plants were gently pulled up from the soil at each site to isolate potassium solubilizing rhizobacteria. The soil sticking to the roots was carefully removed and put in sterilized plastic bags to isolate rhizobacteria. Immediately, the samples were shifted to the laboratory and stored in the fridge at 4°C for further isolation of rhizobacteria.

3.2.2 Analysis of soil samples

Soil samples collected for the isolation of KSB were analysed for physical and chemical properties of soil, such as soil texture, pH, available K, total K, available P, organic matter, organic carbon, and electrical conductivity. The soil analysis was conducted using the standard protocols described by Nyoki & Ndakidemi (2018).

Figure 3.1 Sampling sites of paddy rhizosphere soil in the coastal area of Kota Kuala Muda, Kedah, Malaysia.

3.2.3 Isolation and screening of rhizobacteria from the rhizosphere

Bacteria were isolated from the rhizosphere soil collected from the root zone of rice crop by serial dilution using spread plate technique on nutrient agar. The soil samples were serially diluted in saline solution (0.85% w/v NaCl) (Yahaghi et al.

2018) up to $10^{-6,}$ and 50 µl of soil suspension was spread in Petri plates containing nutrient agar (Fatima et al., 2009). Rhizobacteria were screened based on colour, colony shape, size, and margins, etc. Pure colonies were obtained by following the streak plate technique. Colonies were repeatedly streaked on nutrient agar until a single pure colony was obtained. These pure colonies were stored in the fridge at 4°C for further use.

3.2.4 Screening of rhizobacteria for K solubilization

All isolated rhizobacteria were subjected to test their capability for potassium solubilization. Modified Aleksandrov agar containing 0.2 % insoluble mica powder as an insoluble source of potassium was used. The Aleksandrov medium was amended with bromothymol blue (Parmar & Sindhu, 2019; Rajawat et al., 2016).

The Petri plates containing autoclaved, modified Aleksandrov agar were spot inoculated with the newly grown bacterial cultures (Zhang & Kong, 2014). The Petri plates were incubated at 28±2°C for four days. Evaluation of potassium solubilization by different rhizobacterial isolates was based upon the conversion of medium colour from blue to yellow (Rajawat et al. 2016). The bacterial strains that converted the medium colour from blue to yellow were considered positive for potassium solubilization.

3.2.5 Qualitative estimation of plant growth promoting properties of KSB strains

Forty-two potassium solubilizing rhizobacteria were studied for their other plant growth promoting properties.

3.2.5(a) Phosphorus Solubilization

The capability of rhizobacteria for phosphorus solubilization was determined using National Botanical Research Institute's Phosphate (NBRIP) (Nautiyal, 1999). The sterilized NBRIP agar was poured into the Petri plates and kept at 28±2°C for 24 hours. The next day, the NBRIP plates were spot inoculated with 24 hours old grown KSB strains and incubated at 28±2°C for five days. The appearance of clear hallo zones around the colonies was deemed a positive strain for phosphorus solubilization from an insoluble source of phosphate.

3.2.5(b) Indole Acetic Acid Production

Indole Acetic Acid (IAA) production by KSB strains was determined by using Salkowski reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid) (Ehmann, 1977). IAA production was assessed by growing the strains in nutrient broth (Hi-Media) modified with 0.2 mg/ml L-tryptophan (Bharucha et al. 2013). The flasks containing 100 ml nutrient broth amended with L-tryptophan were autoclaved and left for cooling. After cooling, 0.1 ml of 24 hours old grown bacterial cultures were added to each flask and incubated on a rotary shaker at 180 rpm for 72 hours at 28±2°C. After incubation, the culture was centrifuged at 8000 rpm for 15 minutes. One ml of cell-free supernatant was mixed with two ml of Salkowski reagent and kept in the dark for twenty minutes (Gordon & Weber, 1951). The appearance of a red/pink colour in the mixture was considered as positive for IAA production, and no change in colour was deemed to be negative for IAA production.

3.2.5(c) Siderophores Production

Siderophores production by KSB was determined using a universal Chrom Azurol S (CAS) agar plate assay (Schwyn & Neilands, 1987). To prepare CAS agar, 60.5mg of CAS was mixed in 50 ml of distilled water, and 72.9 mg of Hexadecyl Trimethyl Ammonium Bromide (HDTMA) was mixed in 40 ml of distilled water. These two solutions were mixed. Ten ml of 1mM$FeCl_3.6H_2O$ solution prepared in 10 mM HCl was added to the mixture of CAS and HDTMA. Above Chrom Azurol S (CAS) solution was added into 900 ml of King’s B agar. The CAS agar was poured into sterile Petri plates. The CAS agar plates were spot inoculated with freshly grown KSB strains and incubated at 28°C for 72 hours. The siderophore production by the KSB strains was evaluated based on the change in the colour of CAS agar. The change in CAS agar colour from green to orange was deemed positive for siderophores production. No alteration in the colour of CAS agar around the colonies was considered negative for siderophores production.

3.2.5(d) ACC deaminase activity

ACC deaminase (ACCd) activity of isolates was tested using the process described by Penrose & Glick (2003). Dworkin & Foster (DF) minimal salts medium (Dworkin & Foster, 1958) was utilized to test the ability of KSB to use 1-aminocyclopropane-1-carboxylic acid (ACC) as the only source of nitrogen. All the potassium solubilizing strains were grown in 5 ml of Tryptic Soybean Broth (TSB) and incubated at 28±2°C at 180 rpm for 24 hours. After twenty-four hours, the bacterial culture was centrifuged at 3000xg for 5 minutes at 4°C. The supernatant was discarded, and cell pellets were washed with sterilized 0.1 M Tris-HCl (pH 7.5). The washing procedure was repeated twice. The cell pellets were suspended in 1 ml of 0.1 M Tris-HCl (pH 7.5). The bacterial cells were spot inoculated on DF salts minimal medium in Petri plates with ACC, without ACC, and with (NH4)2SO4 (0.2% w/v) serving as a negative and positive control. The spot inoculated plates were incubated at 28±2°C for 72 hours, and the growth of KSB strains was compared between negative and positive controls. The KSB strains were chosen depending on growth by using ACC as a nitrogen source.

3.2.5(e) Ammonia Production

Peptone water was prepared and autoclaved at 121°C for 15 minutes. After cooling, peptone water was poured into test tubes, and fresh bacterial culture was inoculated into peptone water. Test tubes were incubated at 28±2°C for 72 hours on a rotary shaker at 180 rpm. After 72 hours, 2 ml of culture medium was taken in centrifuge tubes, and 1 ml of Nessler's reagent was mixed. The development of a brown/yellow colour was deemed ammonia producing strain.

3.2.5(f) Nitrogen Fixation

The qualitative assessment of nitrogen fixation by KSB strains was led, as depicted by Baldani et al. (1986). Jensen's Nitrogen free medium, autoclaved at 121°C for 15 minutes, was poured into Petri plates and left to solidify properly. After solidification, the strains were spot inoculated on the Petri plate containing Jensen's Nitrogen free medium and incubated for two days at 28±2°C. The change in medium colour from green to blue was considered the strains as positive for nitrogen fixation.

3.2.5(g) Exopolysaccharides Production

ATCC No. 14 agar was used to test the KSB strains for exopolysaccharides production (Mu' minah et al., 2015). The autoclaved medium at 121°C for 15 minutes was poured into Petri plates and left overnight to solidify properly. Freshly grown bacterial culture was spot inoculated on ATCC No.14 medium and incubated for four days at 28±2 °C. The formation of mucoid colonies on the medium was considered to be positive for exopolysaccharides production.

3.2.6 Osmoadaptation assay on nutrient agar plates

Salinity tolerance of 13 KSB strains, which were positive for all tested PGP properties, was evaluated by conducting an osmoadaptation assay using nutrient agar plates. Nutrient Agar was modified with varying NaCl (2, 4, 6, and 8% (w/v) concentrations (Upadhyay et al. 2009). Twenty-four hours of freshly grown bacteria were inoculated on Petri plates containing amended nutrient agar and incubated for 48 hours at 28±2°C. Control plates having no NaCl inoculated with bacterial cultures