Characterization of Endophytes for Stress Tolerance, Isolated from *Olea ferruginea*

QURAT UL AIN

(M.Phil.)

Department of Plant Sciences,
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan

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By

QURAT UL AIN

DEPARTMENT OF PLANT SCIENCES
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2018

DEDICATION

I dedicate my thesis to my Father who is been a rock of stability throughout my life, who strongly support me at every step of the way.

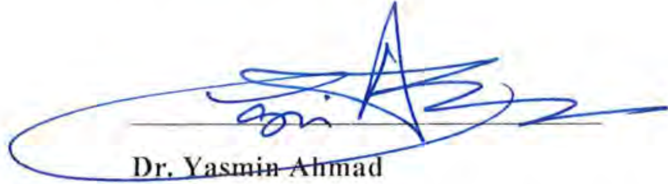
APPROVAL CERTIFICATE

This is to certify that the dissertation entitled "Characterization of Endophytes for Stress Tolerance, Isolated from *Olea ferruginea*" submitted by **Qurat ul ain** is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan, as satisfying the dissertation requirement for the degree of Master of Philosophy (M.Phil.) in Plant Sciences (Plant-Microbe Interactions).

Supervisor

Dr. Hassan Javed Chaudhary
Assistant Professor
Department of Plant Sciences
Quaid-i-Azam University Islamabad

External Examiner



Dr. Yasmin Ahmad
CSO, Crop Diseases Research Program
Department of Botany
National Agriculture Research Institute
Islamabad

Chairman

Dr. Tariq Mahmood
Department of Plant Sciences
Quaid-i- Azam University Islamabad

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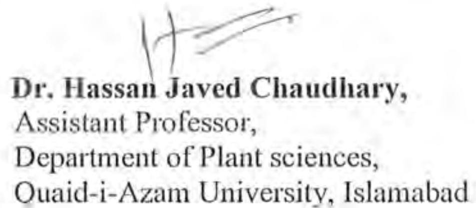

Dr. Hassan Javed Chaudhary,
Assistant Professor,
Department of Plant sciences,
Quaid-i-Azam University, Islamabad

DECLARATION

I hereby declare that no part of this thesis has been previously submitted to this or any other University as part of the requirement for a higher degree. The contents of this thesis are the results of my own work unless otherwise acknowledged in the text or by reference. The research work presented in this thesis was carried out by me in the **Plant-Microbe Interactions lab,** Department of Plant Sciences, Quaid-i-Azam University Islamabad.

Qurat ul ain

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LIST OF ABBREVIATIONS

Abbreviations	
NaCl	Sodium chloride
RWC	Relative water content
IST	Induced systematic tolerance
PGPB	Plant growth promoting bacteria
PGPR	Plant growth promoting rhizobacteria
IAA	Indole acetic acid
EC	Electrical Conductivity
KCl	Potassium chloride
NaCl	Sodium Chloride
$MgSO_4$	Magnesium sulfate
OD	Optical density
LSD	Least Significant Difference
LB	Luria-Bertani
EPS	Exopolysaccharides
CAT	Catalase
RNA	Ribonucleic acid
SOD	Super oxide dismutase
POD	Peroxidase
DNA	Deoxyribo nucleic acid
CRD	Complete randomized design

Abstract

Among all abiotic stresses salinity stress considered as a serious and chronic threat to agriculture as it adversely affects the physiological and biochemical processes of plants. Present study was conducted to screen and characterize salt tolerant endophytes and evaluate their effect for reducing the effect of salinity on the physiological and biochemical aspects of two varieties of maize EV 1097 and Haq Nawaz gold. Six strains Q1, Q2, Q3, Q4, Q5 and Q6 isolated from *Olea ferruginea* were screened at different salt concentrations (6% 10% 12% and 17%) to check their salt tolerant ability and plant growth promotion traits (Indole, Ammonia, Catalase, Protease, Cellulase, Exopolysaccharide, Amylase, Pectinase production, Phosphate solubilization and Zinc Solublization) . Out of these six strains the most effective strains of PGPB Q2 and Q4 were evaluated in co inoculation for their PGP activities at 250mM salt level in a pot trial. Each treatment contain three replicates and kept in green house. After 21 days of sowing, stress was applied to plants and Electrical conductivity maintained for 10 days to observe symptoms of stress on plants. The plants was harvested after 31 days of sowing for estimation of Relative water content, proline, chlorophyll, antioxidant, catalase and electrolytic leakage of plants. Results revealed that salinity stress significantly reduce plant growth, leaf area, fresh and dry weight of plant but inoculation with PGPBs reduce the inhibitory effect of salinity by increasing relative water content, production of antioxidant enzymes, proline and chlorophyll content. Thus co-inoculation with PGPBs could be a useful powerful approach to induce salt tolerance in maize plants and thus improving their growth and salt-stress conditions.

Key words: Salinity, Endophytes, PGP traits, Stress amelioration, Growth promotion

INTRODUCTION

1.1 SALINITY STRESS:

Environmental problems like pollution, shortage of water resources salinization of soil and water is mark-able aspects of 21st century. Day by day reduction in cultivated land occurs due to increased human pollution and this is considered as devastating threat for sustainable agriculture (Shahbaz and Ashraf, 2013). A number of abiotic stresses like flood, high temperatures, drought, salinity and high winds have bad effects on the cultivation and production of agronomic crops. Among all of these environmental stresses, salinity stress is considered as most threatening stress that causes drastic decrease in crop productivity and quality in cultivated land area (Shrivastava and Kumar, 2015).

Salinization is defined as the addition of water soluble salts in the soil column more than their normal level that badly effects environmental health and economic welfare of the country by reducing agricultural production (Rengasamy, 2006). A soil which has 4 dSm^{-1} electrical conductivity (approximately 40 mM NaCl) in the root zone at 25 °C as well as has exchangeable sodium of 15% is defined as saline soil. At this ECe, significant reduction in crop yield occurs, however in some crops decrease in yield occur at lower EC (Jamil *et al.*, 2011).

In the soil salts are present in the form of ions and these ions are produced by weathering of mineral elements in the soil. Moreover their artificial source is through water irrigation and input of fertilizers. Low precipitation is also a reason for accumulation of ions in the soil causes salinity of soil (Blaylock et al., 1994). Water soluble salts are present in all types of soil and essential nutrients are absorb by plants in the form of soluble salts, but excess amounts of these salts retard the plant growth.

Soil salinity badly disturbs the soil structure and texture and makes the soil hard and water-resistant. It causes the decrease in the air and water holding capacity of soil, as a result, plants are unable to get enough moisture and oxygen to grow (Toor and Shober, 2015). It has been estimated that 33% of irrigated agronomic lands and 20% of total

cultivated land all over the world are stressed by high salinity. Moreover, various factors like, evaporation, weathering of rocks, low precipitation, poor cultural practices and irrigation with saline water results in the annual 10% increase in saline soils. About 1-2% of world's irrigated land is decreasing in the arid and semi-arid regions due to salinization (Kasim *et al.*, 2016). Salinization is documented as the leading threat to human health and environmental resources in many countries, affecting 7% of earth's continental extent which is almost 1 billion/ha globally that represents, approximately 20 times the size of France (Yensen, 2008). Human activities can exacerbate the problem of salinity e.g. in dry and hot regions of the world, irrigation methods are used to grow crops. Inadequate irrigation practices leads to secondary source of salinization of land and water that effects 20% of the agricultural land globally (Cheng *et al.*, 2007).

The decline in fertile lands and continuously increasing population, specifically in the under developing countries of the world is the alarming situation. It's vital to challenge these stresses to feed continuously growing population. Scientists predicted that more than 50% of the arable soil would be salinized after 32 years (Jamil *et al.*, 2011) and global food production should increase 50% by the year 2050 to maintain food supply to the increasing population at current level. Therefore, more struggles are necessary to increase the productivity per unit area.

1.1.1 Impact of salinity on plants:

Saline soils have poor soil physical conditions so they are recognized to inhibit the growth of plants (Paul, 2013). Crops grown on salt stressed soils suffer from nutrient (N, K, Fe, P, Ca and Zn) imbalance, high osmotic and oxidative stress and toxicities. High salt concentration in the soil causes growth retardation in most of the crop plants. Moreover it depends on the plant growth stages, nature and intensity of salt, the salt resistant or avoidable mechanism of the plant tissue and also depend upon cultivars (Munns and James, 2003). Most of the crop plant have ability to tolerate salinity up to their threshold level, and beyond this yield reduction occur as the level of salinity increases (Khan and Gul, 2006). Salinity not only causes the loss of productivity of most agricultural crops and low economic returns, but also, disturbs ecological balance of the ecosystem and effects soil structure and function.

Effects of salt stress are the consequences of many metabolic processes and interactions between biochemical, morphological and physiological processes (Singh and Chatrath, 2001) (Akbarimoghaddam *et al.*, 2011) and it disturbs nearly all phases of plant growth and development e.g. seed germination, vegetative and reproductive growth of plant.

Management practices of soil and water have eased agricultural production on salinity effected soil but an extra advantage from these methodologies seems challenging (Zahir *et al.*, 2008). Yield of the food, fodder and forage is also lessening every year due to salinization and desertification (Shahid, 2013). There is a need to increase in grain yields of main crop plants for example wheat, rice, and maize to about 50% to fulfil the requirements of food for the continuously increasing population (Godfray *et al.*, 2010).

Salt stress level up to 0.25 M NaCl badly effects maize crop limit its growth and causes wilting of seedlings (Menezes-Benavente *et al.*, 2004). Salinity stress causes disturbance in potassium uptake by interference of sodium ion which disrupts stomatal undulations which results in severe decrease in relative water content and causes necrosis in (Fortmeier and Schubert, 1995).

Secondary effect of salinity stress comprises excessive release of ROS (reactive oxygen species) which causes oxidative malfunctioning in the cells. Singlet oxygen, hydrogen peroxide, hydroxyl radicals and superoxide radicals are the commonly present ROS in the plant (Del Río and López-Huertas, 2016). ROS causes many kinds of destruction in the macromolecules, comprising the photosynthetic pigments, lipids, protein and in the end the cellular organelles (Gill and Tuteja, 2010). ROS by-products are present in plant cells, but when abiotic stresses occurs on plant scavenging ROS starts to over produce and accumulate in plants cellular compartments causing imbalance of ROS (Karuppanapandian *et al.*, 2011). The biphasic model of soil salinity shows that the osmotic stress and ion toxicity cumulatively causes adverse effects on physiology and are actually reason for the low yield of cereal crops (Fortmeier and Schubert, 1995). Sodium chloride is a principal salt species in most of the saline soils, and its effect is shown as low productivity or even plant death. Soil salinity disturbs uptake of water by the roots more difficult which results in high salt concentration in the plants which leads to toxicity

(Munns and Tester, 2008). High level of salt causes both ionic and hyper osmotic stress, which leads to modification in plants mechanisms including specific ion toxicity, low water potentials, ionic imbalance (Tester and Davenport, 2003), protein synthesis, lipid metabolism and photosynthesis (Parida and Das, 2005). K+ act as a cofactor for many enzyme activities and its high concentration in cell is mandatory for binding transfer RNA (tRNA) to ribosomes and consequently synthesis of proteins (Zhu, 2002). Salinity stress causes ion toxicity by replacement of potassium ion (K+) by sodium ion (Na^+) in different biochemical reactions, which on reaction with chloride ion (Cl^-) promoted alterations in proteins structure and causes oxidative stress (Chinnusamy *et al.*, 2006). Excess amount of sodium ion in plants cell wall causes osmotic stress and eventually cell death (Munns, 2002).

Elements like sodium, boron, chlorine have very toxic effects on plants, and if toxic elements more than threshold are present in soil then sensitive plants may be affected badly even at relatively little concentrations of salt. As several salts are important as plant nutrients, thus high amount of salt in the soil cause interference in the nutrient uptake or disturb the nutrient balance in the plant (Blaylock, 1994). Salinity reduce rate of photosynthesis through reduction in stomatal conductance, chlorophyll content, leaf area and to a lesser extent through a decrease in photosystem II efficiency (Netondo *et al.*, 2004).

1.1.2 Plants responses to salinity stress:

Some plants have ability to reduce negative effects of salinity by adopting several mechanisms comprising regulation and compartmentalization of ions, production of antioxidative enzymes, generation of plant hormones and by altering photosynthetic pathway (Cheeseman, 1988). Many techniques have been established in order to reduce the lethal effects of salinization, like plant genetic engineering (Wang *et al.*, 2003), and in recent times the use of plant growth-promoting bacteria (PGPB) (Dimkpa *et al.*, 2009)

1.1.3 Various strategies to Ameliorate salinity stress:

Plant scientists have implemented several approaches to overwhelm the salt stress. To check the genomic variability of the current germplasm to find stress tolerant

varieties that can produce adequate yield on saline soil is one major aspect (Ashraf et al., 2006). This methodology involves to understand plant growth responses at various stages of life under saline situations equally stated in certain food crops like wheat (Ali *et al.*, 2002), maize (Khan *et al.*, 2003), sorghum (Azhar and Khan, 1997), soybean (Kamal *et al.*, 2003), rice (Shannon *et al.*, 1998) and cotton (Azhar and Ahmad, 2000). These provide a pathway for breeders to look forward for economically important plants with increased salt tolerance. It is difficult to do screening for salinity tolerance under field conditions, due to physio-chemical properties seasonal fluctuations in rainfall and spatial heterogeneity of soil.

Salt stressed soils can be managed by implementing different strategies such as by lowering water table levels, draining of excess soluble salts from root zone by means of fresh irrigation water, disposing salt crust containing soil at the surface, upgrading by application of chemicals such as acids, gypsum and organic matter (Murtaza *et al.*, 2009) changed farm management practices and by different biological methodologies like development of salt tolerant varieties, use of plant growth regulators, and Plant growth promoting bacterial inoculation in plant seeds and seedlings (Athar and Ashraf, 2009). Even though the implementation of these methods can improve yield of plants under salt stress, but application is frequently inadequate due to accessibility of H_2O reserve and cost of good water quality. Developing low cost, efficient and easy-going methods for environmental stress management is a main challenge. Broad research is being carried out worldwide, to develop approaches to ameliorate abiotic stresses, through development of drought and salt resistant varieties, shifting the crop calendars and resource management practices etc. (Sahay *et al.*, 2012).

1.1.4 Salinity effected areas in Pakistan

Salinity is a very serious problem in many arid and semi-arid regions of Pakistan. About 400 thousand-hectare land in Pakistan is become unproductive every year by soil salinity (Ghafoor *et al.*, 2004). This fact conveys extra significance to the present study, as the country can ill afford to not use these salt-affected soils for agriculture purposes. It's a need of the day to develop effective ways and means to use these soils/lands for agricultural purposes.

1.1.5 Endophytes

Microorganisms found everywhere even at very extreme conditions like dessert and high temperature (Vidali, 2001). According to (Sikora *et al.*, 2007) endophytes are kind of microbes that has the ability to colonize plant tissues internally, whether it is advantageous, disadvantageous or else neutral to its host. Mostly endophytes are symbiotic and beneficial to its host. These endophytes obtain their food from different excretory substances of roots and in return they release active metabolites through various metabolic activities which aid to promote plant growth, development and vigor (Li *et al.*, 2008). As endophytes resides inside the plant so they are more secure and safe from biotic and abiotic stresses and have less competition than rhizospheric bacteria (Rosenblueth and Martínez-Romero, 2006; Mei and Flinn, 2010).

1.1.6 Endophytes-Plant interaction

Microorganisms found in the environs of plant act together by various ways, based on enormous signals and signal perception mechanisms adapted by both partner (Mei and Flinn, 2010). Microbes can sense and generate signals due to which the entire population spread out as a biofilm on the root surface and start an intensive action. Thus a particular microbial density is attained. This phenomenon is known as quorum sensing. The rhizospheric bacteria chemotactically attracted to plant roots via root exudates, stick to, colonize the root surface and become endophytes (Ross *et al.*, 2004). These plant endophytic interaction results in plant growth promotion by supplying nutrients, fix atmospheric nitrogen (Iniguez *et al.*, 2004), produce phytohormones, control the activity of plant pathogens, etc. As compare to rhizospheric bacteria endophytic microbes have less competition because they are present inside and are more protected from environmental fluctuations (Lodge *et al.*, 1996).

Endophytic bacteria are very important for plants, environment as well as human being.

1.1.7 PGPB: abiotic stress mitigation tool in crops

As salinity causes ionic and osmotic stress, leads to ionic inequity that can weaken the selectivity of root plasma membranes and causes deficiency of potassium (Ahmad *et al.*, 2006). High amounts of toxic salts build up in the apoplasm of leaf consequences of the loss in the cell turgor and dehydration that destroys leaf cells and tissues (Affenzeller *et al.*, 2009) leading to decrease in growth and yield. Though some physical and chemical means are cost effective but they are also hazardous to environment in one or another way that's why other means are focus now days. Among which PGPB are the most studied microorganisms, which are eco-friendly, low in cost and also easily practicable (Tairo and Ndakidemi, 2013). Growth promoting bacteria not only enhance productivity of crops but also increase soil health by interactions of soil microorganisms and plant roots (Lugtenberg *et al.*, 2002).

Role of microbes for controlling abiotic and biotic stresses is gaining importance and former studies purpose that application of PGPB has become a hopeful substitute to improve toxic effects of stress on plant development caused by soil salinity (Yao *et al.*, 2010). PGPB has a great influence on plant growth promotion as they aid to provide nutrients as well as control diseases of plants. These advantageous bacteria colonize the rhizosphere/endorhizosphere of plants and by various direct and indirect mechanisms helps to enhance growth of plants (Nia *et al.*, 2012).

The term induced systematic tolerance (IST) is used for plant growth promoting bacterial induce chemical and physical changes that help to tolerate abiotic stresses. PGPB have role to aid plant growth indirectly by protecting plant via reducing plant pathogens, or directly by increasing the nutrient uptake through phytohormones production (e.g. auxin, gibberellins and cytokinin) by lowering level of ethylene in plant (Kohler *et al.*, 2006).

1.1.8 Alleviation of salinity stress by Microbes: Mechanism

So many types of endocellular and intracellular microorganisms colonized plants in their natural habitat (Gray and Smith, 2005). Rizospheric microorganisms, predominantly advantageous bacteria and fungi helps to improve plant development and

growth under stress conditions, thus, augment yield both indirectly and directly (Dimkpa *et al.*, 2009). Plant-endophytic bacteria interactions enhance tolerance against abiotic stresses e.g. drought (Malinowski and Belesky, 2000) and salinity. Nitrogen is an important source for plants to process their metabolism and some plant growth-promoting bacteria (PGPB) provide a direct stimulation on plant development by providing plants with fixed nitrogen as well as phytohormones and iron that have been sequestered by bacterial siderophores and soluble phosphate (Hayat *et al.*, 2010). Some microbes indirectly protects the plants from many soil-borne diseases, caused by pathogenic fungi (Shrivastava and Kumar, 2015). Among PGPB, rhizobia are the most deliberate PGP microorganisms and are, practicable, economic and eco-friendly (Tairo and Ndakidemi, 2013).

The beneficial effect of Rhizobia in legumes is well known. Lately, it has also reported that rhizobia can improve yield and growth of non- legume plants including wheat (Mehboob *et al.*, 2011), rice (RHIZOBACTERIA, 2014), maize (Hadi and Bano, 2010). They can colonize roots of non-legumes and carry out various direct and indirect mechanisms to increase their growth. By direct mechanism they can synthesize numerous growth hormones in plants such as, cytokinins (Senthilkumar *et al.*, 2009) gibberellins (Boiero *et al.*, 2007), abscisic acid and auxins (Zahir *et al.*, 2010) and secrete many other chemicals beneficial to plant growth such as siderophores (Huang and Erickson, 2007). exopolysaccharides (Zafar-ul-Hye *et al.*, 2013), ACC-deaminase (Duan *et al.*, 2009) phosphatases (Afzal and Bano, 2008) phytase (Glick, 2012) phosphohydrolases (Gügi *et al.*, 1991) etc.

By mobilizing nutrient present in the soil rhizobia also enhance availability to the plant and improving the soil structure (Barea and Richardson, 2015). Indirectly, rhizobia improve plant health by improving the self-defense of plant through induction of systemic resistant (Reddy, 2012) against pathogens, diseases, harmful insects and viruses (Huang and Erickson, 2007). Rhizobia have also been reported to improve the growth and yield of legumes (Ahmad *et al.*, 2012)(El-Akhal *et al.*, 2013) and non-legumes (Afzal and Bano, 2008)(Bano and Fatima, 2009) under salt stressed conditions. There are extensive reports of rhizobia for enhancement of root proliferation, plant growth

promotion and number of primary roots, even under high salinity level (Naz and Bano)(El-Akhal *et al.*, 2013). In regards of above discussion, the current experiment was carried out to evaluate application of endophytic microbes to ameliorate salinity stress in maize plants.

1.1.9 Maize

Maize (***Zea mays* L.**) is considered as the third supreme cereal crop after wheat and rice, and it is grown globally under a diverse environmental conditions. There are about 50 species of maize plant with diverse type of textures, colours (commonly found in yellow, white and red types) grain sizes and shapes. Maize is an annual crop and its reproduction take place through seed. Maize grains usually take nearly 125 to 155 days from seedling to harvest. The plant normally requires 2-3 L of water day but consumption may differ from 2.0-4.3L per day during vigorous growing period. Maize is a distinctive C4 plant and bright days are prerequisites for rapid photosynthetic activity. The long overcast period is unsafe for the development of crop, but a scarce sunlight and haze of shower is considered as a perfect for its growth and development.

Although well drained and fertile soil is recommended for maize growth however it can be cultivated in a variety of soils. Optimum pH range of soil should be 6.5-7.5 and soil should have enough water holding capacity because maize is really sensitive to water logging. Maize is a summer-growing crop required warm day time temperature (26°C-31°C) and cool nights (Lim, 2013). Temperature beyond 45°C and under 8°C causes retardation in the development of plant. (Birch *et al.*, 2003).

1.1.9.1 Maize classification

Kingdom: Plantae

Order: Poales

Family: Poasceae

Genus: Zea

Species: *Z. mays*

1.1.10 Maize production in world

In 2000 it was recorded that North America is a major producer of world's maize production that is approximately 50%. USA is a 2nd largest producer of world's production with 42% production than China contributes about 18% and Europe almost 10% (Farnham *et al.*, 2003). Maize is an important grown crop in Pakistan and is the fourth chief crop after rice, wheat and cotton. In KPK maize covered 56% of the total area and 63% of total production while Punjab contributes 39% of the total area and 30% of the production whereas Baluchistan and Sindh contribute 5% of the total area and 3% of the total production. Annual production of maize crop in Pakistan is 4.04 million tons of maize grains is achieved in its wide range of cultivation at about 0.95 million hectares (Hammad *et al.*, 2011).

1.1.10.1 Commercial Uses:

Maize is known as most productive crop with an global yield of more than 3 tonnes per hectare (Paliwal *et al.*, 2000)(Farnham *et al.*, 2003). Maize is considered as highly nutritious and high energy value crop. It can use as food in various forms, as grains, dry fodder and green chop. It is an important ingredient in food or drinks like Corn syrup in maize meal or soft drinks, or for industrial purposes. Starch one of the main component of corn used throughout the world as food substances, also in its chemically modified or in native form. Maize starch is agitated into alcohol, including fuel ethanol while the maize starch is used at a large level in the paper industry. The protein and oil are used as by-products of starch production and used in food engineering.

1.1.11 Olea ferruginea

Olea ferruginea is an evergreen ethnomedicinal plant with a height of up to 15m. It is known as resistant to drought and frost and can easily planted in dry temperate, arid, semi-arid and also in wastelands and marginal where the soil condition is not good for other plants (Siddiqui *et al.*, 2011). Its wood is very strong and resistant to fungus, so it is used for manufacturing of building materials and agricultural tools. Recently (Amin *et al.*, 2013) reported antifungal and antibacterial activities of leaf extract of *O. ferruginea*.

1.1.12 Objectives

The aims of the current study are as follows.

1. Screening and characterization of plant associated microbes with potential to endure salt stress conditions.
2. To assess plant growth promoting (PGP) traits of endophytic bacteria
3. To analyse biochemical characteristics of endophytes.
4. To analyse the induction of PGPB mediated salt tolerance and enhancement of plant growth promotion in maize.
5. To introduce eco-friendly technique in agriculture sector for enhanced growth of maize crop under axenic conditions.

MATERIALS AND METHODS

2.1 STRAINS ACQUISITION

Six previously isolated bacterial strains (Q1, Q2, Q3, Q4, Q5, and Q6) were used in this study to test their potential to combat with salinity stress tolerance. The strains were obtained from Plant-Microbe Interactions lab QAU Islamabad.

2.2 SALINITY TOLERANCE:

The tolerance of six bacterial strains against salinity stress was evaluated by growing them in TSB media supplemented with increasing concentration of Sodium chloride (NaCl) ranging between (6%, 10%, 12% and 17%). Sodium Chloride was added in the medium to stimulate salinity stress. The samples were then incubated at 28°C with shaking at 120rpm for 24 hours and the bacterial growth was checked by measuring optical density of each sample at 600 nm.

2.3 GROWTH PHASE UNDER SALINITY STRESS

The effect of NaCl on growth kinetics of all strains was determined by growing them in TSB medium with different concentrations of NaCl ranging from (6%, 10%, 12% and 17%). The samples are then incubated at 28°C. The bacterial growth was recorded by taking OD after every 24-hour interval for 7 days.

2.4 MOLECULAR IDENTIFICATION OF BACTERIAL STRAINS

DNA of all strains was extracted by following protocol. Add 570µl TE buffer into an Eppendorf tube. A loop full of bacterial culture was added to Eppendorf tube. 30µl of 10% SDS and 6µl of proteinase K added to pellet and mixed by using micropipette. Incubate the Eppendorf tube at 37°C for 60 minutes. After incubation add 100µl of 5M NaCl (sodium chloride) to pellet and then, 80µl CTAB/NaCl solution was added and sample was mixed thoroughly. The sample was incubated for 10 minutes at 65°C. After incubation adds 500µl chloroform: Isoamyl alcohol (24:1) solution and invert the samples to obtain milky solution. Then centrifuged the sample at speed of 14,000 rpm for 5 minutes. Transfer the aqueous layer in new Eppendorf tube and add 500µl phenol: chloroform: isoamyl alcohol in ratio of 25:24:1 to the supernatant. Centrifuge the samples again for 3-5 minutes at maximum rpm. After centrifugation transfer supernatant to

another Eppendorf and add 600μl isopropanol. Then centrifuged the samples at maximum rpm for 3 minutes. The supernatant was discarded and 500μl of 70% ethanol was added to DNA pellet and centrifuge at maximum rpm for 3-5 minutes. Then supernatant was removed and pellet was allowed to get dry. Precipitated DNA was stored in 70μl TE buffer at -20 °C.

2.5 POLYMERASE CHAIN REACTION FOR 16S

The 16S rRNA gene was amplified using universal primers 27 F (5'-AACTGAAGAGTTTGATCCTGGCTC-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). The amplification reaction was carried out in a final volume of 50μL containing 5μL of Taq polymerase buffer, 1μL of each dNTP, 4μL of $MgCl_2$, 2μL of each primer, and 0.7μL of Taq polymerase and 1.5μL of DNA sample. PCR was performed in total 40 cycles using thermocycler according to the following program:

1. 5 min at 96°C
2. 1 min at 96°C,
3. 1 min at 56°C,
4. 1 min at 72°C
5. Final cycle of 10 min at 72°C.

The amplified PCR product has been sent for commercial sequencing.

2.6 AGAROSE GEL ELECTROPHORESIS

PCR product was then run on GE chamber using 1.5% agarose gel prepared by adding 1.5g agarose in 100ml 1x TAE buffer in which 10μl EtBr for 40 minutes at 80 volts and 500 milliampere.

2.7 BIOCHEMICAL CHARACTERIZATION OF BACTERIAL STRAINS:

2.7.1 Gram Staining

To observe bacterial morphology following method are followed

- A single colony of bacterial strains was placed on glass slide and fixed it by gentle heating.
- Primary stain (Crystal violet; 1.24g in 100 ml dH_2O) were added and stay on slide for 30 seconds.
- As a moderator Grams Iodine (0.33g iodine and 0.67g Potassium Iodide in 100ml of water) was added directly to primary stain after 30 seconds.
- The slide was gently washed with distilled H_2O and after by Ethanol (v/v) and then again by distilled water.
- Then add safranin (Secondary stain) was then added to the slide for 1 minute.
- The slide was then air dried and observed under microscope.

2.8 DETERMINATION OF ANTIBIOTIC SENSITIVITY

All six strains (Q1, Q2, Q3, Q4, Q5, and Q6) were verified for their potential to grow in the presence of eleven different antibiotics on agar media using the standard antibiotic working concentrations. LB plates separately added with Norfloxacin, Tobramycin, Cloxacillin, Piperacillin, Carbenicillin, Ceftazidime, Ciprofloxacin, Amikacin, Colistin, Nitrofurantoin, Cefoxitin were used in the assay. The strains were cultured in LB broth at 28±2 °C for 24 hours. After one day, 0.1 ml from this culture was swabbed on petri plates containing LB medium. Plates were supplemented with different antibiotics and incubated overnight at 32 °C. After that bacterial susceptibility was determined by measuring the hallow zone around each disc.

2.9 EXOPOLYSACCHARIDE PRODUCTION

To check ability of bacterial isolates for production of exopolysaccharide bacterial strains were streaked on ATCC medium no. 14 (Sucrose 20g, yeast extract 0.5g, $FeCl_3$ 2mg, $CaSO_4.2H_2O$ 0.1g, K_2HPO_4 0.8g, KH_2PO_4 0.2g, MgSO4.7H2O, $Na_2MoO.2H_2O$ pH 7.2) under sterile conditions and incubate them at 28 °C for three days. Slimy layer around bacterial colonies considered as positive for Exopolysaccharide production. Colonies of bacteria that form thick slime layer are further screened for their exopolysaccharide producing potential. For these bacterial strains were grown in 50ml

were harvested by using 500μl 1mM EDTA and centrifuged for 10 minutes at 9000 rpm. Supernatant was separated and mixed with chilled acetone with a ratio of 1:3 and centrifuge for 2 times 30 minutes at speed of 15000 rpm. Deposition of exopolysaccharide was taken and washed with distilled H_2O. Biomass was dried at 60°C for 24 hrs to get dry weight.

2.10 PRODUCTION OF IAA

Method of (Bric *et al.*, 1991) was used to check production of IAA. Freshly grown bacterial cultures were grown in nutrient broth media for 72 h at 36 – 38°C. Then these cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was from each culture mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent. Change of pink to red colour indicates IAA production.

2.11 PHOSPHORUS SOLUBILIZING ACTIVITY

For the qualitative estimation of phosphorus Pikoviskaya's media was used. Bacterial strains were spot inoculated on Pikoviskaya's agar plates. The plates were incubated for 7 days at temperature of 28°C. Development of clear halo zone indicated the capability of strains to solubilize phosphorus on plates (Premono *et al.*, 1996).

Solubilization index will calculated the ratio of the total diameter both colony and halo zone divided by colony diameter.

$$SI = \frac{Colony\ diameter + Halo\ zone\ diameter}{Colony\ diameter}$$

Solubilization efficiency were noted according to following formula

$$SE = \frac{Diameter\ of\ solubilization\ halo\ zone}{Diametr\ of\ Colony}\ x\ 100$$

2.12 AMMONIA PRODUCTION

Peptone water was used to screen the production of ammonia by bacterial isolates. 24 hours old culture was inoculated in 10 ml peptone water in each tube and incubated at 28±2°C for 48-72 h. Nessler's reagent (0.5 ml) was added to each glass tube.

Development of brown to yellow color indicates positive results for ammonia production (Cappuccino and Sherman, 1992).

2.13 CATALASE

The single bacterial colony was picked by a loop and placed on the glass slide. To check catalase activity by bacterial strains a drop of 30% hydrogen peroxide (H_2O_2) was added. Formation of gas bubbles indicated the presence of catalase enzymes (Yasmin and Bano, 2011).

2.14 ZINC SOLUBILISATION:

Tris minimal medium was used for screening of zinc solubilizing strains. The isolates were inoculated into modified Tris medium, (Dextrose-10.0 g; Zinc phosphate 1g; Ammonium sulphate 0.5g; Yeast extract 0.5g; Potassium chloride 0.2g; Ferrous sulphate 0.01g; Manganese sulphate 0.01g; Di-potassium hydrogen phosphate 0.25g; Agar 15g in 1000 mL double distilled water) having 0.1% insoluble zinc compounds (ZnO, ZnPO4 and ZnS) (Fasim *et al.*, 2002). Bacterial strains were spot inoculated on above mentioned media and incubated at temperature of 28 ± 2°C for 48-72 hr. Colony diameter and halo zones around the bacterial colonies were measured. Zinc solubilization efficiency (SE) was calculated as described by (Ramesh *et al.*, 2014).

$$SE = \frac{Diameter\ of\ solubilization\ halo\ zone}{Diameter\ of\ colony}\ x\ 100$$

2.15 PECTINASE:

Pectin agar medium (3.2g Ammonium Sulphate, 0.1g of yeast extract, 0.6g of Na_2HPO_4, 0.3g KH_2PO_4, 0.5g pectin from citrus peer and 2g agar in 100ml of distilled water) was used to assayed pectinase production by bacterial culture. For this, a colony of bacteria was placed in the center of the plate having pectin from citrus peer. The plates were then incubated for 48 hours at temperature of 28°C. After 2 days of incubation plates were flooded with Iodine, clear halo zone around colonies show pectinase production.

2.16 PROTEASE PRODUCTION:

Bacterial strains was screened for protease production by spot inoculating single colony of each bacterial strain in the middle of 3% skimmed milk agar plates (0.1g dextrose, 0.2g peptone, 0.5g of yeast extract, 0.1g KH_2PO_4, 0.02g $MgSO_4.7H_2O$, 0.5g skimmed milk, 2.5g Agar in 100ml Distilled H_2O). The plates were then incubated at 30°C for 3-8 days. Clear halo zone around each bacterial colony indicated positive results for protease activity.

2.17 AMYLASE PRODUCTION

Bacterial strains were also screened for amylase production by the method of (Salahuddin *et al.*, 2011). Starch agar medium was prepared by adding 5g starch, 1g yeast extract, 0.1g magnesium sulphate, 7g k_2HPO_4, 5g NaCl, 1g $(NH_4)_2SO_4$ and 15g agar in 1000ml of distilled water. A loop full of bacterial culture was spot inoculated and incubated for 48h at 28°C. After incubation period of two days plates were saturated with iodine solution and formation of halo zone around bacterial colony shows positive result for amylase production.

2.18 GLASS HOUSE EXPERIMENT

2.18.1 Inocula Preparation

Among six halotolerant PGPR strains, two strains Q2 and Q4 were most resistant to stress and in PGP activities was selected to apply on maize crop in a greenhouse to assess their abilities to cope with salinity stress in plants.

2.18.2 Seeds disinfection and inoculation:

Two Maize varieties were obtained from NARC Islamabad. Before sowing seeds (EV1097, Haq Nawaz Gold) were surface sterilized by using 95% ethanol solution for dipping in for 5 min and in 0.2% Mercury chloride ($HgCl_2$) solution for 3 min, and then washed carefully with distilled H_2O to remove traces of $HgCl_2$ (Khalid *et al.*, 2004). For seeds inoculation, bacteria were inoculated in LB broth and incubated for 48 hours at

32°C and 120rpm. After two days of incubation disinfectant seeds were dipped in this culture for three hours prior to sowing.

2.18.3 Experimental design and growth conditions:

For pot studies three different sets were prepared: (Maize Varieties, Bacteria and Salinity stress) and arranged according to CRD (Completely randomized design) under factorial arrangement. Soil samples were collected, air dried, mixed thoroughly, sieved (2.0 mm), and analyzed for physicochemical characteristics. The soil was analyzed for pH, electrical conductivity (ECe), using standard procedures. Inoculated and non-inoculated maize seeds were sown in plastic pots containing 5Kg autoclaved soil, sand, and compost (pH 7.7, EC 1.8 dS/m) mixed in 1:1:1 ratio.

The experiment was laid out in completely randomized design under factorial arrangements with 3 replications. The plants were grown under normal conditions maintaining three plants per pot. Salinity stress was induced after 3 weeks of sowing by adding the aqueous solution of sodium chloride (NaCl) (250mM) to the culture media (soil) for 10 days. Plants were harvested after 31 days of sowing. Harvested plants were washed by tap water once and twice with distilled water. For analyzing different parameters, 3 plants were selected randomly from each plot and the average was calculated.

2.19 TREATMENTS:

The experiment comprised of six following treatments for each genotype.

Table 2.1 Experimental Treatments

T1	Control
T2	Treated with bacteria Q2 (B1)
T3	Treated with bacteria Q4 (B2)
T4	Treated with 250mM salt stress
T5	Treated with 250mM salt stress + Bacteria Q2 (B1)
T6	Treated with 250mM salt stress + Bacteria Q4 (B2)

Same treatments were scheduled for both varieties (EV1097=V1) and (Haq Nawaz Gold =V2) and all conditions were kept same.

2.20 Physiological and biochemical analysis of plants:

Immediately after harvesting plants are then used for following analysis.

2.20.1 Plant length (Shoot & Root length)

Length of freshly harvested shoots and roots were taken by using measuring tape. Each treatment consists of 3 replicates.

2.20.2 Plants fresh weight

Fresh weight of freshly harvested plants was measured by using digital balance.

2.20.3 Leaf Area:

Leaf area of plants was measured by measuring leaf length and width by measuring tape. And then calculated by using following formula

$$Leaf\ area = Leaf\ length\ x\ Leaf\ width\ x\frac{2}{3}$$

2.20.4 Relative water content:

RWC of maize leaves was checked according to (Ahmed *et al.*, 2016). 0.5g of fresh excised leaves was kept in water for almost 4 hours at 4°C to record turgid weight. Dry weight measurement was made by drying leaves in oven at 80°C for two days. RWC can determine by applying following formula:

$$RWC = \frac{(Fresh\ Weight - Dry\ Weight)}{(Turgid\ Weight - Dry\ Weight)}\ x\ 100$$

2.20.5 Electrolyte leakage:

Method of (Lutts *et al.*, 1996) was used to measure Electrolyte leakage of plants samples. From the first primary branch one gram fresh leaf was collected from three plants for each treatment and washed with distilled water to eliminate surface-adhered electrolytes. 20 ml of deionized water was added placed in closed vials and the leaf discs were immersed and incubated at 25°C on a rotary shaker for 24 h, and by using EC meter the electrical conductivity of the solution (Li) was determined. Samples were then autoclaved at 120°C for 20 min to completely disrupt the leaf tissues and the final electrical conductivity (Lf) was obtained after cooling at 25°C. The electrolyte leakage can be determined by using following formula:

$$Electrolyte\ leakage\ (\%) = \frac{Li}{Lf}\ x\ 100$$

2.20.6 Photosynthetic pigments:

0.05g fresh weight of leaf material was homogenized in a mortar with 10 ml of 80% acetone. The homogenate was loaded in Eppendorf tubes and incubated at 4°C for 1 h in the dark to extract the pigment. Then, the extracts were centrifuged for 15 min at 5000 rpm. Aliquots were taken from the supernatant, and chlorophyll a measured by taking optical density at 650 and chlorophyll b levels were measured by spectrophotometry at 665 nm. Five millimeters of 1 M NaOH and 15 ml of diethyl ether

were added to the total volume. Carotene content was measured by spectrophotometry at 450 nm (Joslyn and Mackinney, 1938).

2.20.7 Antioxidant enzymes:

2.20.7.1 Super oxide dismutase (SOD):

To access SOD activity, method of (Beauchamp and Fridovich, 1971) was followed.

For measuring SOD activity two phosphate buffers, Monosodium dihydrogen phosphate (15.6g into 500ml of distilled water) and Disodium hydrogen phosphate (53.65g into 600 ml of distilled water) was used. To make buffer solution of pH 7, 117ml of monosodium dihydrogen phosphate and 183ml of disodium mono hydrogen phosphate were mixed and final volume raised to 600ml by adding distilled water.

Buffer solution pH (pH7.8) was made by 25.5 ml of monosodium dihydrogen phosphate and 275.5ml of disodium mono hydrogen phosphate and final volume was made to 600ml.

Using cool mortar and pestle, 0.5 g of maize plant tissue were grinded in 5ml of solution prepared by adding 1g of PVP and 0.028g Na_2EDTA in 100 ml of phosphate buffer (pH 7). Centrifuge at 4°C for 10 minutes and collect the supernatant and make its total volume up to 8 ml by adding phosphate buffer of pH 7.Then 3 ml reaction mixture was prepared by adding 0.1 mM EDTA, 13 mM methionine, 0.075 mM NBT, 0.002 mM riboflavin and 0.1 ml of enzyme extract in 50 mM phosphate buffer having pH 7.8. Absorbance of samples was recorded at 560 nm.

Using following formula SOD activity was measured and units were expressed as per 100 gram fresh weight.

S_1 = Optical Density of reference

S_2 = Optical Density of blank

S_3 = Optical Density of sample

$S_4 = S_3 - S_2$

$A = S_1 (50/100)$

$Final = S_4/A$

2.20.7.2 Peroxidase (POD):

POD activity was determined by modified method of (Reddy *et al.*, 1985). 1 gram of fresh maize leaves was grounded in 10ml of 0.1M phosphate buffer solution (pH 6.5). Then the samples were centrifuged and supernatant was taken to record absorbance at 430nm for 3 minutes. 1% H_2O_2 solution (500 µl) prepared in phosphate buffer pH 6.5 was taken as blank. The change in optical density per minute at 430nm was taken as one unit of peroxidase.

$$POD = OD\ final - OD\ initial$$

2.20.7.3 Catalase

Catalase activity was assess by method of (Luck, 1974) with some modifications. 0.1g of fresh shoot material was homogenized in 8 ml of 0.067 M phosphate buffer (Disodium monohydrogen phosphate (5.963g) and Monosodium dihydrgen phosphate (5.226g) in 500ml) having pH 7. The extract was centrifuged and used for the enzyme activity by mixing 40µl supernatant thoroughly in 3 ml H_2O_2 (100ml phosphate buffer + 2.65µl H_2O_2). Spectrophotometer was used to check absorbance at 240 nm and the decrease in absorbance by 0.05 units was noted. The phosphate buffer was taken as blank. To decrease the absorbance of enzyme by 0.05 units at 240nm is served as one enzyme unit.

2.20.8 Osmoprotectants:

2.20.8.1 Proline

Method of (Talaat *et al.*, 2015) was used to measure proline content in maize plants. 0.1g fresh shoot material homogenized in 4ml of 3% sulphosalicyclic acid and then kept overnight at 5°C. At very next day suspension was centrifuged for 5 minutes at 3000 rpm to get supernatant. Then 2 ml of supernatant was mixed with acidic ninhydrin

(1.25 g ninhydrin in 20ml of phosphoric acid (6 M) and 30 ml of glacial acetic acid (1M H_3PO_4=3N H_3PO_4) with agitation ,until dissolved). The samples were then incubated at 100°C in water bath for one hour. After incubation samples were cooled and 4ml of toluene was mixed and O.D was recorded at 520 nm. Toulene was taken as blank. Proline (Sigma) was used to make a standard curve. Quantity of proline was calculated using following formula:

$$Proline\ \mu g/g = \frac{k\ value\ x\ Dilution\ factor\ x\ Absorbance}{Sample\ weight}$$

K value= 17.52

Dilution factor= 2

Weight of sample= 0.1g

2.21 STATISTICAL ANALYSIS

Data recorded was analyzed statistically by fisher's analysis of variance techniques using Statistics 8.1. Mean values were also compared using LSD (Least Significant Difference) at 5% probability level.

RESULTS

3.1 SALINITY TOLERANCE

A total of six bacterial strains were screened for their ability to grow in salt stress conditions. The bacterial strains with absorbance of 0.1 at optical density of 600nm were considered tolerant. The strains showed minimum growth at 24 hours interval after stress at all levels (Fig. 1). However, after 48 hours of stress induction, all the strains tolerated salt induced stress at 10% NaCl concentration. Q1, Q4, Q5 and Q6 show resistance at 12% NaCl concentration while Q3 and Q2 reduce their potential at higher salt concentration (12% NaCl). At 17% NaCl concentration no strains shows significant growth. A significant decline in growth was observed as the level of stress increased.

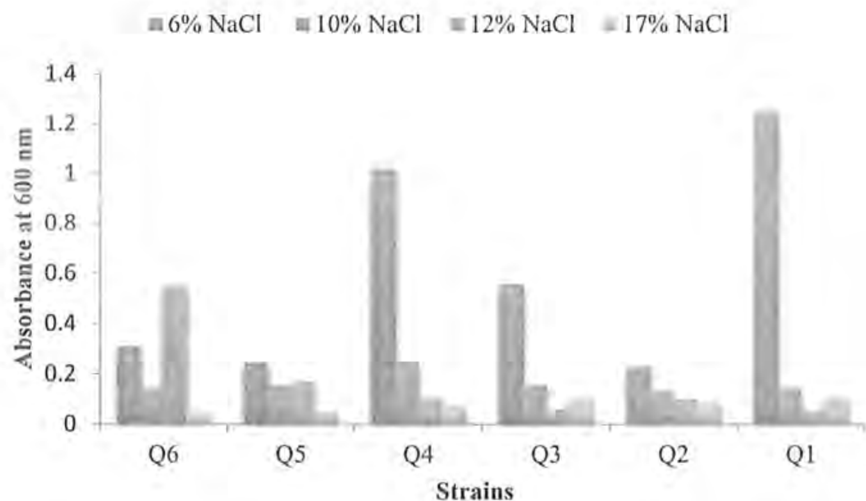


Fig. 3.1 Growth of 24 hrs. old strains at different levels of salt stress

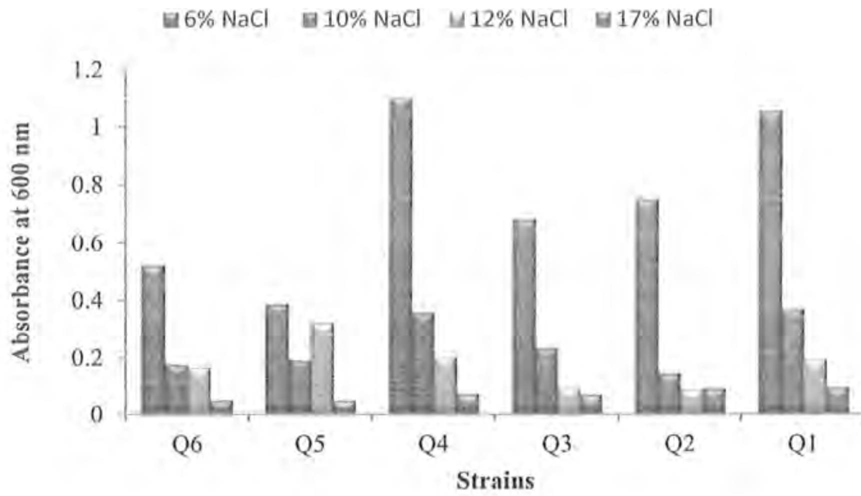
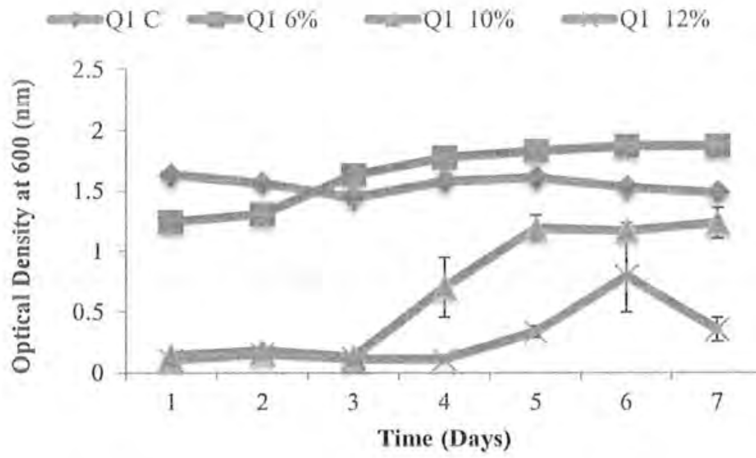
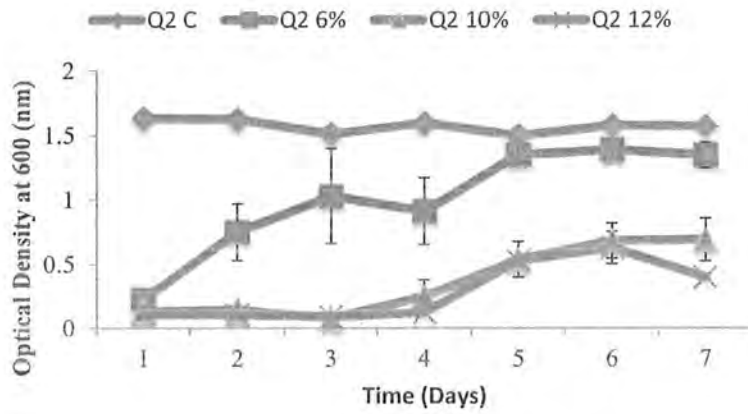


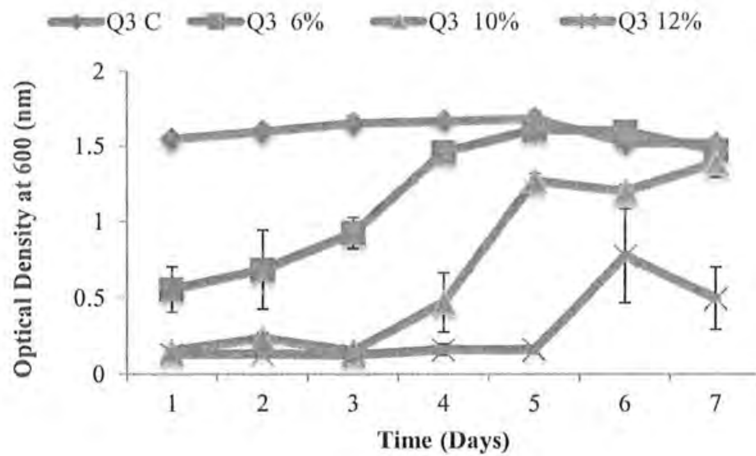
Fig. 3.2 Growth of 48 hrs. old strains at different levels of salt stress

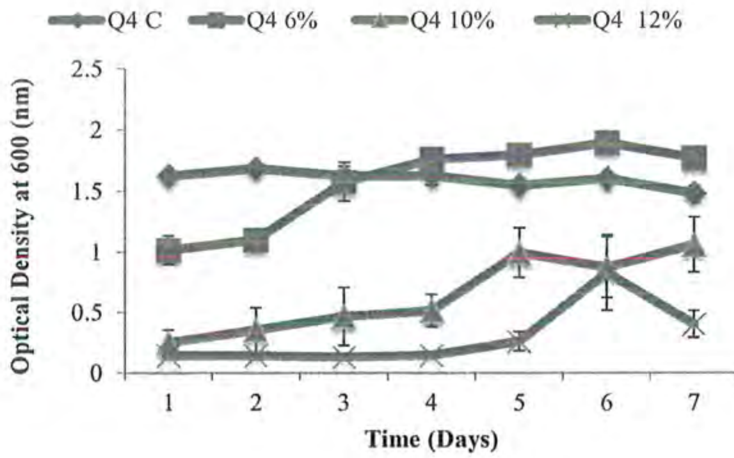
3.2 GROWTH CURVE OF CELLS UNDER SALINITY STRESS

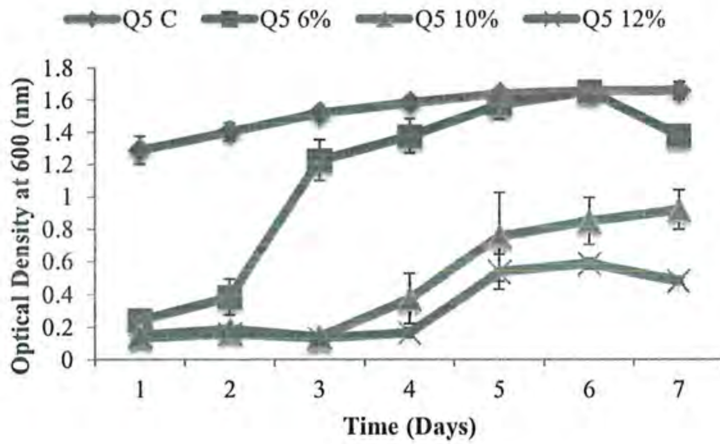
Bacterial growth kinetics was also evaluated under different levels of salt stress. All bacterial strains survived at all levels of salinity stress till 7th days of stress incubation. However, fluctuations in growth rate under stress were also observed.


Q1 C
Q1 6%
Q1 10%
Q1 12%
Optical Density at 600 (nm)
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
Time (Days)


Q2 C
Q2 6%
Q2 10%
Q2 12%
Optical Density at 600 (nm)
2
1.5
1
0.5
0
1
2
3
4
5
6
7
Time (Days)


Q3 C
Q3 6%
Q3 10%
Q3 12%
Optical Density at 600 (nm)
2
1.5
1
0.5
0
1
2
3
4
5
6
7
Time (Days)





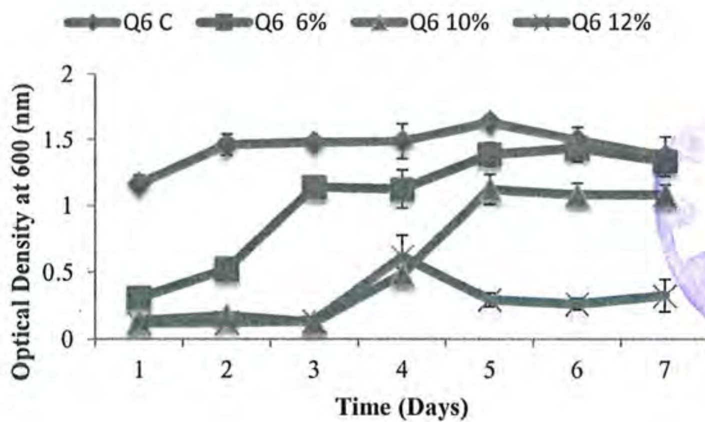


Fig. 3.3 Growth curves of strains at different levels of salt stress

3.3 PLANT GROWTH PROMOTING TRAITS

All halotolerant bacterial strains were characterized for its PGP potential. The response towards different tests is given below in table.

3.3.1 Exopolysaccharide production:

The bacterial strains form slimy, mucoid layer on ATCC medium no. 14 around bacterial colonies which are considered as positive for exopolysaccharide production. Dry weight weighing results represented in table below. Each value is a mean of three replicates.

Table 3.1 Dry weight and Colony colour of EPS production

Sr. No.	Bacterial Code	Colony Colour	Dry Matter EPS (mg/ml)
1	Q1	White Turbid	2.4
2	Q2	White Turbid	2.6
3	Q3	White Turbid	3.3
4	Q4	White Turbid	3
5	Q5	White Turbid	1.13
6	Q6	White Turbid	1.13

3.3.2 Indole acetic acid production

All six strains were screened for IAA producing ability. All strains expect Q5 and Q6 shows positive results for IAA production.

3.3.3 Phosphorus solubilizing activity

Strains were screened for their ability to phosphate solubilization. It is a useful character of endophytes that ensures availability of phosphorus to plants. Halo zone around all bacterial colonies in specific media plates indicated positive results for phosphate solubiliztion.

The phosphate solubilization indexes of the strains Q2, Q3, Q4 were 4.6, 3.3 and 3.2 respectively. However Q1, Q5 and Q6 were negative for phosphate solubilization. Phosphate solubilization efficiency was also noted. Q2, Q3, Q4 showed significant phosphate solubilization efficiency, which was 357, 228 and 216 respectively.

Table 3.2 Comparison of a phosphate solubilization efficiency and Solubilization index of representative isolated strains.

Strain Code	Phosphate SE (%)	Phosphate SI
Q1	-	-
Q2	357	4.6
Q3	228	3.3
Q4	216	3.2
Q5	-	-
Q6	-	-

3.3.4 *Production of Ammonia*

Bacterial strains were screened for ammonia production using peptone water. Ammonia producing endophytes are beneficial for plants as they are considered as a source of nitrogen for associated plants. All strains show positive results for ammonia production. Strains were categorized very good and good according to the colour. All strains except Q3 and Q4 shows brown yellow colour so they were conducted as very good for the production of ammonia. Moreover strains Q3 and Q4 showed yellow colour which were considered as good for ammonia production.

3.3.5 *Catalase*

All strains were screened for catalase enzyme production. Q1, Q2, Q3 and Q4 show catalase production by formation of bubbles along with rapid evolution of oxygen

and considered as strongly positive for catalase production. While Q5 and Q6 are slightly positive for catalase production.

3.3.6 Zinc Solubilization

All six bacterial strains are screened to check their zinc solubilizing ability. Halo zone around colonies shows zinc solubilization by bacterial strains.

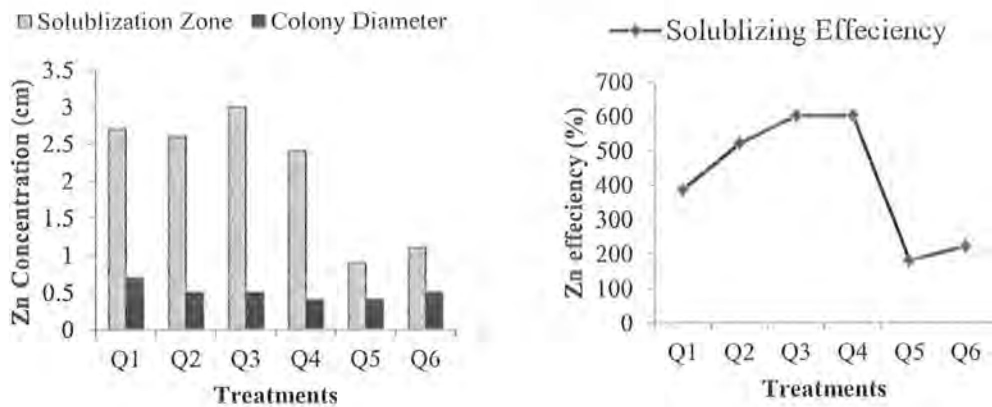


Fig. 3.4 Zinc solubilization index and solubilization efficiency by bacterial strains

3.3.7 Pectinase

The isolates were screened for pectinase production all strains expect Q1 shows positive results by forming clear halo zone around bacterial colonies when flooded with iodine solution

3.3.8 Protease production

All strains are positive for protease production and shows clear halo zone formation around bacterial colonies.

3.3.9 Amylase production

Starch agar medium was used to check out potential of extracellular digestion, by bacterial isolate. Bacteria produce amylase to the outside of their cells to carry out

extracellular digestion. Q6, Q4 and Q1 are slightly positive by forming a clear boundary around bacterial grown culture but remaining three showed no positive result. Results have been compiled in table

Table 3.3 PGP activities of bacterial isolates

Strain Code	Ammonia	Catalase	Protease	Cellulase	Amylase	IAA
Q1	+++	+++	+	-	+	+++
Q2	+++	+++	+	+	-	+++
Q3	++	+++	+	+	-	+++
Q4	++	+++	+	+	+	+++
Q5	+++	++	+	+	-	-
Q6	+++	++	+	+	+	-

3.4 GRAM STAINING

Gram staining results showed that all isolates were gram negative and shows red colour on staining. Shapes of bacterial isolates were noted as

Table 3.4 Shapes of isolates

Isolate Code	Gram Stain	Shape of Strain
Q1	Gram negative	Cocci
Q2	Gram negative	Cocci
Q3	Gram negative	Cocci
Q4	Gram negative	spiral
Q5	Gram negative	cocobacilli
Q6	Gram negative	cocobacilli

3.5 ANTIBIOTIC SENSITIVITY PROFILE

In microbially-assisted plant growth promotion the production of antibiotics is an important feature since antibiotic-resistant strains may have the ability to outcompete other strains in the rhizosphere. All of the six bacterial strains were tested for their ability to resist growth inhibition by the antibiotics Amikacin, Cloxacillin, Nitrofuratoin, Cabrenicillin, Cefoxitin, Ceftazidime, Ciprofloxacin, Piperaillin, Norflaxacin, Colitin sulphate, and Tobramycin using the regular antibiotic working concentrations. Current results revealed that Q2 and Q4 strains were susceptible to all selected antibiotic; however Q1, Q3, Q5 and Q6 had the ability to resist different antibiotics.

Table 3.5 Q1 shows antibiotic sensitivity and resistance against various antibiotics

Q1	Drugs	Symbol	Concentration	Zone diameter (cm)	Diameter Interpretation
1.	Norfloxacin	NOR	10µg	28	Resistance
2.	Tobramycin	TOB	10µg	15	Intermediate
3.	Cloxacillin	OB	5µg	0	Resistance
4.	Piperacillin	PRL	100µg	22	Intermediate
5.	Carbenicillin	CAR	100µg	23	Resistance
6.	Ceftazidime	CAZ	30µg	28	Susceptible
7.	Ciprofloxacin	CIP	5µg	22	Intermediate
8.	Amikacin	AK	30µg	19	Resistance
9.	Colistin	CT	10µg	12	Susceptible
10.	Nitrofurantoin	F	300µg	15	Susceptible
11.	Cefoxitin	FOX	30µg	17	Susceptible

Table 3.6 Q2 shows antibiotic sensitivity and resistance against various antibiotics

Q2	Drugs	Symbol	Concentration	Zone diameter (cm)	Diameter Interpretation
1.	Norfloxacin	NOR	10μg	30	Susceptible
2.	Tobramycin	TOB	10μg	16	Susceptible
3.	Cloxacillin	OB	5μg	7	Resistance
4.	Piperacillin	PRL	100μg	17	Susceptible
5.	Carbenicillin	CAR	100μg	22	Susceptible
6.	Ceftazidime	CAZ	30μg	23	Susceptible
7.	Ciprofloxacin	CIP	5μg	27	Susceptible
8.	Amikacin	AK	30μg	18	Susceptible
9.	Colistin	CT	10μg	12	Intermediate
10.	Nitrofurantoin	F	300μg	16	Susceptible
11.	Cefoxitin	FOX	30μg	14	Intermediate

Table 3.7 Q3 shows antibiotic sensitivity and resistance against various antibiotics

Q3	Drugs	Symbol	Concentration	Zone diameter (cm)	Diameter Interpretation
1.	Norfloxacin	NOR	10μg	20	Susceptible
2.	Tobramycin	TOB	10μg	16	Susceptible
3.	Cloxacillin	OB	5μg	9	Resistance
4.	Piperacillin	PRL	100μg	19	Susceptible
5.	Carbenicillin	CAR	100μg	18	Susceptible
6.	Ceftazidime	CAZ	30μg	28	Susceptible
7.	Ciprofloxacin	CIP	5μg	20	Susceptible
8.	Amikacin	AK	30μg	17	Susceptible
9.	Colistin	CT	10μg	13	Intermediate
10.	Nitrofurantoin	F	300μg	18	Susceptible
11.	Cefoxitin	FOX	30μg	0	Resistance

Table 3.8 Q4 shows antibiotic sensitivity and resistance against various antibiotics.

Q4	Drugs	Symbol	Concentration	Zone diameter (cm)	Diameter Interpretation
1.	Norfloxacin	NOR	10µg	31	Susceptible
2.	Tobramycin	TOB	10µg	15	Intermediate
3.	Cloxacillin	OB	5µg	7	Resistance
4.	Piperacillin	PRL	100µg	22	Susceptible
5.	Carbenicillin	CAR	100µg	21	Susceptible
6.	Ceftazidime	CAZ	30µg	28	Susceptible
7.	Ciprofloxacin	CIP	5µg	22	Susceptible
8.	Amikacin	AK	30µg	18	Susceptible
9.	Colistin	CT	10µg	11	Intermediate
10.	Nitrofurantoin	F	300µg	13	Intermediate
11.	Cefoxitin	FOX	30µg	17	Susceptible

Table 3.9 Q5 shows antibiotic sensitivity and resistance against various antibiotics

Q5	Drugs	Symbol	Concentration	Zone diameter (cm)	Diameter Interpretation
1.	Norfloxacin	NOR	10µg	21	Susceptible
2.	Tobramycin	TOB	10µg	16	Susceptible
3.	Cloxacillin	OB	5µg	0	Resistance
4.	Piperacillin	PRL	100µg	18	Susceptible
5.	Carbenicillin	CAR	100µg	10	Resistance
6.	Ceftazidime	CAZ	30µg	10	Resistance
7.	Ciprofloxacin	CIP	5µg	32	Susceptible
8.	Amikacin	AK	30µg	24	Susceptible
9.	Colistin	CT	10µg	0	Resistance
10.	Nitrofurantoin	F	300µg	16	Susceptible
11.	Cefoxitin	FOX	30µg	18	Susceptible

Table 3.10 Q6 shows antibiotic sensitivity and resistance against various antibiotics.

Q6	Drugs	Symbol	Concentration	Zone diameter (cm)	Diameter Interpretation
1.	Norfloxacin	NOR	10μg	19	Susceptible
2.	Tobramycin	TOB	10μg	15	Intermediate
3.	Cloxacillin	OB	5μg	0	Resistance
4.	Piperacillin	PRL	100μg	16	Susceptible
5.	Carbenicillin	CAR	100μg	0	Resistance
6.	Ceftazidime	CAZ	30μg	8	Resistance
7.	Ciprofloxacin	CIP	5μg	24	Susceptible
8.	Amikacin	AK	30μg	21	Susceptible
9.	Colistin	CT	10μg	0	Resistance
10.	Nitrofurantoin	F	300μg	15	Intermediate
11.	Cefoxitin	FOX	30μg	18	Susceptible

3.6 MAIZE RESPONSE TO SALINITY STRESS

A greenhouse experiment was done to evaluate the *in vivo* activity of stress tolerant bacterial strains Q2 and Q4, on maize plants. Plants were subjected to 250mM NaCl stress in both inoculated and un-inoculated conditions and further analyzed for following parameters.

3.6.1 *Effect of salt tolerant strains on shoot length of maize*

Differential response to salt stress in both varieties was observed in terms of growth parameters. Salt stress significantly impaired maize growth in terms of all growth parameters. However, significant increase in plant growth was found in inoculated plants. Moreover, inoculation of plants with bacterial strains Q2 and Q4 without salt stress significantly increased maize growth than non-inoculated control.

Analysis of variance of shoot length of maize plant showed that in variety EV1097 salinity stress decreased (11%) shoot length as compared to control. Similar effects were observed in variety Haq Nawaz Gold in which shoot length reduced (0.69%). Though application of PGPB stimulated shoot length of both varieties of maize under stress and non-stress conditions. In Variety EV1097 inoculated stressed plants exhibited a considerable improvement in shoot length by 15% and 54% by Q2 and Q4 respectively. In variety Haq Nawaz Gold Q2 increase 25% and Q4 increase shoot length 41% as compared to their respective stressed non-inoculated plants.

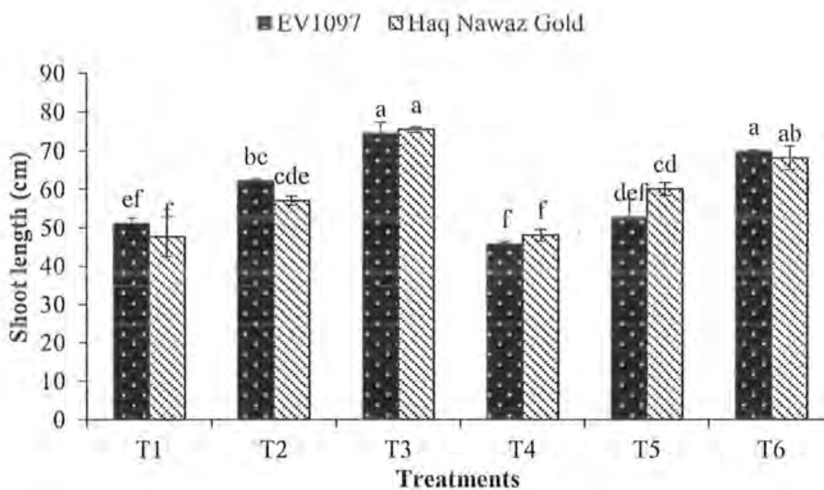


Fig. 3.5 Effect of Q2 and Q4 on Shoot length of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.6.2 *Effect of salt tolerant strains on root length of maize*

Under salt stress condition drastic reduction in root length was observed in variety EV1097 (38%) and variety Haq Nawaz Gold showed about (6%) decreased root growth as compared to their respective controls. PGPE inoculated salinity stressed plants show increased root length 66% and 83% by Q2 and Q4 respectively in Variety EV1097 and 32% by Q2 and 29% by Q4 in variety Haq Nawaz Gold. Moreover 7% and 5% increment found by Q2 and Q4 respectively and 72% and 27% root length increase in non-stressed inoculated plants in variety Haq Nawaz Gold by Q2 and Q4 respectively as compared to their non-inoculated plants.

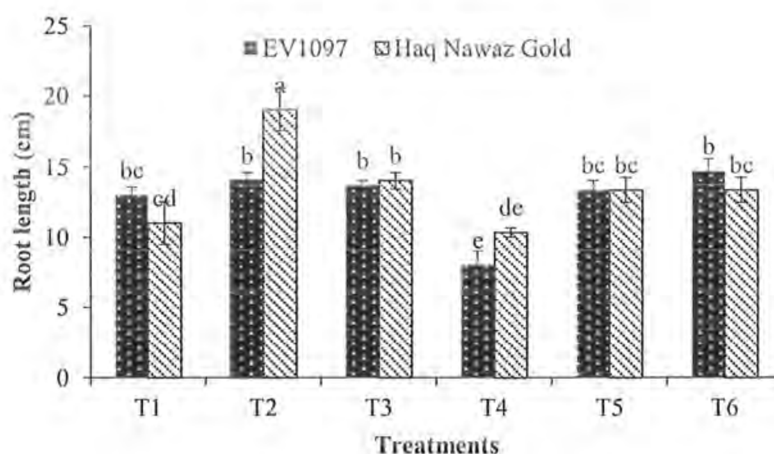


Fig. 3.6 Effect of Q2 and Q4 on Root length of Zea mays L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.6.3 *Effect of salt tolerant strains on fresh weight of maize*

In non-stressed conditions bacterial strains significantly increase fresh weight of both varieties of maize as compared to un-inoculated treatment. Fresh weight increases up to 26% by Q2 and 119% by Q4 in Variety EV1097 and 15% by Q2 and 78% by Q4 in variety Haq Nawaz Gold. Salinity stress effects severely un-inoculated plants as 26% and 23% decrease in fresh weight was observed in Variety EV1097 and variety Haq Nawaz Gold, respectively as compared to their non-stressed non-inoculated plants. The use of salt tolerant PGPR mitigate the stress effectively as the fresh weight of bacterial treated stressed plants was boosted to 29% by Q2 and 290% by Q4 in Variety EV1097 and 54% by Q2 and 69% by Q4 in variety Haq Nawaz Gold as compared to non-inoculated stressed plants.

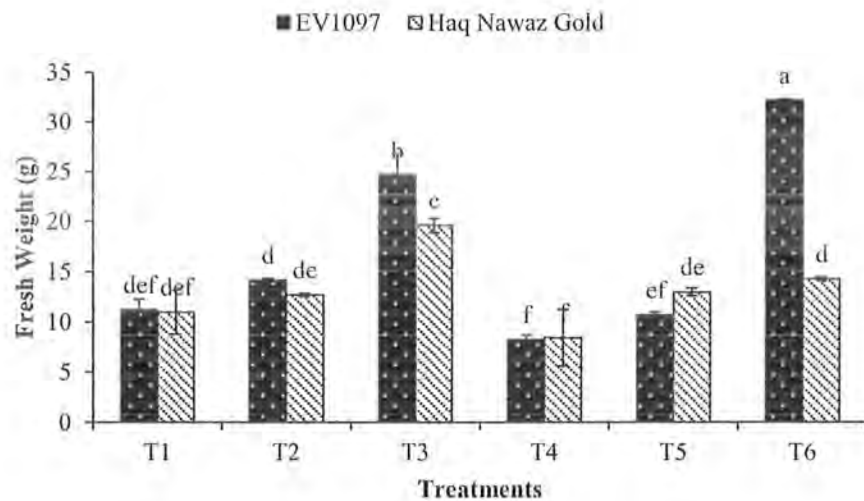


Fig. 3.7 Effect of Q2 and Q4 on Fresh weight of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.6.4 Effect of salt tolerant strains on relative water content of maize

Inoculation with PGPE significantly enhanced water status of stressed and non-stressed plants in both varieties. Inoculated plants showed 9% and 12% increment by Q2 and Q4 respectively in EV 1097 and 14% by Q2 and 9% by Q4 increase in RWC was found in variety Haq Nawaz Gold as compared to their un-inoculated control. Similarly inoculated stressed plants in variety EV 1097 showed increment of 130% and 131% by Q2 and Q4 respectively and 14% by Q2 and 38% by Q4 in variety Haq Nawaz Gold as compared to their stressed non inoculated plants. Moreover non inoculated stressed plants exhibited a significant decrease in RWC by 55% and 8% in EV 1097 and Haq Nawaz Gold genotype respectively.

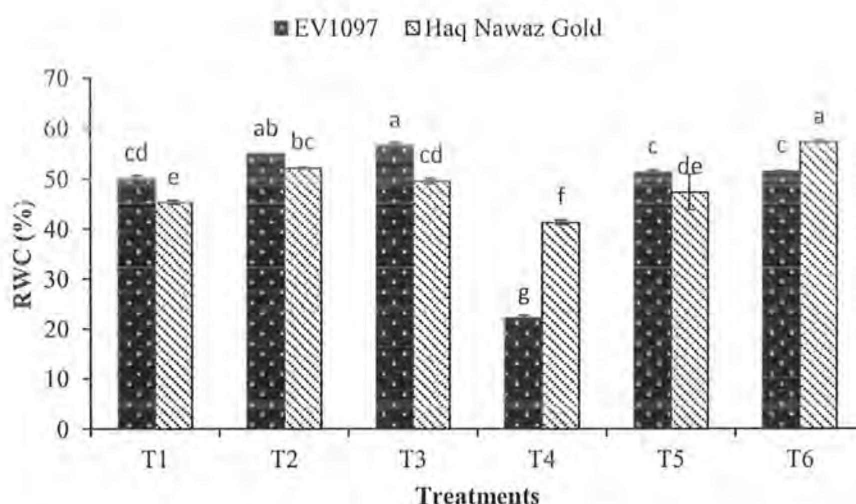


Fig. 3.8 Effect of Q2 and Q4 on Relative water contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.6.5 *Effect of salt tolerant strains on leaf surface area of maize*

In terms of leaf area, a decreasing trend was found in un-inoculated control and non- inoculated stressed plants. In variety EV 1097 leaf area reduce 9% as compared to their non-inoculated control. Same results were found in variety Haq Nawaz Gold which shows decrease in leaf area about 10%. Statistical analysis showed that un-inoculated control and stressed treatments are statistically similar. No significant difference found between treatments. But a significant increase in leaf area was found in PGPE treatments in both stressed and non-stressed conditions. Bacterial inoculation with stressed conditions increases leaf area 84% by Q2 and 332% by Q4 in Variety EV1097 and 78% by Q2 and 97% by Q4 in variety Haq Nawaz Gold. PGP applied alone increase leaf area 57% and 218% by Q2 and Q4 in EV1097 and 43% and 100% by Q2 and Q4 respectively and Haq Nawaz Gold.

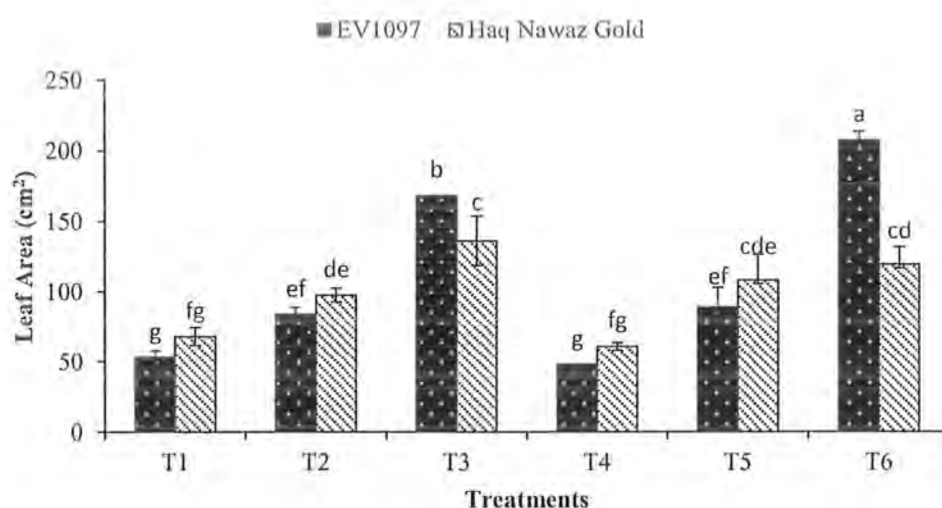


Fig. 3.9 Effect of Q2 and Q4 on Leaf area of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.7 ELECTROLYTE LEAKAGE

Salinity stress caused significant increase in electrolyte leakage in both genotypes EV 1097 & Haq Nawaz gold but bacterial inoculation reduced adversity of leakage. In EV 1097 Q2 decrease electrolyte leakage up to 42% and Q4 reduce 50% EL. In Variety Haq Nawaz gold leakage reduce by Q2 and Q4 to about 21% & 83% respectively. 64% increase in electrolyte leakage observed in Variety EV1097 and 51% in variety Haq Nawaz gold as compared to their non-stressed non-inoculated control. Bacterial inoculated non-stress plants also shows significant decrease in EL as compared to their non-inoculated control. Statistical analysis shows that all treatments are significantly different from each other.

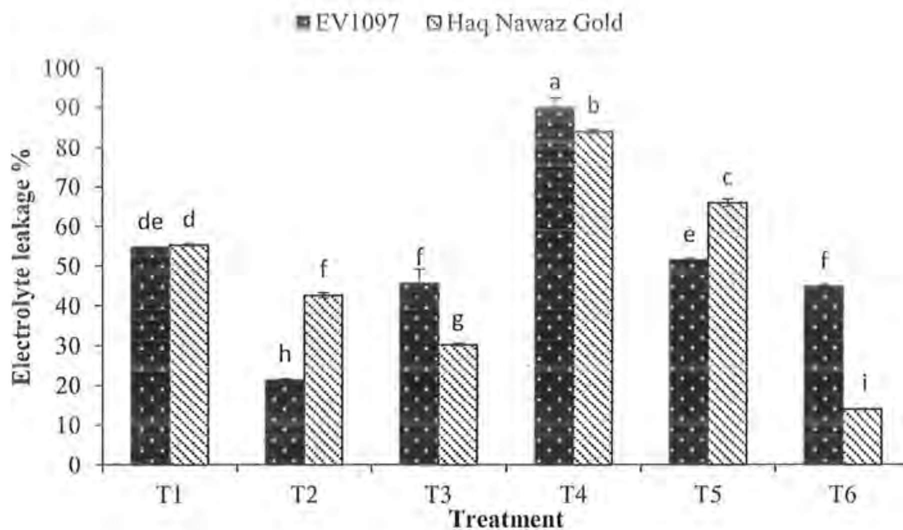


Fig. 3.10 Effect of Q2 and Q4 on Electrolytic Leakge of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.8 BIOCHEMICAL ANALYSIS OF PLANTS

3.8.1 Chlorophyll a Content

Salinity stress significantly reduced amount of chlorophyll a in both genotypes. In Variety EV1097 amount of chlorophyll a content reduce 60% and in Haq Nawaz Gold 35% reduction occur as compared to their respective un-inoculated control. Bacterial inoculation improves chlorophyll content in stressed conditions to 172% by Q2 and 138% by Q4 in Variety EV1097 and 193% by both bacteria in Haq Nawaz Gold. Non stressed Bacterial inoculated plants also showed an increase in chlorophyll content than non-inoculated control.

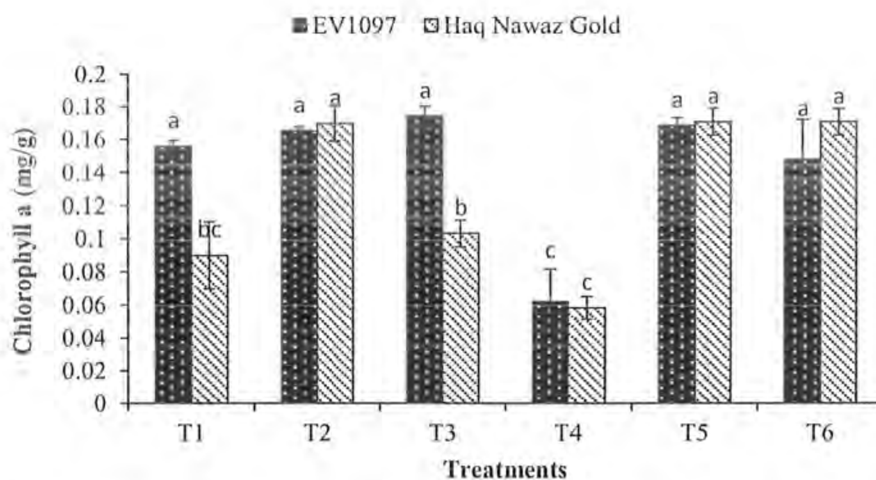


Fig. 3.11 Effect of Q2 and Q4 on Chlorophyll a contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.8.2 Chlorophyll b Content

In Haq Nawaz gold there is a no significant difference between non stressed bacterial treatments and their respective control while genotype EV1097 shows increase amount of chlorophyll b content in bacterial treatments as compared to their control. EV1097 shows 420% increase in chlorophyll a content by bacteria Q2 and 178% by bacteria Q4. Furthermore, Inoculation with PGPB significantly enhanced chlorophyll b content than non-inoculated stressed plants. The improvement was 852% by Q2 and 138% by Q4 in Variety EV1097 and 233% by Q2 and 246% by Q4 in Variety Haq Nawaz Gold. Stress decrease amount of chlorophyll 42% and 65% in Variety EV1097 and Haq Nawaz Gold respectively.

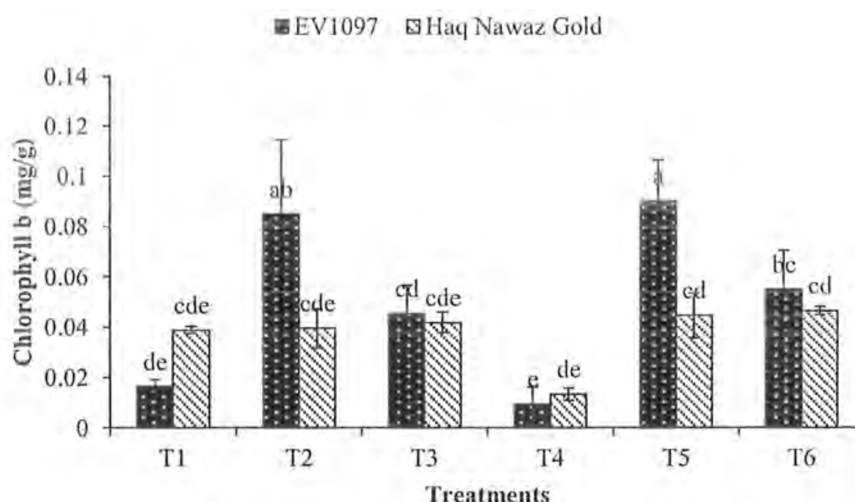


Fig. 3.12 Effect of Q2 and Q4 on Chlorophyll b contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.8.3 Carotenoids

In carotenoids almost similar trend was found in treatments as in Chlorophyll b with few variations. In Variety EV1097 there is no significant difference between Q4 inoculated treatments and their respective control. But in Variety Haq Nawaz gold Q4 improves carotenoid contents as compared to their non-inoculated control. There were low carotenoids content in both non stressed varieties by Q2 strain as compared to their non-inoculated control. PGPB inoculated stress plants shows increase amount of carotenoids content as compared to their non-inoculated plants. In Variety EV 1097 53% decrease rate was found and 82% in Variety Haq Nawaz Gold. The application of Q2 mitigates salinity stress and 40% increment recorded in V1 and 726% in V2 by Q2. Similarly Q4 efficiently cope with salinity stress 160% in V1 and 426% in V2 by Q4 as compared to their non-inoculated stressed treatments.

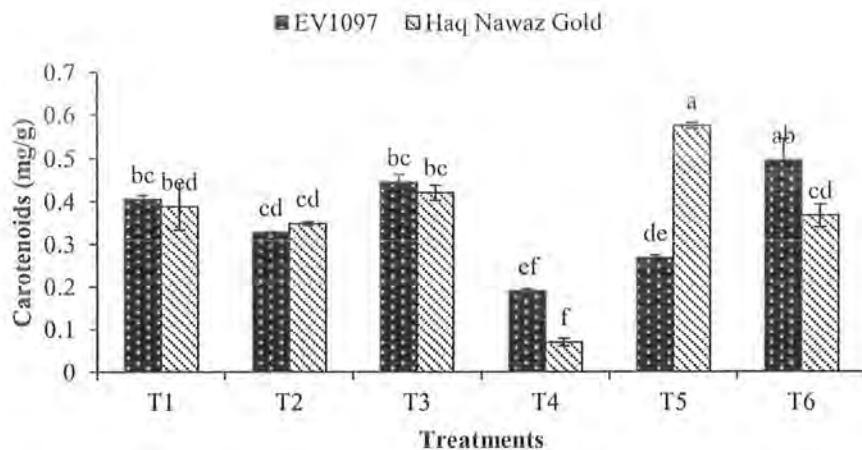


Fig. 3.13 Effect of Q2 and Q4 on Carotenoids contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.9 ENZYMATIC ANTIOXIDANT STATUS

3.9.1 Superoxide dismutase activity

As the salinity stress applied the SOD enzyme activity reduce 4% in variety EV1097 and 64% in Variety Haq Nawaz Gold as compared to their respective control. Application of salt tolerant strains enhanced SOD activity to 93% by Q2 and 104% by Q4 in variety EV1097 while 251% by Q2 and 446% by Q4 increase in SOD activity was found in Variety Haq Nawaz Gold.

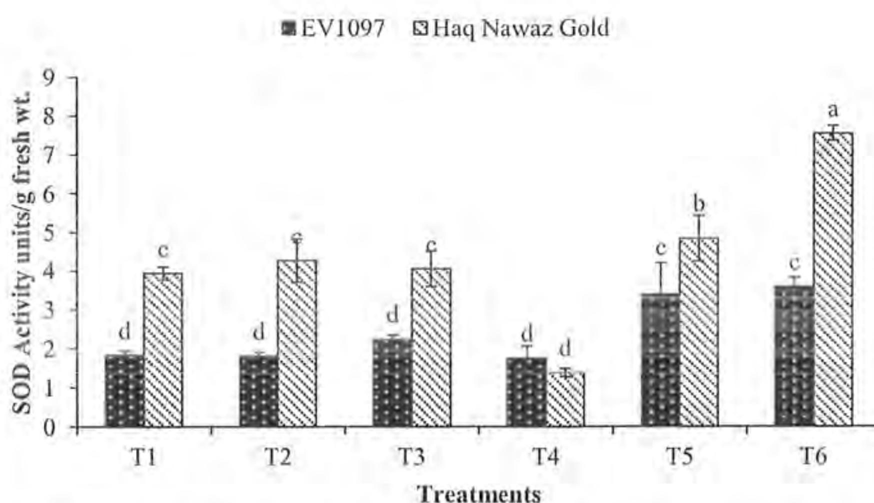


Fig. 3.14 Effect of Q2 and Q4 on Superoxide dismutase contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.9.2 Peroxidase activity

In peroxidase activity salinity stress reduce peroxidase activity in both genotypes as compared to their respective control. About 38% decrease in POD activity was found in Variety EV1097 and 22% in Haq Nawaz Gold genotype. To improve the oxidative damage in response to salinity stress as compared to un-inoculated stressed treatment, the strains significantly enhanced POD activity in EV 1097 to 219% by Q2 and 118% Q4 and in Haq Nawaz Gold 188% by Q2 and 29% by Q4.

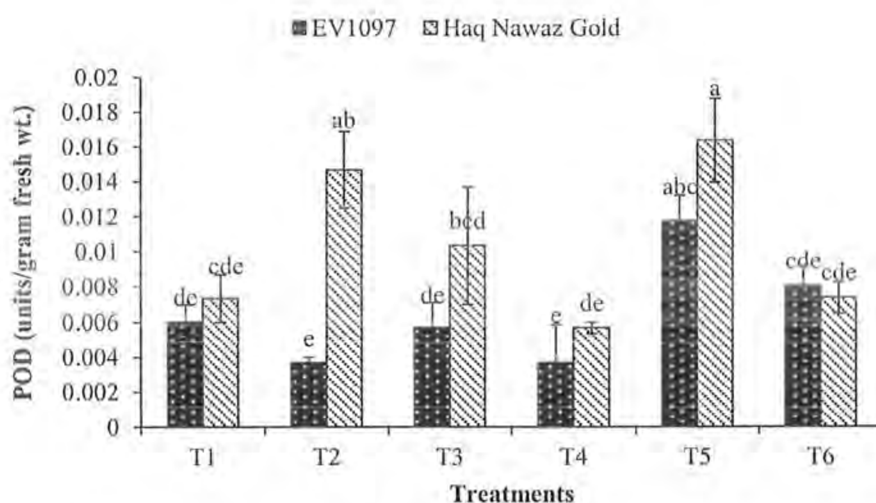


Fig. 3.15 Effect of Q2 and Q4 on Peroxidase activity of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.9.3 Catalase

Salinity stress drastically reduces catalase enzyme activity in stressed non-inoculated plants to 42% and 89% in EV 1097 and Haq Nawaz Gold respectively. PGP activity improves catalase production in both varieties. In EV1097 Q2 improves catalase production 34% and in Haq Nawaz Gold production was increased to one thousand fold as compared to their respective control. Similar results was shown by Q4, it increase enzyme production 102% in Variety 1 and 565% in Variety 2 as compared to their respective non inoculated control. PGPRs enhance enzymatic activity in inoculated non-stressed treatments as well but in Haq Nawaz Gold there is a no significant difference between Q4 inoculated treatment and their respective non-inoculated control.

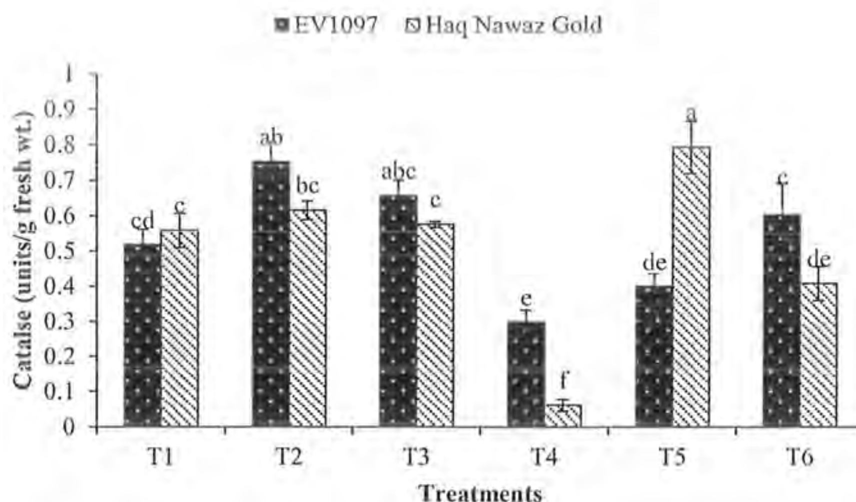


Fig. 3.16 Effect of Q2 and Q4 on Catalase contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

3.10 PROLINE

Both maize genotypes couldn't produce much proline in stress conditions but there is a significant production of proline in inoculated stressed plants and non-stressed plants as compared to their non-inoculated stressed plants. PGPRs help the plants to tolerate salinity stress by producing proline. In Variety EV1097 Q2 shows 119% increase in proline production while Q4 shows 40% increment of proline production. In variety Haq Nawaz gold Q2 improves proline content 49% and Q4 increase 50% as compare to their non-inoculated stressed control. Statistical analysis shows significant difference between all treatments.

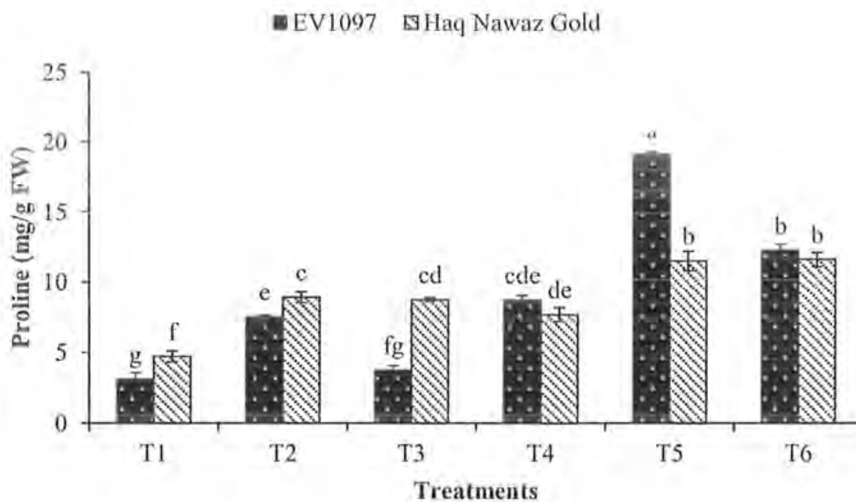


Fig. 3.17 Effect of Q2 and Q4 on Proline contents of *Zea mays* L. under salinity stress, T1= Control, T2= Varieties+ Q2, T3= Varieties + Q4, T4= Stress, T5= Q2+Stress, T6= Q4+stress

DISCUSSION

Salinity stress negatively effects the growth, physiology and ionic balance of crop plants and is one of the main contributing reason to lower yield in arid and semiarid regions. The application of bacteria to promote growth and productivity under stress conditions previously documented by (Ahmad *et al.*, 2006)(Ahmad *et al.*, 2012). Therefore, characterization and screening of salt tolerant endophytes and their applications in agriculture would definitely ensure food security by enhancing productivity. Furthermore, PGPB can overwhelmed deleterious effects of pesticides and chemical fertilizers (Praveen Kumar *et al.*, 2014). They promote growth and enhance development by various mechanisms both directly and indirectly. The current study was directed to identify salt tolerant bacterial strains that can tolerate saline conditions but also helps plants to promote their growth by reducing negative effects of salinity. In the current study, six strains were screened to check their salt tolerant ability and all these strains can tolerate 12% salt concentration but the decline in growth rate was observed with gradual increase in salt concentration. (Tank and Saraf, 2010) reported the PGP effect of salt tolerant strains on tomato plant that are able to survive at 6% NaCl.

Increase in plant growth by PGPB is primarily due to activity of growth promoting hormones and other plant growth promoting qualities (Glick, 2005). Abiotic stresses in plants can be best cope with inoculation of bacterial strains that possesses more than one plant growth promoting trait (Yang *et al.*, 2010). All six strains showed one or more PGP traits and out of these Q2 and Q4 show most promising results for IAA production, phosphate solubilization, zinc solubilization, ammonia production, catalase production, and other extracellular enzymes production. IAA is reported for enhancement of stress tolerance as well as plant growth promotion (Marulanda *et al.*, 2009). Salinity stress disturbs ionic imbalance which results in nutrient deficiency, while inoculation of PGPB can maintain nutrient balance of plants by enhancing plant-microbe interactions. So, on the basis of characteristics, effective PGPB can be used for plant studies under harsh environmental situations.

In the present study results shown that salt stress suppress all the traits of maize plants. Though, inoculation with endophytic bacteria isolated from the *O. ferruginea*

significantly decrease the negative effects of salt stress. Both strains showed more promising results and show enhancement in growth and quality parameters of maize than without their application. The enhancement in growth, nutrient and chlorophyll contents of plant in response to bacterial inoculation might be due to the production of IAA (Zahir *et al.*, 2010), phosphate solubilization (Afzal and Bano, 2008) exopolysaccharides (Hussain *et al.*, 2014)(Zafar-ul-Hye *et al.*, 2013), the availability and uptake of nitrogen and phosphorus by the plants (Zahir *et al.*, 2009).

Furthermore, PGPB inoculation also makes plants resistant against viruses and other harmful pathogens (Huang and Erickson, 2007) and improved root respiration (Volpin and Phillips, 1998) by improving plant physiology. In the present study, it was observed that high salt stress caused a drastic reduction in the plant height, plant weight and leaf area. Decline in growth and development of crops due to salt stress has extensively been reported in the literature (Zahir *et al.*, 2009)(Bano and Fatima, 2009)(Naz *et al.*, 2013).

Salinity stress causes osmotic stress which results in toxicity of specific ions in plants causing a decrease in growth and yield. So, decrease in growth of maize observed in present experiment might be due to the accumulation of high concentration of ions in leaves. Salts results in the dehydration of the cells by their accumulation in the apoplast and hinder the photosynthesis process by amassing in the chloroplast (Munns and Tester, 2008). But, bacterial inoculation significantly enhanced growth and yield of plants compared to non-inoculated maize plants. Moreover, auxins production by applied bacteria may be involved in enhancing plant height (Zahir *et al.*, 2010) because IAA increase the cell elongation and cell division in the plants (Perrot-Rechenmann, 2010) resulting in enhanced height of bacterial inoculated plants than non-inoculated plants.

LWC describes the proficiency of plants to sustain water status, so it is frequently used to check the effect of salinity on plants growth under salinity conditions. In current study, low relative water content was observed in salt stressed plants than inoculated plants. Several other scholars also observed decreased RWC of plants exposed to salinity (Srivastava *et al.*, 1988)(Kaya *et al.*, 2003). As saline conditions increased plants ability to absorb water decreases that results in decline in RWC (Heidari *et al.*, 2011) (Amirjani,

2011). High salts concentration have decrease the sap flux flow which have decreased the root hydraulic conductivity and in result reduced relative water content of plant (Vysotskaya et al., 2010). Bacterial strain inoculation results in the improvement of RWC has been described in several studies (Bano and Fatima, 2009)(Ahmad *et al.*, 2012).

Outcomes of current study also showed that bacterial inoculation increase relative water content both in stressed and non-stressed plants. Rhizobia promotes root hairs and root volume (Glick, 2012) that enlarged the roots that aid in more water uptake under salinity conditions (Yu *et al.*, 2007).

Under biotic and abiotic stresses plant membranes are subject to changes often associated with the loss of integrity and increases in permeability. So, the cell membranes ability to control the movement of ions in and outside of cells is used to test tissues injuries and damage. In our findings it was observed that membrane damage is high in stress treated plants while bacterial inoculation significantly decreases the rate of electrical conductivity in both stressed and non-stressed plants.

Data about photosynthetic pigments clearly showed that salinity stress decrease chlorophyll contents in maize plants compared to non-stressed plants (Bano and Fatima, 2009). Osmotic stress disturbs the uptake and assimilation of nutrients and minerals that might cause decrease in chlorophyll content (Soliman *et al.*, 2012). Under high salinity stress insufficiency of mineral elements especially nitrogen, may cause inhibition in the formation of chlorophyll molecules (Huang *et al.*, 2004). Furthermore, high accumulation of salts in the cells of leaves causes high ROS production and forced the chloroplast to destroy all protein component of chloroplast (Muneer *et al.*, 2014). Among all the treatments, PGPB inoculation exhibited significant improvement in chlorophyll "a", "b", and carotenoid contents in both stressed and non-stressed conditions compared to other un-inoculated non-stresses and stressed control. These results are also supported by findings of (Hussain *et al.*, 2014) who investigated the effect of rhizobial inoculation on maize under stressed conditions and found the variable response of maize plants to isolates of different species of rhizobium. Increase in chlorophyll content due to the application of bacterial inoculum might be due to enhanced solubilization and mobilization of minerals (Zahir *et al.*, 2009) especially N, which is an vital constituent of

chlorophyll (Swan, 1971), that eventually resulted in the high chlorophyll contents (Bojović and Marković, 2009).

Activities of antioxidant enzymes and resistant to stress strongly correlated with each other. Salinity stress created an osmotic imbalance and induced oxidative stress. The stressed plants adapted to osmotic stress by enhancing proline content, and the increase was further augmented more than two fold in PGPR-inoculated plants. PGPR-induced proline production was also observed under normal conditions, demonstrating that PGPR inoculation enables the plant to overcome osmotic stress much better, and on salinity conditions PGPR inoculation further assisted the plants in overcoming this stress. Stress conditions such as drought extreme temperature , salinity and insect attack cause the accumulation of proline in many plants (Mansour, 2000).

Proline reported as energy source and hydroxyl radical scavenger (Munns and Tester, 2008). Superoxide dismutase known as first enzyme in the series which scavenge reactive oxygen species induced during biotic and abiotic stresses and was significantly stimulated in inoculated and stressed maize leaves. PGPR-inoculated plants under normal conditions had a similar percentage increase, and in stress conditions, a more than two fold increase in SOD was observed.

Similarly, POD is involved in the detoxification of H_2O_2 produced by the scavenging action of SOD. (Heidari and Golpayegani, 2012) reported that application of rhizobacteria under drought stress enhanced the photosynthetic pigments and antioxidants in basil plants. Pseudomonades sp. under water stress significantly increased CAT activity. The greater activities of defense-related enzymes contributed to bio protection of plants against insects and pathogens.

The activity of CAT enzyme was increased with the increase of salinity stress from control to bacterial inoculated plants both in stressed and in non-stressed conditions. A quick and continued rise in catalase activity might show that catalase is a main enzyme involved in detoxification of H_2O_2 in maize under salinity stress.

Ability of rhizobia is well known for producing advantageous hormones, secretions and enzymes, both qualitatively and quantitatively, differs from species to

species and even strain to strain (Mehboob *et al.*, 2012). It is also possible that some strains have more abilities to secrete beneficial secretions and hormones to greater extent compared to other which improved their potential for enhancing the growth and yield of maize.

CONCLUSION

It is inferred from the conducted research that selected endophytic strains enzymes production. Application of the salt tolerant endophytes under salinity stress not only help the plants to resist against salinity stress but also enhance its growth by its growth promoting abilities like IAA, exopolysaccharide production, zinc solubilization, phosphate solubilization and other extracellular enzyme activities. All these mechanisms improve plant growth morphologically and also show improvement in physiological and biochemical attributes. Hence, it is suggested that application of PGP endophytes could be a significant input to reduce oxidative and osmotic stress caused by salinity and promotion of maize growth under salinity stress.

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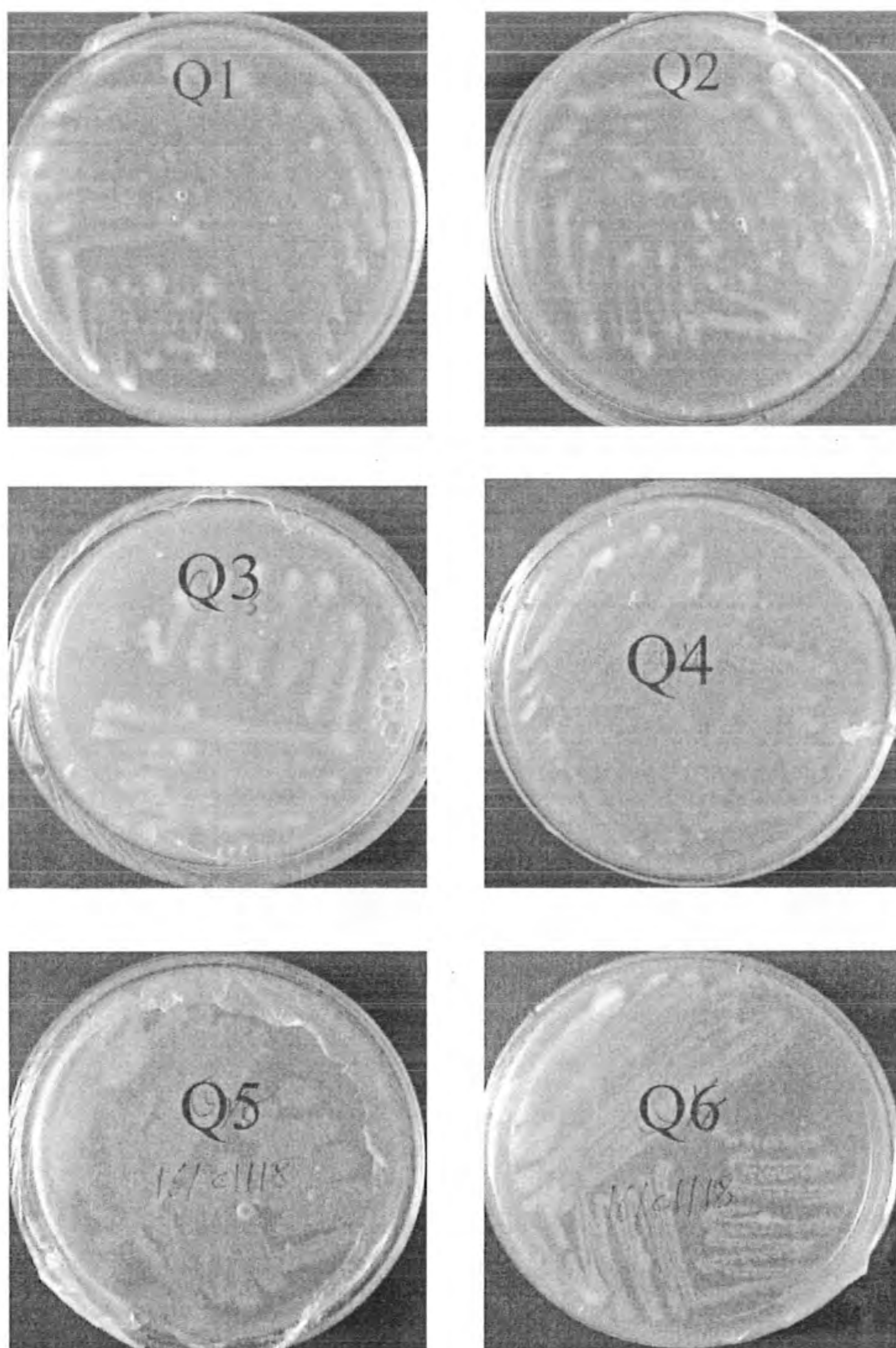


Plate 1 Exopolysaccharide production

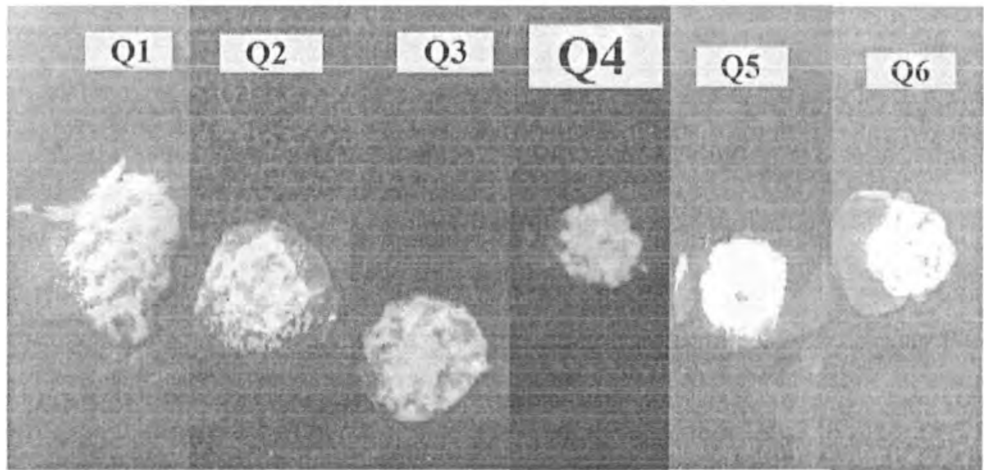


Plate 2 Catalase production

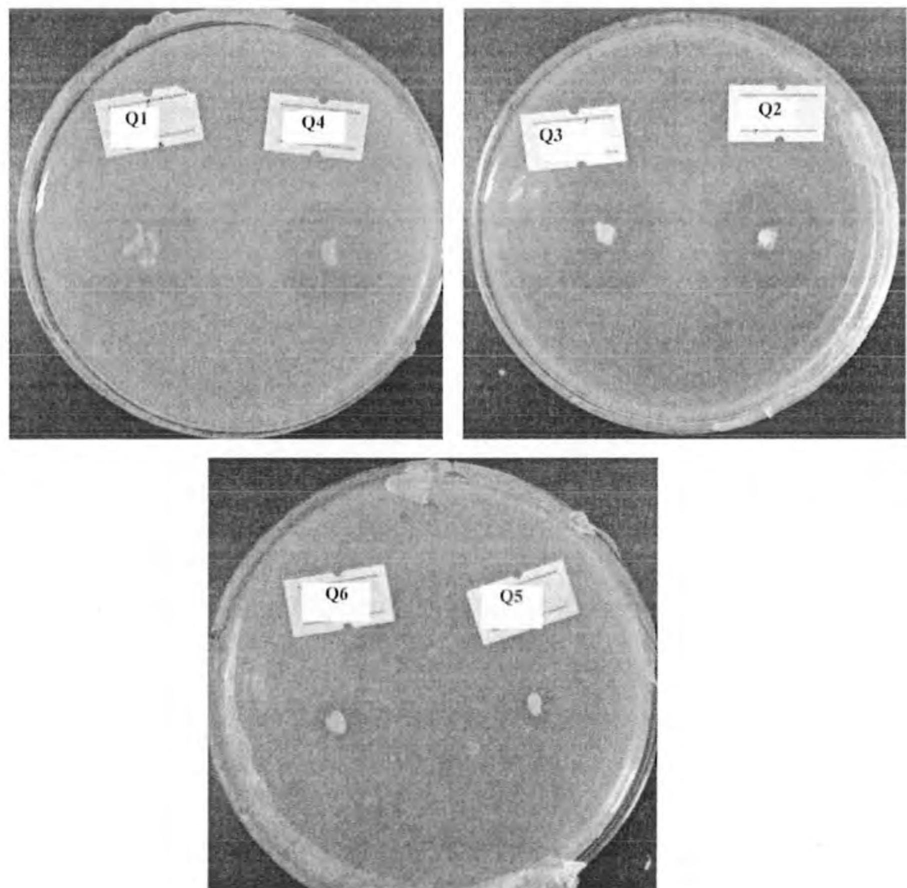


Plate 3 Zinc solubilization

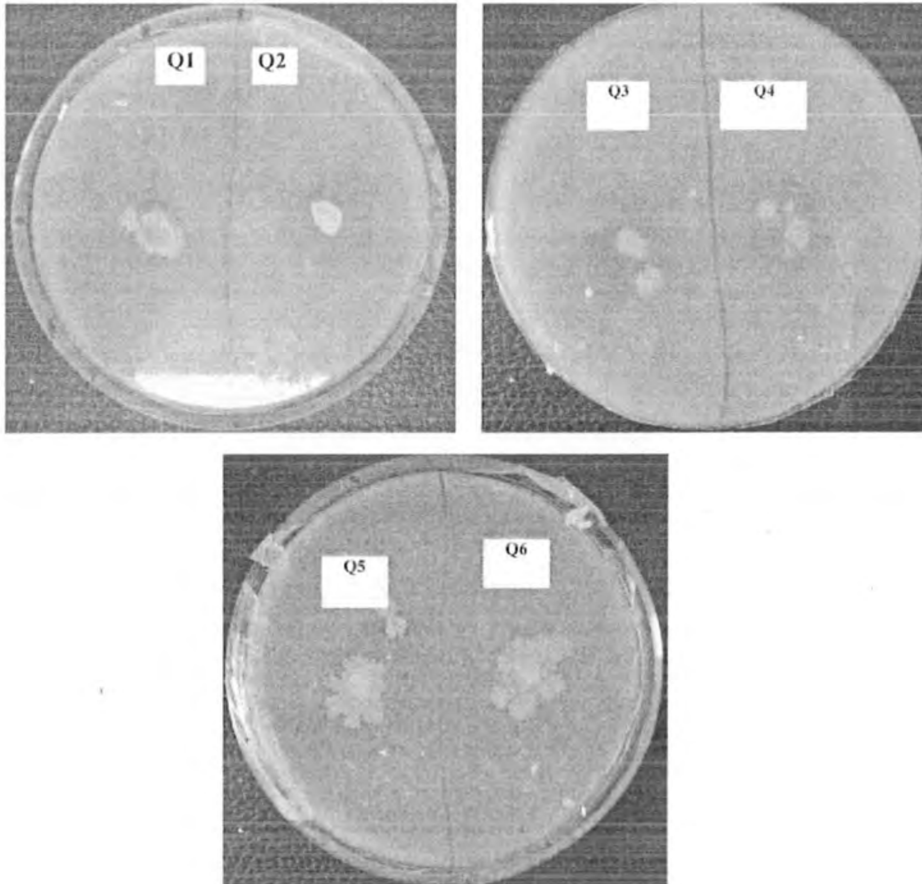


Plate 4 Phosphate solubilization

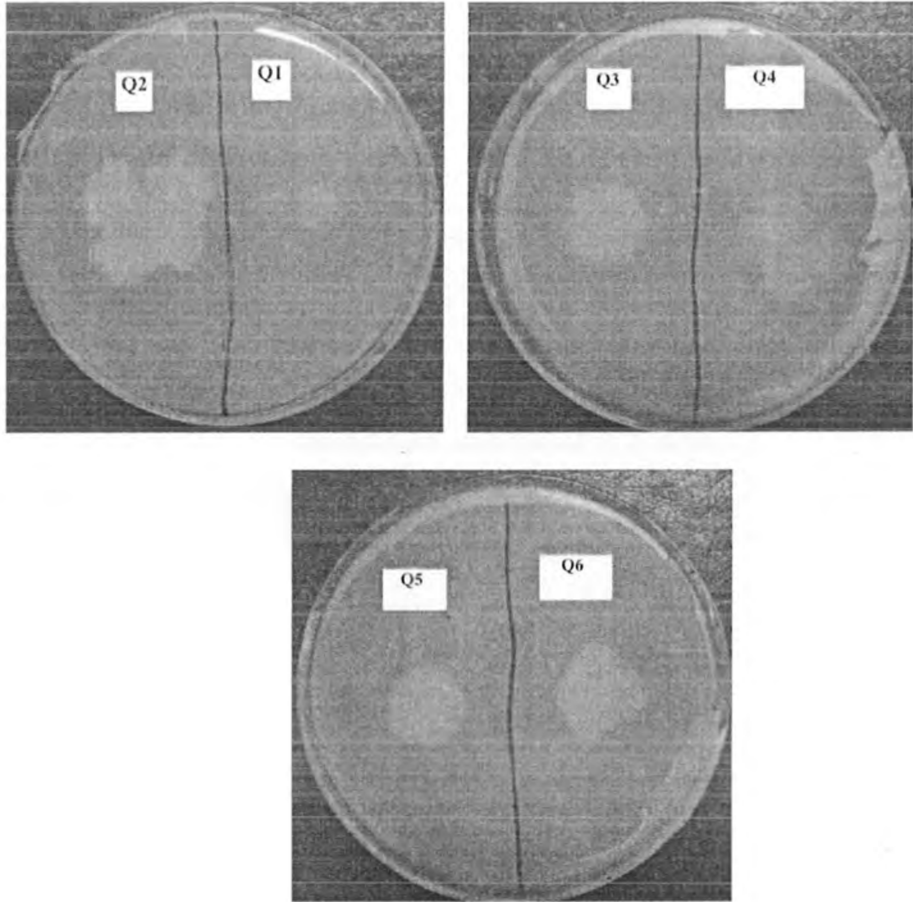


Plate 5 Protease

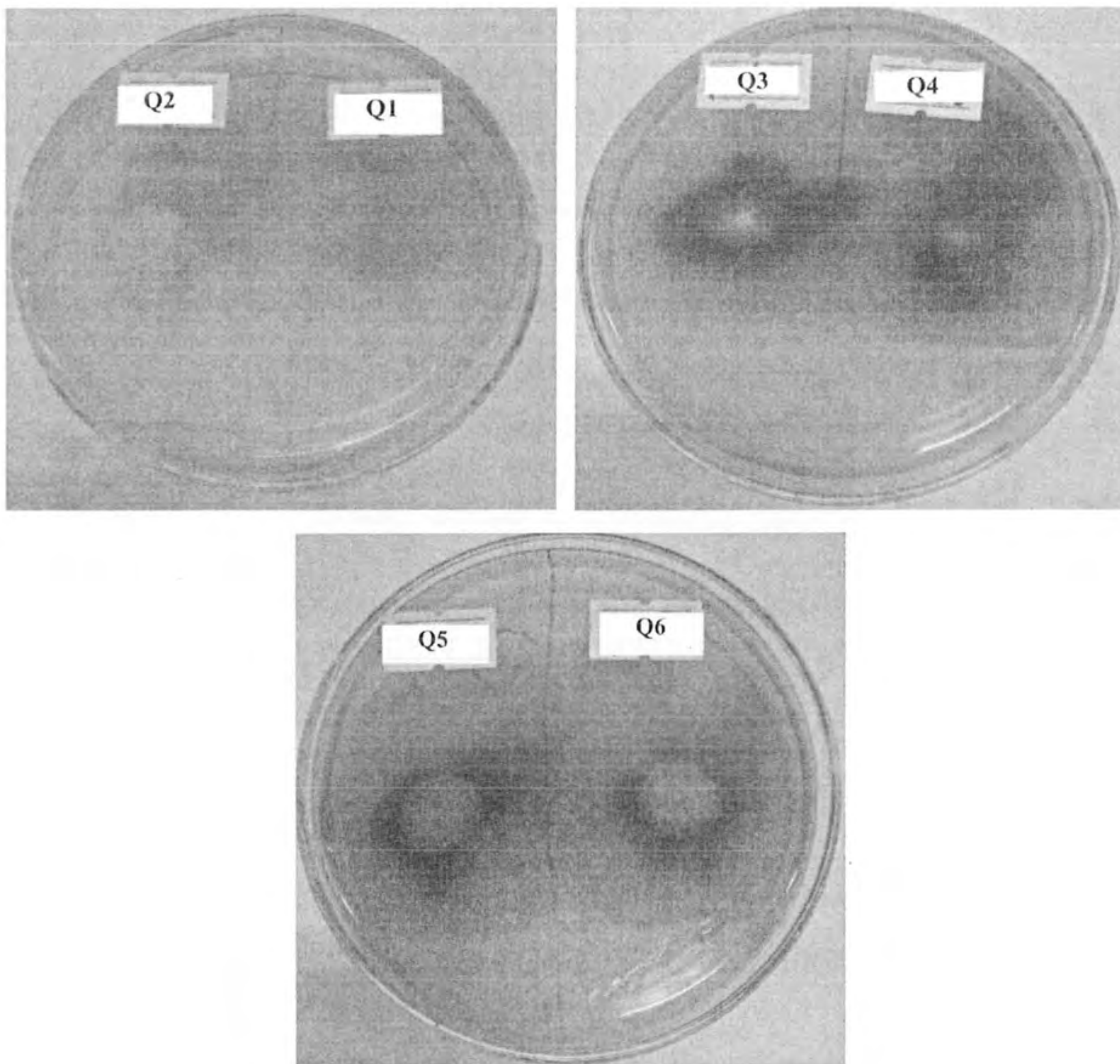


Plate 6 Pectinase

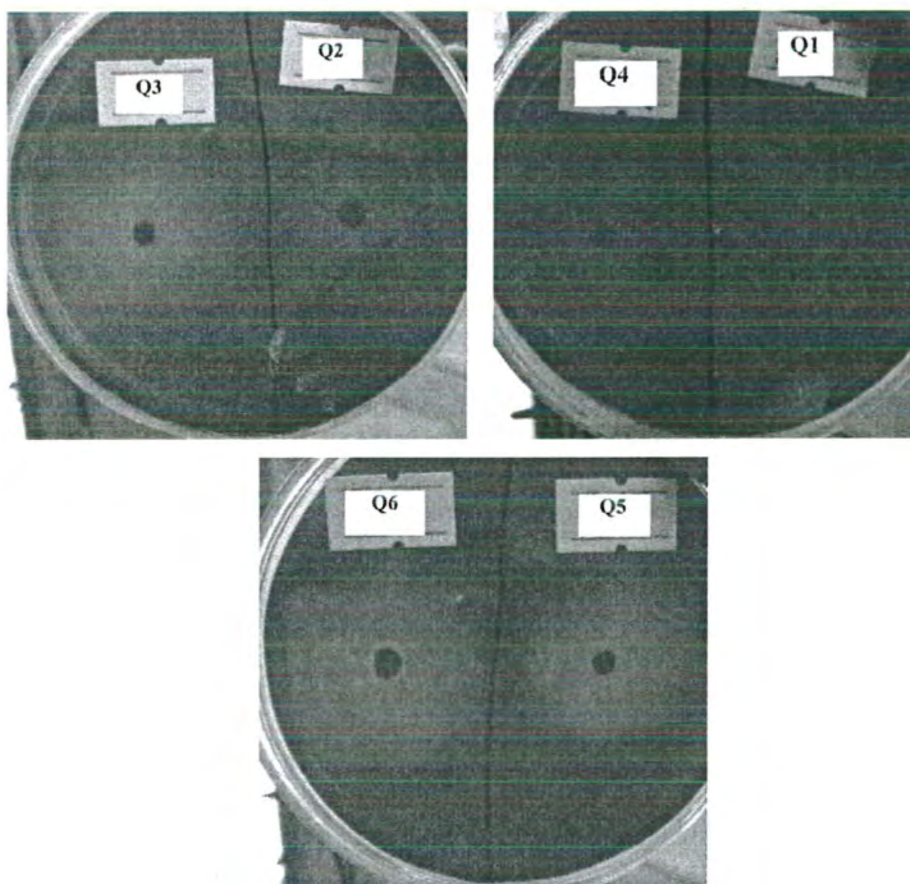


Plate 7 Cellulase

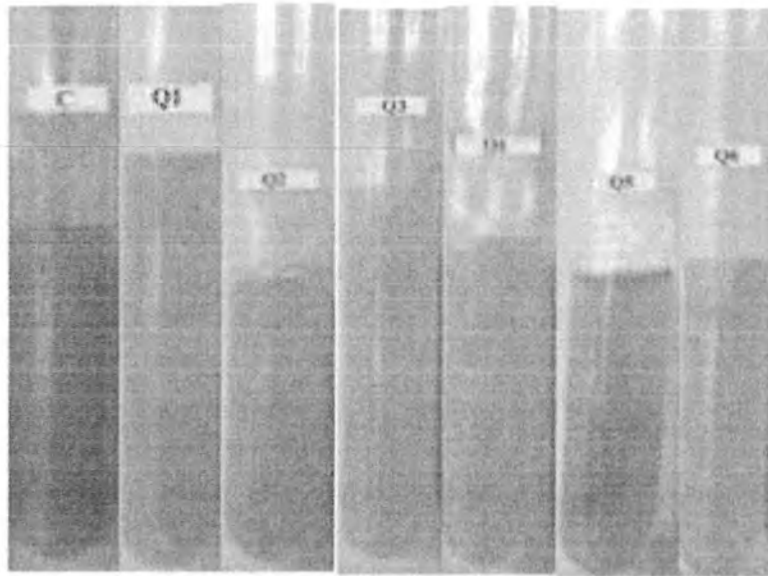


Plate 8 Brown to yellow colour indicates positive result for Ammonia production.

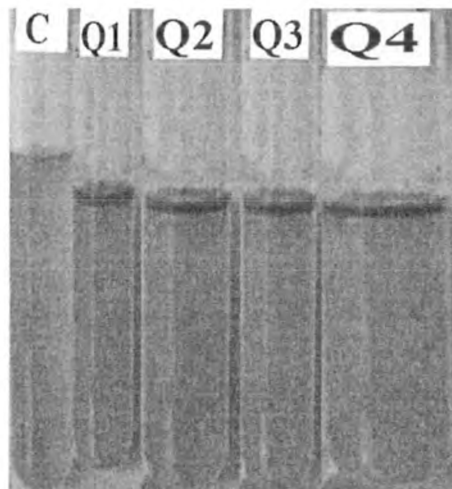
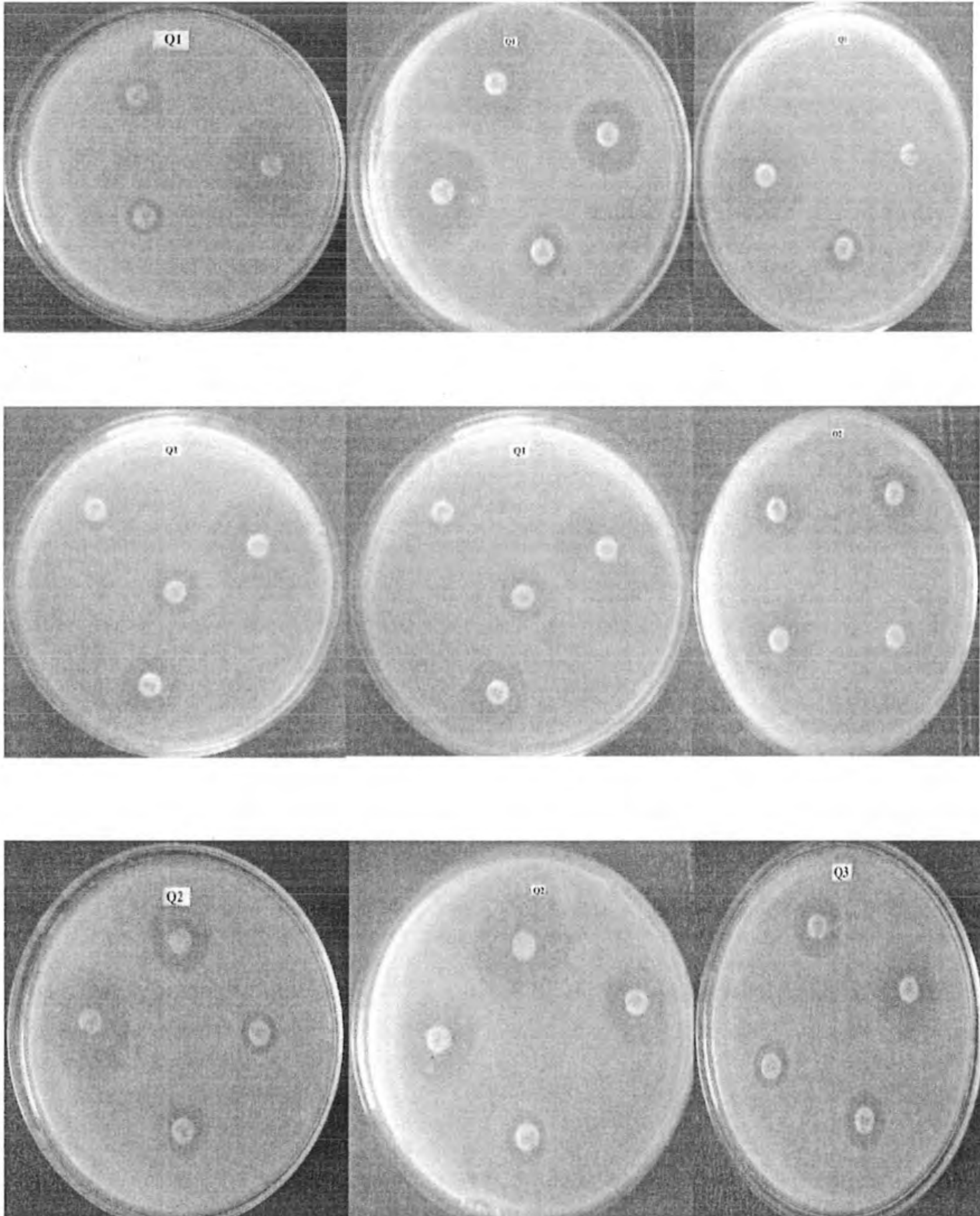
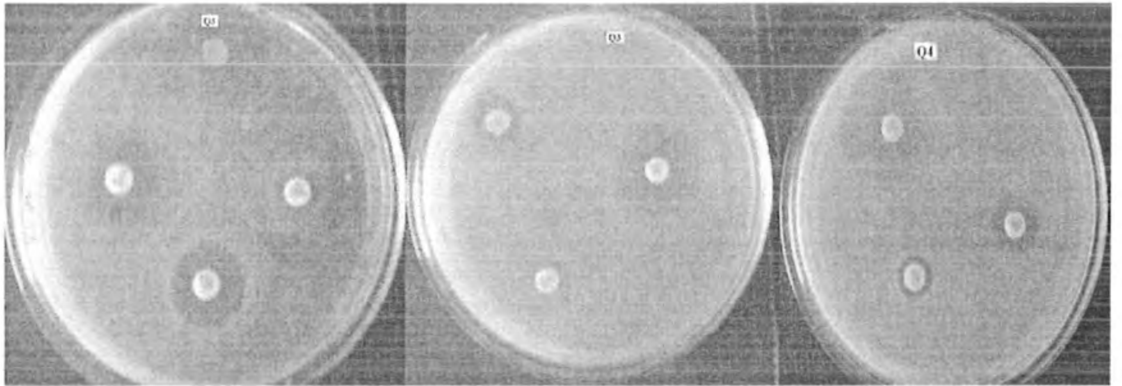
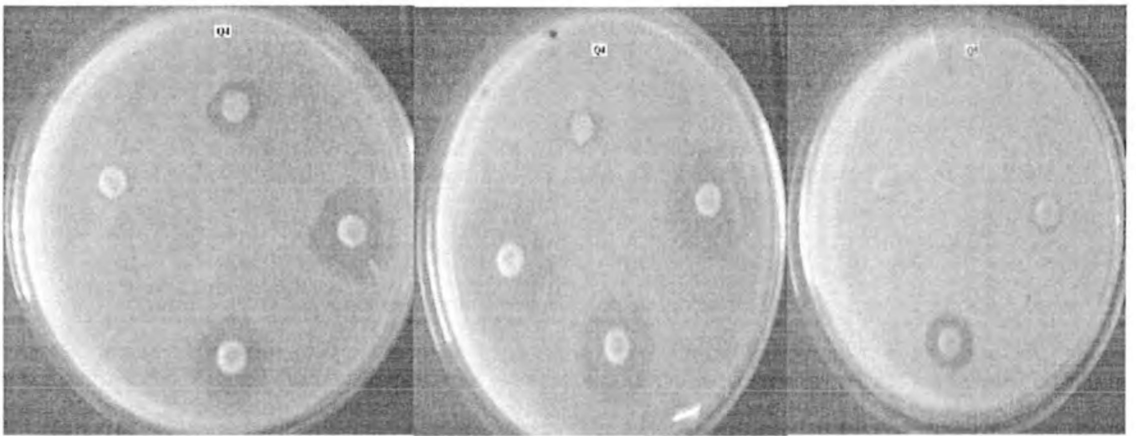
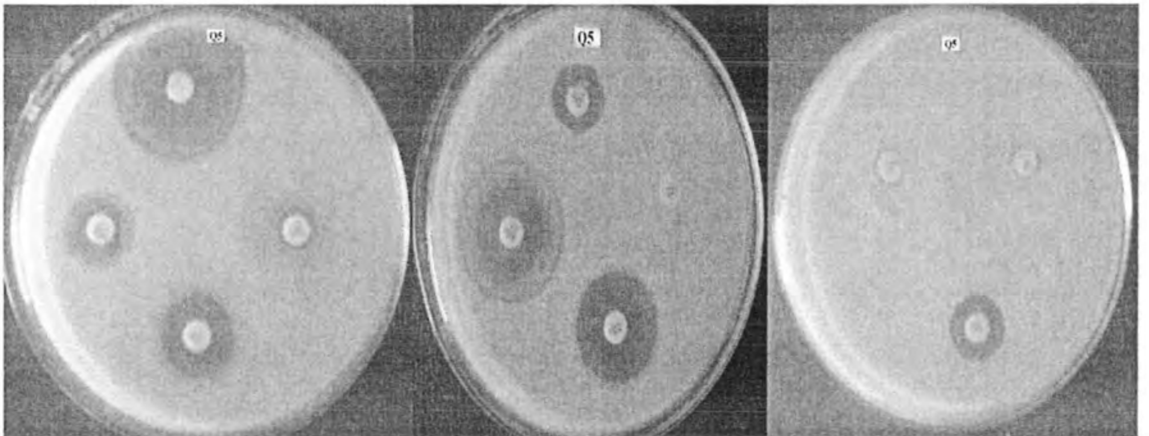


Plate 9 Formation of pink colour ring indicates IAA formation


Q1
Q2
Q3







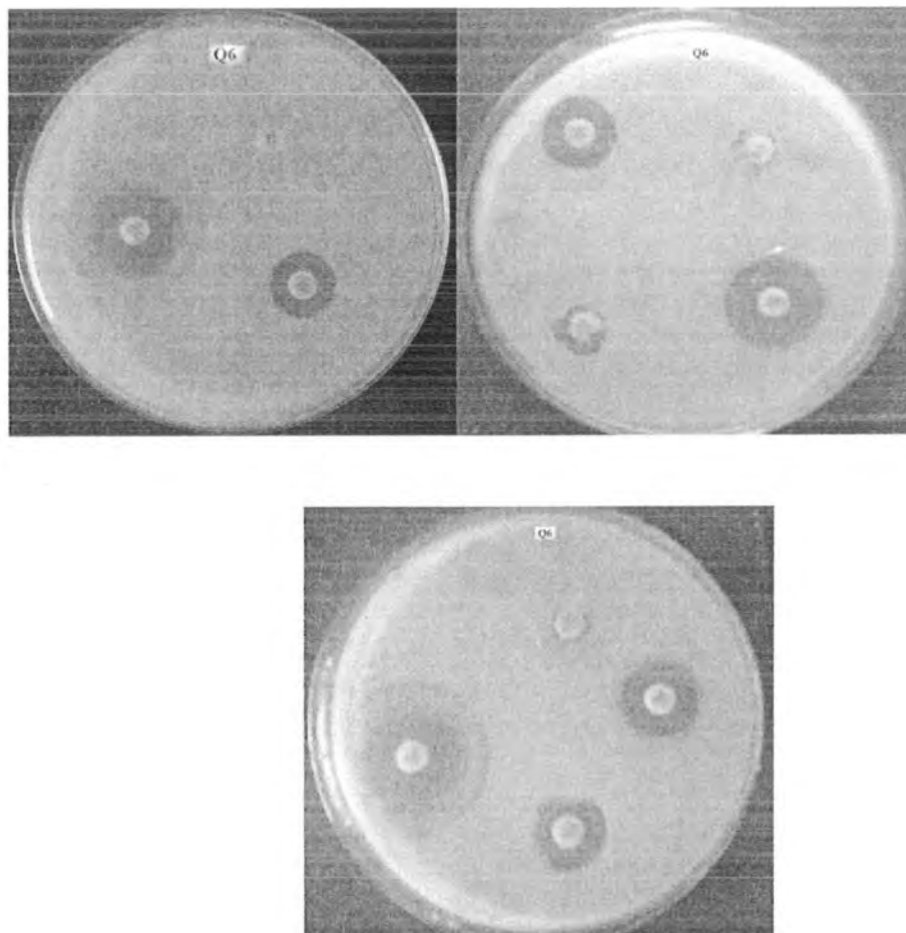


Plate 10 Different plates of antibiotics with zone of inhibition Norfloxacin, Tobramycin, Cloxacillin, Piperacillin, Carbenicillin, Ceftazidime, Ciprofloxacin, Amikacin, Colistin, Nitrofurantoin, Cefoxitin

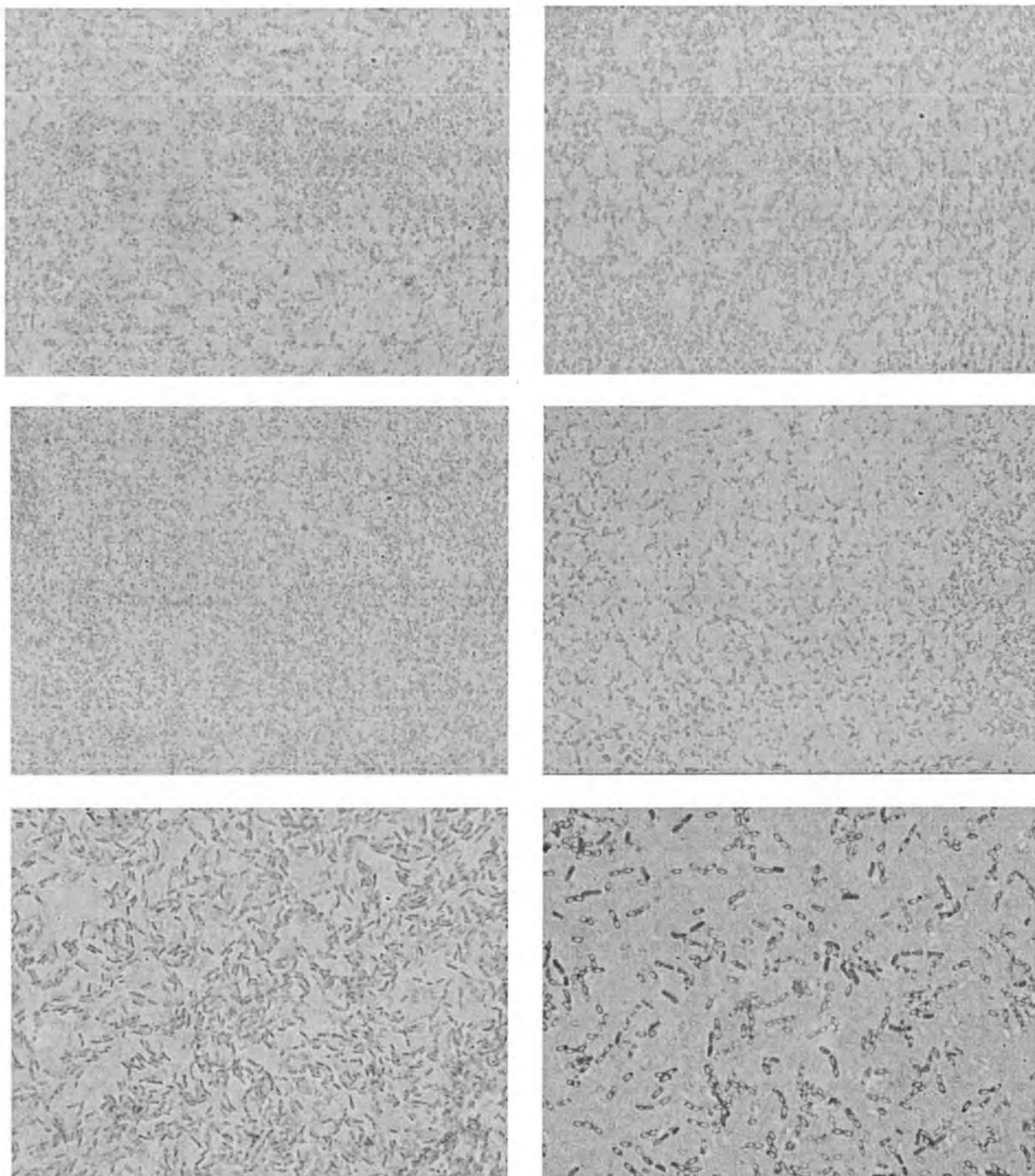
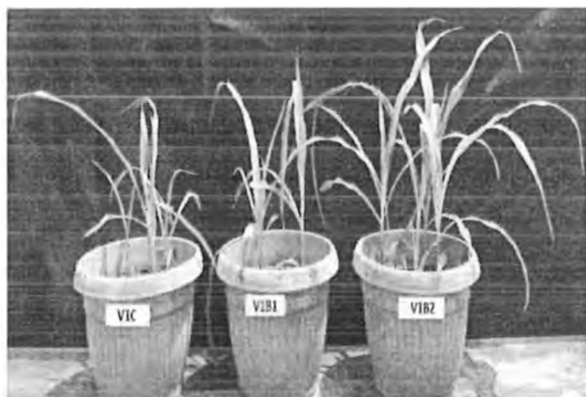
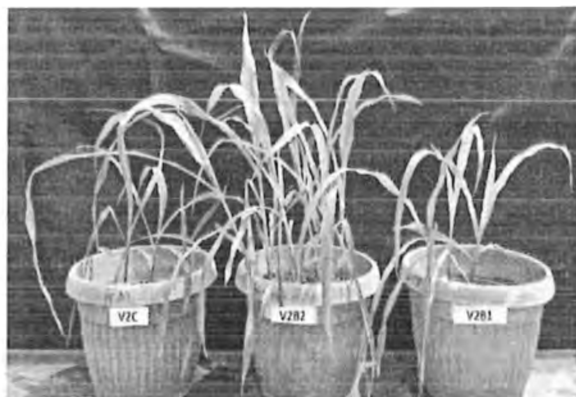
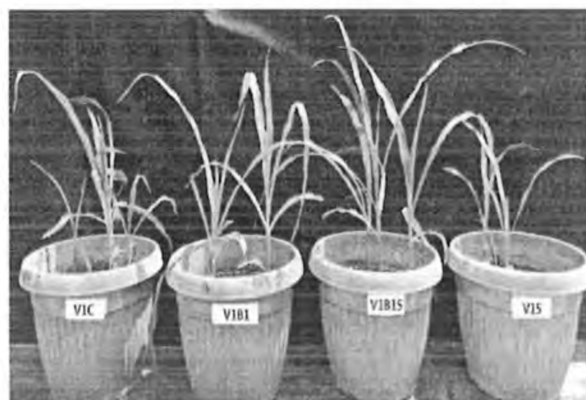
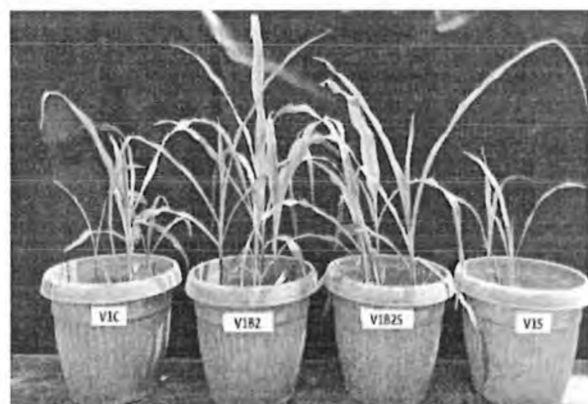


Plate 11 Microscopy of bacterial strains; a): Q1, b): Q2, c): Q3, d): Q4, e): Q5, f): Q6









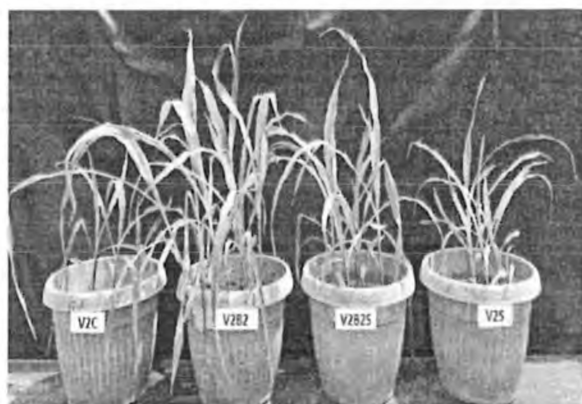


Plate 12 Effects of PGPB on plant growth of *Zea mays* L. before giving salinity stress (250mM).

V1 (EV1097); V2 (Haq Nawaz Gold); B1(Q2); B2(Q4); S (Salinity Stress)

Plate 14 Effect of salinity stress on maize plants with and without PGPB.

V2 (Haq Nawaz Gold); B1(Q2); B2(Q4); S (Salinity Stress)

Plate 13 Effect of salinity stress on maize plants with and without PGPB.

V1 (EV1097); B1(Q2); B2(Q4); S(Salinity Stress)