Effect of bacterial polysaccharides in growth regulation of maize (*Zea mays* L.)

A thesis submited in partial fulfillment of the requirement for the degree of M. Phil in Plant sciences

by

Hafsa Naseem

Reg. No: 02041111019

M.Phil Plant physiology

Department Of Plant Sciences,

Quaid-i-Azam University, Islamabad

2013

Effect of bacterial polysaccharides on growth regulation of maize (*Zea mays* L.)

BY

Hafsa Naseem

Reg. No: 02041111019

M.Phil. Plant physiology

Department Of Plant Sciences,

Quaid-i-Azam University, Islamabad

2013

Oh, Allah Pak open our Eyes,
To see what is beautiful,
Our minds to know what is true.

DEDICATED TO

My Most Beloved MOTHER
&
Affectionate FATHER

Ashfaq Ahmed

Who taught me the first word to speak,
first alphabet to write & first step to take,
Who are always in my mind & in my heart

To my dearest, caring and loving brothers

M. Bilal & M. Hasham

&

To my world, my sisters

DECLARATION

By submitting this dissertation, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless the extent explicitly otherwise stated) and I have not previously in its entirety or in part submitted it for any qualification.

Hafsa Naseem

CERTIFICATE

This is to certify that this dissertation entitled **"Role of bacterial polysaccharides on growth regulation of maize (*Zea mays* L.)"** submitted by **Miss. Hafsa Naseem** is accepted in its present form by the Department of Plant Sciences, Faculty of Biological sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the Degree of Master of Philosophy in plant physiology.

Supervisor

Prof. Dr. Asghari Bano
Chairperson
Department of Plant Sciences
Quaid-i-Azam University
Islamabad

External Examiner

Prof. Dr. Manzoor H. Soomro
Chairman
Pakistan Science Foundation
Islamabad Pakistan

Chairperson

Prof. Dr. Asghari Bano
Chairperson
Department of Plant Sciences
Quaid-i-Azam University
Islamabad

Date: 01-04-13

CONTENTS

List of Abbreviations

Abbreviations	Full Name
BSA	Bovine Serum Albumin
Ca	Calcium
$CaSO_4.2H_2O$	Gypsum
$CaCl_2.2H_2O$	Calcium Chloride
CAT	Catalase
Cl^{-1}	Chloride
$CuSO_4$	Copper Sulphate
°C	Centigrade
C_2H_5OH	Ethanol
EPS	Exopolysaccharide
EDTA	Ethylene diamine tetra acetate
g	Gram
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
HNO_3-$HClO_4$	Nitric chloric acid-Perchloric acid
H_3BO_3	Boric acid
HCO_3^{-1}	Bicarbonate
H_2SO_4	Sulphuric acid
K	Potassium
Kb	Kilo base
KCl	Potassium Chloride
K_2HPO_4	Di Potassium hydrogen phosphate
KH_2PO_4	Potassium di hydrogen phosphate
K_2CrO_4	Potassium dichromate
KDa	Kilo Dalton

ml	Milliliters
$MgSO_4$	Magnesium Sulphate
$MnSO_4$	Manganese Sulphate
nm	Nanometer
Na	Sodium
Na_2CO_3	Sodium Carbonate
NaCl	Sodium chloride
NaOH	Sodium Hydroxide
NBT	Nitrobenzene Tetrazolium
NH_4NO_3	AmonniumNittrate
OD	Optical density
PBS	Phosphate Buffer Saline
PSA	Phenol-Sulfuric acid
P	Phosphorous
POD	Peroxidase
RAPD	Randomly amplified polymorphic DNA
Rpm	Revolution per minute
SOD	Superoxide dismutase
µg ml^{-1}	Microgram per millilitre
ml	Microlitre
µS/cm	Microsiemen per centimeter
%	Percentage

LIST OF TABLES

LIST OF FIGURES

In the name of ALLAH,
The Beneficent, the Merciful

ACKNOWLEDGEMENT

All praise to Allah Almighty, who bestowed upon me the rigor and courage to completemy research work successfully. Special praise to Holy Prophet Muhammad (Peace Be UponHim), who declared it an obligatory duty of every Muslim to seek and acquire knowledge.

I am deeply grateful to my supervisor, Professor Dr. Asghari Bano, Dean Faculty ofBiological Sciences as her knowledge, technical expertise, and guidance enabled me tocomplete this challenging task. She remained a source of inspiration throughout theresearch and with her brilliance and empirical approach; she will remain my role modelfor rest of my life.

The staff members of Plant sciences were also very facilitating. They always provided me help andrequired resources. Especially thanks to Dr. Muhammadi, to give me insight of this research approach.

I have no words to express my gratitude for my family especially my parents. Withouttheir love, care, encouragement and support I would not be able to achieve any goal inmy life. The concern and encouragement of my brothers, Muhammad Bilal and Muhammad Hasham love and care of my sisters Sara, Iqra and Khansa that remained the source of motivation throughout my research.

I am also grateful to my uncle Mushtaq Ahmed and Riyaz Ahmed, my first cousins Usman, Abdul Rehman,, Nadia, Hadia, Maria, Sadia, Fayaz Ahmed, my aunt, Bhabi Shamim Akhtar for their sincere support duringwriting this manuscript.

My lab fellows Razia, Afeefa, Farah, Naeem Khan, Almas, Sadia, Tahira, Saba,Faiz ul Saba, Asad, Nasrullah Khan, Shehzad, M. Naeem were always very helpful and supportive.

I am especially thankful to my seniors Miss Humaira, Miss uzma, Miss Asia, Miss Rabia, and Miss Irum Naz, Mr. Faizan, Mr. Samiullah, Mr. Asim, Mr. Tanoor and Mr. Nadeem, for their sincere helpand moral support.

I would also like to thank Dr. Tariq from Plant biochemistry & molecular biology lab,Dr. Samad from Plant Genomic Lab and Dr. Tanveer from general lab of biochemistry, for provisionof equipment facility.

Hafsa Naseem

ABSTRACT

Drought is one of the major abiotic stress affecting crop yields. Bacterial populations of stressed soils are well adapted to stress and can be implicated as bio inoculants to impart tolerance to crops grown in stressed ecosystems. Production of exopolysaccharides (EPS) is a major mechanism in bacteria to cope with drought stress.

The present study deals with the isolation and characterization of exopolysaccharides produced by the PGPR from arid (Ghotki Sindh) and semiarid (Kallar saydan) regions of Pakistan, and to investigate the drought tolerance potential of these PGPR on maize when used as bio inoculant alone and in combination to their respective EPS. Three bacterial strains S1 (***Proteus penneri***), S2 (***Pseudomonas aeruginosa)*** and S3 (***Alcaligenes faecalis)*** were selected as exopolysaccharides (EPS)producing bacteria on the basis of mucoid colony formation. All these strains were gram negative, motile and positive for catalase. Strain S1 was positive for oxidase and was phosphate solubilizer, while S2 and S3 were negative. The isolated strains were sequenced by using 16SrRNA. Biochemical characterization (solubility; quantification of total sugar, protein and uronic acid; and emulsification activity) and Attenuated Total Reflection Fourier Transformed Infra-Red spectroscopy ATR-FTIR of exopolysaccharides was done for determining functional groups of EPS.

Drought stress induced by withholding water supply had significant adverse effects on growth of maize seedlings. Seed bacterization of maize with EPS producing bacterial strainsin combination to their respective EPS improved soil moisture contents, plant biomass, root, and shoot lengthand leaf area. The inoculated plants showed higher levels of relative water content, protein and sugars under drought stress. As compared to un-inoculated seedlings, inoculated seedlings showed significantly lower activities of proline and antioxidant enzymes, superoxide dismutase (SOD) peroxidase (POD) and catalase (CAT) under drought stress.

The strain S2 (***Pseudomonas aeruginosa)*** isolated from semi-arid region of Kallar Saydan was most potent PGPR under drought stress. In all cases inoculum used in combination to their respective exopolysaccharides showed greater potential to drought tolerance as compared to inoculums alone. So exopolysaccharides production is the main mechanism and slower the desiccation of rhizosphere soil during water shortage.

Exopolysaccharides keep the soil moist and helps the plants to cope with long period of drought. Inoculation studies of maize with EPS producing bacteria under drought stress improve the soil moisture content and relative water content RWC of leaves.

INTRODUCTION

1.1. Exopolysaccharides producing bacteria

Rhizosphere-inhabiting bacteria are extensively studied for their role in agriculture, and called plant growth promoting rhizobacteria (PGPR), because they have very good effects on overall health of the plant. Plant and microbe association attain much importance in recent decades (Afzal *et al.* 2008). They mainly belong to ***Pseudomonas*** and ***Bacillus*** genera. Many of the rhizobia are known to produce large amounts of EPS material both in the rhizosphere and when grown in pure culture. While EPS production by ***Rhizobium*** spp. has been fairly well-documented, the specific physiological role of rhizobial EPS in vitro is unknown (Ann Staudt, 2009). Many fluorescent ***Pseudomonas*** strains e.g. (***Pseudomonas aeruginosa***) by colonizing the rhizosphere soil protect the roots through the production of antibiotics and protect the plants by prevention of microbial infections (Jenni *et al.* 1989). There is a need for in-depth studies on the effectiveness of Pseudomonas spp. as PGPR across the globe.

A number of dairy bacteria are also capable of synthesizing exopolysaccharides (Marshall *et al.* 2001). It is also believed that exopolysaccharides production is the ability of lactic acid bacteria (Cerning, 1990; Sikkema *et al.* 1998; Cerning *et al.* 1999; De Vuyst *et al.* 1998; Ricciard *et al.* 2000). List of bacterial species that produce exopolysaccharides.

Bacterial strains	**References**
L. lactis subsp. *Cremoris* H414	Gruter *et al.* (1992)
*L. lacti s*subsp. *Cremoris* SBT0495	Nakajima *et al.* (1992)
L. lactis subsp. *Cremoris* B40	Van Castern *et al.* (1998)

L. lactis subsp. *Cremoris* B39	Van Castern *et al.* (2000b)
L. lactis subsp. *Cremoris* B891	Van Castern *et al.* (2000a)
L. lactis subsp. *Cremoris* ARH74	Yang *et al.* (1999)
S. thermophilus CNCMI 733	Doco *et al.* (1990)
S. thermophilus EU21	Marshal *et al.* (2001b)
*S. thermophilus Sfi*6	Stingele *et al.* (1996)
S. thermophilus Sfi12	Lemoine *et al.* (1997)
S. thermophilus Sfi39	Lemoine *et al.* (1997)
S. thermophilus Sfi20	Navarini *et al.* (2001)
S. thermophilus OR901	Bubb *et al* (1997)
S. thermophilus Rs	Feber, zoon *et al.* (1998)
*S. thermophilus*Sts	Feber*et al.* (1998)
S. thermophilus MR-1C	Low *et al.* (1998)
S. thermophilus EU20	Marshal *et al.* (2001a)
S. thermophilus S3	Feber*et al.* (2001b)
S. thermophilus SY 89, SY 102	Marshal *et al.* (2001b)
S. thermophilus Sc136	Vincent *et al.* (2001)
*Lb. Acidophilus*LMG9433	Robijin *et al.* (1996a)
Lb. delbruekii subsp. *Bulgaricusrr*	Gruter *et al.* (1993)

Lb. delbruekii subsp. *Bulgaricus* 291	Feber *et al.* (2001a)
Lb. helvaticus TY1-2	Yamamoto *et al.* (1994)
Lb. helvaticus NCDO 766	Robijin*et al.* (1995b)
Lb. helvaticus TN-4	Yamamoto *et al.* (1995)
Lb. helvaticus Lh59	Stingele *et al.* (1997)
Lb. helvaticus 2091	Staaf *et al.* (1996)
Lb. helvaticus Lb161	Staaf *et al.* (2000)
Lb. helvaticus K16	Yang *et al.* (2000)
Lb. paracasie 34-1	Robijin *et al.* (1996b)
Lb. rhamnosus C83	Vanhaverbeke *et al.* (1998)
Lb. sakei (formerly *Lb. sake)* 0-1	Robijin *et al.* (1995a)
*Lb.*subsp. G-77	Due*nas-Chasco *et al.* (1998)

1.2. Bacterial Exopolysaccharides

The EPS usually designated for exopolysaccharides, which is the important material for biofilm formation by bacteria. This name of exopolysaccharides was proposed by Sutherland in (1972), he provides the general term for different types of exopolysaccharides (EPS) found outside the bacterial cell wall. Exopolysaccharides (EPS) are most important part of extracellular matrix that often represents 40-95% (Flemming *et al.* 2001) of bacterial weight. Bacteria produce exopolysaccharides (EPS) in two forms: (1) slime exopolysaccharides (EPS) and (2) capsular exopolysaccharides (EPS) (Vanhooren *et al.* 1998). Exopolysaccharides (EPS) are found in a wide variety of complex structures (Kumon *et al.* 1994). Exopolysaccharides are heterogeneous mixture

composed of: (1) polysaccharides (2) proteins (3) nucleic acids and (4) lipids (Wingender *et al.* 1999). Exopolysaccharides are long chain molecules, having molecular weight of 10-30 KDa (Kumar *et al.* 2007). Production of exopolysaccharides by bacteria is favored by high carbon and low nitrogen ratio (Kimmel *et al.* 1998). Exopolysaccharides are water soluble polymers composed of monomers of wide range (Sutherland, 1977). Some exopolysaccharides are neutral, but most of them are polyionic either due to presence of uronic acid (d-galacturonic acid, d-glucuronic acid and d-mannuronic acid) or due to prensence of ketal linked pyruvate group, inorganic parts like sulphate and phosphate may also cause polyionic nature of exopolysaccharides (Sutherland *et al* 1990). Bacterial exopolysaccharides are often acidic polymers due to the presence of some functional groups like (hydroxyl group, carboxyl group and phosphoric acid) that gives the exopolysaccharides high affinity for metal ions (Mittleman and Geesey, 1985). Exopolysaccharides are organic macromolecules formed by the presence of similar building blocks that polymerize as repeated units. The major organic parts are carbohydrates and proteins (Nielsen and Jahn, 1999).

Important role of exopolysaccharides exhibited are (1) Protective (2) surface attachment (3) biofilm formation (4) microbial aggregation (5) plant-microbe interaction and (6) bioremediation (Manca*et al.* 1985). Some physical and chemical properties of exopolysaccharides are useful in industries for stabilizing, thickening, coagulating, gelling, suspending, film forming and water retention capability in different industries like detergents, textile, paper, paints, adhesive, beverages and food (Sutherland, 1996). Also used in petroleum industry, mining industry and oil recovery purposes (Sutherland, 1998). Due to enzymatic activities of exopolysaccharides, they help in heavy metal transformation and degradation of organic recalcitrant (Hullebusch *et al.* 2003; Pal and Paul, 2008). Exopolysaccharides of bacteria are hydrated compounds with ~ 97% of water in polymer matrix due to which EPS provide protection against desiccation (Wingender*et al.* 1999; Hunter *et al.* 2005; Bhasker and Bhosle, 2006). Bacterial exopolysaccharides also attracted the food industry since 1980s, where EPS used as food additives in a large number of products and in fermentation (Robert *et al.* 1995; Sutherland, 1998). Some exopolysaccharides are also beneficial for health as they assist

in immune stimulation (Oda *et al.* 1982; Nakajima *et al.* 1995) also helpful against ulcer (Nagaoka *et al.* 1994).

Dairy bacteria produce exopolysaccharides that can be classified as: - (De Vuyst *et al.* 1998).

Group of EPS	Subgroups of EPS
Homopolysaccharides	α-D-glucans, i.e. dextran β-D-glucans fructans polygalactans
Hetropolysaccharides	Produced by ***Lactobacillus delbruekii*** subsp. ***Bulgaricus*** and ***Streptococcus thermophilus*** with complex chemical structures.

Keeping in view of the commercial importance of the EPS, the present investigation deals with the isolation of exopolysaccharides, and their chemical and physical characterization isolated from the drought stressed areas.

1.3. Isolation and characterization of EPS

There are many methods developed for the extraction of exopolysaccharides, but different methods are used in different conditions. Some requirements that are important for good method of EPS extraction include (1) minimum cell lysis, (2) no disruption in EPS characteristics and (3) ability to extract all the polysaccharides (Nielsen *et al.* 1999).

Different methods that have been reported are divided into 3 categories (1) physical method, (2) chemical method and (3) combination of physical and chemical (Nielsen *et al.*1999). Physical method includes the extraction of exopolysaccharides by

using physical forces i.e. by centrifugation, filtration, dialysis and sonication technique (King *et al.* 1990), also by cation exchange (Frolund*et al.* 1996a) and by heating (Karapanagiotis*et al.* 1989; Morgan *et al.* 1990). While chemical method includes the use of different chemicals i.e. formaldehyde (H_2CO), ethylenediaminetetraacetic acid (EDTA), sodium hydroxide (NaOH) and ethanol (C_2H_5OH) (Liu and Fang, 2002). Chemical methods give more yield than physical methods but the chances of contamination and cell lysis are greater in chemical method (Nielsen *et al.* 1999). In physical method although yield was less than chemical method but chances of contamination and cell lysis are small which make it effective method (Comte *et al.* 2006). But the combination of both physical and chemical methods makes it more reproducible and effective to get higher yield without any contamination and cell lysis (Nielsen *et al.* 1999).

1.4. ATR-FTIR spectroscopy

Attenuated Total Reflection Fourier Transformed Infra-Red spectroscopy ATR-FTIR spectroscopy gives us information at molecular level for inorganic and organic constituents of bacterial exopolysaccharides. It permits to investigate the functional groups in close proximity that are dipolar. ATR-FTIR also shows dissociation of protons of the functional groups and random coil confirmation at different ph i.e. R-helical protein confirmation at pH < 2.6 and unordered confirmation at pH > 6 (Braun *et al.* 1987). ATR-FTIR spectroscopy is in-situ, non-invasive method of spectroscopy that helps in the investigation of exopolysaccharides constituents and functional group chemistry and conformational changes without disturbing intermolecular associations of the constituents e.g. protein-polysaccharide association (Anselm *et al.* 2004).

The most important advantage is its use in biological studies of almost any biological material.

- In FTIR spectroscopy sample is required in very small amount.
- This technique of FTIR spectroscopy is a very sensitive and rapid technique and easy to use

- Since the computer is already used for Fourier-transform, so it is easy to perform many scans to improve the signal- to-noise ratio
- Instruments used for FTIR spectroscopy are not so expensive as compared to NMR, X-Ray and CD spectroscopic instruments; also operation of its equipments is simple.
- Interpretation of the spectra obtained can also be learned easily, it's not so difficult
- Kinetic and time resolved studies are also possible (Mantcsch *et al.* 1986; Mendelson *et al.* 1986; and Haris *et al.* 1999).

1.5. Drought stress

Drought stress effect the various physiological and biochemical process i.e. photosynthesis, respiration, translocation, uptake of ions, carbohydrate and nutrient metabolism and growth promoters which reduces growth of plant (Jaleel *et al.* 2008; Farooq *et al.* 2008). Reactions of plants to drought stress at various organizational levels are different that depends on intensity of stress, time duration, plant species and its stage of growth (Chaves *et al.* 2002; Jaleel *et al.* 2008b). It is very important to understand the response of plant to drought stress, also to make stress tolerant crops (Reddy *et al.* 2004; Zhao *et al.* 2008). About one-third of the world's arable land effected due to shortage of water and crop production suffer from drought stress (Kramer *et al.* 1980). As integral part water plays an important role in plants growth initiation and maintainance of other process involved in development and finally the economy of country effected. During a long period of drought (1998-99) about 48.8mm rainfall that allowed only 4.8 t/ha grain yield.

1.6. Maize (*Zea mays L.*)

Maize is one of the earning crops in the world and also most versatile in the world. The grain of maize is used for corn starch, corn syrup, corn flakes, corn oil dextrose, grain cake, lactic acid and acetone preparation used by textile, foundry, fermentation and food industries. Due to development of poultry and livestock industry, the consumption of

maize also increased in feed. Humans used it in diet in both fresh and processed form (Hallauer *et al.* 1988). The global demand of maize will increase from 526-784 million tons from 1993-2020 (Rosegrant *et al.* 1999). Maize gets an important position in the cropping system of Pakistan. In Pakistan after wheat and rice, maize is at third position in grain production. It is grown in almost all provinces of Pakistan, but most familiar in maize production are KPK and Punjab. Maize is the source of food not only for humans but also for livestock and poultry and used as a good forage crop. It is grown on an area of 87,000,000 ha with average yield of 12.23 million bushels (FAO, 2008). In Pakistan the average yield of maize is very low as compared to developed countries (Anonymous *et al.* 2000). The total area of world under the maize crop was 147.6 million ha, have a grain production of 701.3 million metric tons and yield of 4752 kg/ha during 2006-07. World area under maize crop was 147.6 million hectare with a grain production of 701.3 million metric tons and overall yield of 4752 kg per hectare during 2006-07. In Pakistan maize was grown on 1051.7 thousand hectares with annual production of 3604.4 thousand tones and average yield of 3427 kg per hectare. Maize crop is extensively grown as grain for humans and fodder for livestock consumption. Maize is a cereal crop, C4 species and high yielding. It uses moisture more efficiently. Maize uses 500-800mm of water during its life cycle that is 80-110 days (Critchley *et al.* 1991). Requirement of water for maize is (135mm/month) at the time of seedlings that is (4.5mm/day) and this requirements of water increases up to (195 mm/month) that is (6.5mm/day) during hot and windy conditions (Jamieson *et al.* 1995). To cope with the shortage of water drought tolerant maize varieties are reliable options. Some physiological parameters can also be used as criteria for selection of maize varieties that should be simple, measureable heritable and related to plant growth and yield (Richads *et al.* 1978). Agaiti-2002 had higher germination of 50.22 and 49.90%, respectively after 36 h as compared to the rest of cultivars. Maize is not a reliable crop in dry land conditions, although it has origin in semi-arid areas (Arnon *et al.*1972). In Pakistan it is grown twice a year (in spring and autumn). Apparently it is more tolerant to drought stress in its early stages of growth than fully developed stage (Dhillon *et al.* 1995). It has been found that drought stress reduce the photosynthesis of plant, leaf area, leaf chlorophyll and grain yield of maize (Jun-Chen

et al. 1996). Nitrogen requirement of maize plant is 9-11 kg for the production of 1t biomass (Annuare *et al.* 1995).

AIMS AND OBJECTIVES

Objectives of present study include:

- Isolation and characterization of efficient EPS producing bacteria from arid (Gotki Sindh) and semi-arid (Kallar Sayedan) areas of Pakistan
- Isolation and quantification of exopolysaccharides
- ATR-FTIR spectroscopy of exopolysaccharides
- Analysis of different growth and physiological parameters of maize grown under drought stress with inoculation of EPS producing bacteria alone and in combination of their respective exopolysaccharides.

REVIEW OF LITERATURE

2.1. EPS producing bacterial interaction with plant roots

Previously documented reports indicate that rhizospheric bacteria i.e. ***Pseudomonas, Azospirillum*** and ***Rhizobium*** that form association with plant roots are found in the mucigel of the root or may be found inside the extra cellular matrix self-produced by bacteria (Fujishige *et al.* 2006).Attachment of bacteria to the plant root is also a first step in biofilm formation by bacteria on roots of the plants. Biofilms formed by the bacteria around the roots are made up of bacterial populations or bacterial communities that encased inside the polymeric extracellular matrix (e.g. bacterial exopolysaccharides) formed by bacteria itself, they adhered to the external surfaces that contain sufficient moisture. This biofilm formation strategy of bacteria in real life is common in bacterial life (Costern *et al.* 1995). In attachment process first phase is a reversible, weak, and unspecific binding phase. In this phase 3 components are involved (1) lectin proteins, (2) Ca^{+2}-binding protein called rhicadhesin and (3) bacterial polysaccharides. Lectin proteins of legumes are present at the tip of root hairs recognized the specific carbohydrates that are present on surface of bacteria and bind to them (Matthysse *et al.* 1998). Generally it is accepted that different plant factors that are involved in the process of attachment the most important are plant lectins that act as receptors for the attachment of bacterial exopolysaccharides (Ridge *et al.* 1998; Hirsch *et al.* 1999; and Rudiger *et al.* 2001).

There are different polysaccharides in which at least four different types are known to play a role in the formation of symbiosis association of bacteria to the plants these four types are (1) cyclic glucans, found in the periplasmic space of bacteria and also found in culture supernatants; (2) bacterial exopolysaccharides, form weak association with outer membranes and found in the extracellular medium; (3) capsular polysaccharides, that are bound to the outer membranes and (4) lipopolysaccharides, are the structural components of the outer membranes. It is also believed that bacterial surface exopolysaccharides also play a key role in the in the attachment of agro bacterium to the plant cell.

2.2. Survival of EPS producing bacteria under drought stress

Many bacterial organisms and plants have an ability to survive in the long period of desiccation up to the extent of 99% water loss of cell (Welch *et al.* 2000). Some exopolysaccharides (EPS) producing bacteria like Pseudomonas have ability to survive even under drought stress due to production of their exopolysaccharides (EPS) (Sandhya*et al* 2009 a, b), these exopolysaccharides (EPS) protect these bacteria from desiccation in drought stress by enhancing the water retention and by regulation of organic carbon source's diffusion (Hepper*et al.* 1975; Roberson *et al.* 1992; Chenu *et al.* 1993; Chenu and Roberson. 1996). It might be possible in the plants to tolerate the drought stress by increasing exopolysaccharides producing bacterial population density in the root zone of plants. Most of the strains of rhizobia that form nodulation to important cereal crops are very sensitive to desiccation of soil (Osang *et al.* 1982). Inoculation of ***Azospirillum*** strain has alleviation effect for low moisture content of soil on wheat plants under drought stress (El-Komy *et al.* 2003). Specific root colonization has growth promotion effect on wheat and maize seedlings that is more evident under low moisture content of soil (Alvarez *et al.* 1996). In association of plant and ***Azospirillum*** many biochemical changes which results in growth promotion and tolerance of plant in drought stress when soil moisture content is low (Pereyra *et al.* 2006). The phenomenon of adaptation to drought stress is a complex process in which physiological and biochemical changes takes place. Physiological changes that take place during plant microbe interaction and make them able to survive under unfavorable conditions are (1) formation of cyst (Sadasivan *et al.* 1987; Bashan *et al.* 1991), (2) formation of flock (Neyra *et al.* 1995), melanin production (Givaudan *et al.* 1993), synthesis of poly-β-hydroxybutyrate (Okon *et al.* 1992), synthesis of exopolysaccharides (Del Gallo *et al.* 1990) and protection inside ectomycorrhizal spore caps (Li *et al.* 1987). Immediate response of rhizobacteria under drought stress has morphological changes (Shoushtari *et al.* 1985). It is well known that some free-living rhizobia (saprophytic) are capable of survival under water stress or low water potential (Fuhrmann *et al.*1986).

2.3. Changes in root and shoot of maize under drought stress

Most sensitive to drought stress is the cellular growth. In maize weight of shoot decreased while weight of roots increased under drought stress (Aggarwal *et al.* 1983; Morize t*et al.* 1983). It has been studied that under drought stress fresh and dry weight both are reduced in maize by 58 and 40% respectively (Shiralipour*et al.* 1984). Stress also decreased the shoot length and fresh weight in maize (Thakur *et al.* 1984). Drought resistance varieties of sorghum have deeper root penetration, heavier and numerous roots both primary and secondary roots than susceptible varieties (Bhan *et al.* 1974). The effect of drought stress on rate of cell division in the meristem of primary roots of maize was studied and observed that drought stress caused meristematic cells to be longer and lessen the division of cell. Highest cell division rate was 8.2 cells/mm per hr at 0.8 mm from the apex of cells under drought stress as compared to the 13 cells/mm per hr at 1.0 mm in control (Sacks *et al.* 1997).

2.4. Changes in the physico-chemical properties of soil under drought stress when inoculated with EPS producing bacteria

A key parameter that helps in determination of available water, oxygen, and soil nutrients to the plants as well as microorganisms is the water potential of that soil (Postma *et al.* 1989; Blum *et al.* 1992; Van Gestel *et al.* 1993). The interaction among the soil, roots of plants, bacteria and water in the rhizosphere cause changes in physic-chemical and structural properties in the rhizosphere soil (Tisdall *et al.* 1982; Haynes *et al.* 1990). Microorganisms of soil play an important role in the maintenance of quality and soil health (Jeffries *et al.* 2003). Polysaccharides produced by bacteria can attach to the particles of soil and form aggregates (1) macro aggregates of >250 µm) and (2) micro aggregates of <250 µm) in diameter (Edwards *et al.* 1967; Tisdall *et al.*1982; Oades 1993). To keep soil structure maintained is important for the agriculture because it affect a number of processes that have great impacts on the yield of crop. But drought stress change the physico-chemical and also biochemical properties of soil and make it unsuitable for good yield of crop and activity of soil bacteria. In the soil availability of water control the production of polysaccharides and proteins by bacteria in the soil, also

make it possible to consume them. Water availability in the soil also affects the soil structure (Roberson *et al.* 1992). The role of exopolysaccharides producing bacteria ***Azospirillum*** in soil aggregation was reported by Bashen *et al.* (2004). Plants treated with exopolysaccharides producing bacteria ***Azospirillum*** show resistance to water stress (Bensalim *et al.* 1998) this was because of improving the soil structure by exopolysaccharides (Sandhya *et al.* 2009). Under drought stress inoculation of sunflower with exopolysaccharides producing rhizobacterial strain YAS34 show increased in (RAS/RT) root adhering soil per root tissue (Alami *et al.* 2000). Exopolysaccharides are the active constituents of soil organic matter (SOM) (Gouzou *et al.*1993). The association of rhizobacteria with plant roots through these extra-cellular polymers also cause (1) formation of rhizosheath RS and (2) strict adherence of soil to the plant roots. The important role of this rhizosheath RS is (1) active site for soil-microbial process (2) responsible for soil nutrient recycling (3) nutrient availability to the plants (4) responsible for the flow of water and nutrients to the plant roots and (5) regulation of ions (Tisdall *et al.* 1994; Amellal *et al.* 1998). In addition to all these functions of rhizosheath (RS), the portion of roots with attached RS when ploughed after crop harvesting, helps in the formation of micro aggregates, mentainence of microaggrigates, the indispensible structure of soil for physico-chemical properties and fertility of soil for agriculture (Amellal *et al.* 1998; Watt *et al.* 1993). It is supported by experiments that the soil amendments with exopolysaccharides of bacteria cause increase in the aggregation of soil particles (Cheshire *et al.*1979; Martens *et al.*1993).

2.5. Effect of EPS producing bacteria on growth of maize

Maize show high sensitivity towards the drought stress during growing season while during flowering and grain filling period it is especially vulnerable to the drought of soil. Plants inoculated with exopolysaccharides producing microbes show high resistance to drought stress than non-inoculated plants (Bensalim *et al.*1998). Plants growing in the arid and semiarid areas inoculated with beneficial bacteria shows increase in tolerance to the drought stress (Marulanda *et al.*2007). ***Pseudomonas putida*** strain GAP-P45 is exopolysaccharide producing bacteria can form biofilm on surface of roots of sunflower seedlings and give tolerance to sunflower under drought stress. The plants

inoculated with ***Pseudomonas putida*** strain GAP-P45 exopolysaccharides producing bacteria showed increased soil aggregation, root adhering soil and high relative water content of leaves (Sandhya *et al* .2009). It was observed that there is a correlation between the amount of exopolysaccharides (EPS) produced by ***Bradyrhizobium*** strain of cowpea and tolerance of these bacteria (Hartel *et al.*1986). Adaptation of plant to the environmental stresses lead to the adjustment of metabolites in the plant body that causes the several compatible solutes (i.e. sugar, polyamines, betaines, proline, polyhydric alcohols, quaternary ammonium compounds and other amino acids (Yancey *et al.*1982). Also associated with the production of stress proteins e.g. dehydrins with 9-200 KDa in size (Close *et al.*1996). Variation in the production of proteins is the indication of plant response to different stresses. Lettuce (*Lactucastiva* L.) inoculated with the PGPR ***Pseudomonas mendocina*** augmented antioxidant enzyme like catalase in drought stress (Kohlar *et al.* 2008). In antioxidant system antioxidant enzymes i.e. (SOD) superoxide dismutase, (POD) peroxidase and (CAT) catalase and many other compounds such as carotenoids and ascorbate form the principal defense system in oxidative damage (Larson *et al.* 1988; Burke *et al.* 1991). Maize is more susceptible to drought stress than sorghum and water contents of leaf decline more in maize than sorghum having low values of leaf water potential (Nagy *et al.*1995). Relative water content of leaves (RWC) is the index of plant water status that helps in the evaluation of tolerance to drought stress, and reduction in the (RWC) cause closure of stomata that further lead to decreased in CO_2 assimilation in plants (Gindaba *et al.*2004). Relative water contents of leaves in different cultivars differ greatly under drought stress, and membrane permeability of leaf cell increased significantly (Liu *et al.*1995). The maize inoculated with bacteria ***Pseudomonas*** spp. strains has increased plant biomass, relative water contents of leaves, leaf water potential, root adhering soil/root tissues ratio, stability of soil aggregates and decreased the leaf water loss (Chen *et al.*1994). Plants inoculated with exopolysaccharides (EPS) producing bacteria showed higher concentration of sugars, proline, and free amino acids under drought stress conditions. But the starch, protein content and electrolyte leakage were decreased under same conditions with plant inoculation with (EPS) producing bacteria. Same plants inoculated with exopolysaccharides producing bacteria showed decreased activity of (APX) ascorbate peroxidase, (CAT) catalase and (GPX) glutathione

peroxidase enzymes under drought stress that is the indication of less effect of stress on the maize seedlings inoculated with (EPS) producing bacteria (Sandhya *et al.* 2010).

2.6. Phosphates solubilization by microbes

Several species of microorganisms having the ability of phosphorus solublization have been isolated from the rhizospheric soil of the crops. Out of these (PSMs) only 20 to 40% phosphorus solubalizers microorganisms are culture able in the laboratory conditions. These (PSMs) can be used as a biofertilizers in the soil for solubalization of soil phosphorus. Due to this phosphorus solubilizing ability of bacteria they increase the soil nutrients and have great importance. The application of these (PSMs) has a great impact on yield increasing of several crops (i.e. cereals, legumes, vegetables, oils, fibers) and other crops (Kundu BS *et al.*2009). The ability of (PSMs) to convert the insoluble and unavailable phosphorus in the soil to the soluble phosphates (i.e. orthophosphate) is important for the improvement of plant yield (Rodriguez *et al.* 2006; Chen *et al.*2006). The most efficient (PSMs) belong to the ***Bacillus, Pseudomonas*** and ***Rhizobium*** genera of microbes (Rivas *et al.*2006). In all the microorganisms found in the rhizosphere soil, the (PSMs) have an alternative to the biotechnology used for crop production to fulfill the phosphorus requirement of plants. These (PSMs) in addition to phosphorus solubalization also promote the plants development by enhancing the growth (Zaidi *et al.*2009). This ability of phosphorus solubalization and growth promotion of (PSMs) together 50% lessens the application of phosphorus to the crops (Yazdan *et al.*2009). Inoculation of seeds or soil with (PSMs) have largely used for solubalizing the applied and fixed phosphorus in the soil and improved the growth of plants (Nauyital *et al.*2000). Due to these (PSMs), solubility of calcium phosphate was mostly reported but the solubilization of other forms of phosphates (i.e. $AlPO_4$ and $FePO_4$) was not investigated. The ability of phosphorus solubalization of (PSMs) can be determined by their ability to release some metabolites i.e. organic acids, these organic acids form chelation with cation bound to the phosphate through their hydroxyl and carboxyl group, that latter converted into the soluble form. It may also involve the production of proton extrusion in addition to organic acids (Nahas E *et al.*1996).The organic acids (mainly gluconic acids and keto-gluconic acids) produced by (PSMs) to solubilize the phosphorus (Duebel A *et al.*2000),

additionally these organic acids also lower the ph of rhizospheric soil (Khan AA *et al.* 2009).

2.7. Antioxidants Enzymes activity under stress

At cellular level most of the damages take place due to oxidative damage under drought stress, this injury is the result of imbalance between the production of reactive oxygen species (ROS) and their detoxification. At cellular level plants produce antioxidants enzymes (CAT) catalase, (SOD) superoxide dismutase, (POD) peroxidase, (GPX) glutathione peroxidase, (APX) ascorbate peroxidase, these antioxidant enzymes detoxify the reactive free radicals (Simsova-Stoilova *et al.*2008). Detoxification of O^{2-} to H_2O_2 catalyzed by SOD (Dionisio-Sese *et al.*1998). Catalase is present in the peroxisomes of cells and protects the cells by catalyzing the decomposition of H_2O_2 into O_2 and H_2O (Foyer *et al.*2000). POD peroxidase is an enzyme is present in higher plants and catalyzes the process of oxidation of phenolic and endolic co substrates and protects the plant cells from damage (Asada *et al.*1992; Borsanni *et al.* 2001). The action of antioxidant enzymes was changed under stress condition. Damage in the leaves is the indication of stresses e.g. increase in the peroxidation of membrane lipids, due to decrease in the level of antioxidant enzymes (SOD, POD, CAT) activities (Del Longo *et al.* 1993; Chen *et al.*1994; Qu *et al.*. 1996). It was found that in maize drought stress at booting stage of female inflorescence decrease the activity of SOD enzyme and increase the peroxidation of membranes permeability takes place (Song *et al.*1996). Similar results were obtained by (Li *et al.*1998).

2.8. Protein accumulation under drought stress

In plants accumulation of osmolytes like proteins help in maintaining water status of cell, sub-cellular structures and protection of membranes and proteins from osmotic stress (Ashraf *et al.*2007). Stress proteins play their role in plants to tolerate the stress, these proteins are the water soluble (Wahid *et al.* 2007).Increased level of amino acids is the indication of drought tolerance in plants. It may be caused by the hydrolysis and breakdown of proteins in response to osmotic adjustment of the cellular contents (Greenway and Munns 1980).

2.9. Sugar accumulation under drought stress

In sugar family, sucrose was known to function in osmoprotection, stabilization of cellular membranes and maintenance of turgor in plants (Mundree *et al.*2002). Bacteria consist of large amount of ionic polysaccharides in the periplasmic space of bacterial cell, that were too large that diffuse through porin proteins and help to maintained the turgor pressure (Change *et al.*, 2007; Sandhya *et al.*2009a, b). Soluble sugars are the important osmolytes that helps in the osmotic adjustment in plants under drought stress. An increased level of soluble sugars was observed in plants under drought stress (Deka `nkova` *et al.* 2004).

2.10. Proline accumulation under drought stress

In plants increased level of proline is the indication of drought tolerance. Proline is found in large concentration in higher plants increases in stress response (Kavi Kishore *et al.*2005). Large number of studies showed that accumulation of soluble sugar, proline and antioxidant increase the tolerance to drought stress (Lei *et al.* 2006; Ren *et al.*2007; Xiao *et al.* 2008; Xu*et al.* 2008). There was an evidence of compatible solutes accumulation in the plants in high concentration that help in the inactivation of the enzymes or due to water deficiency loss in membrane integrity (Schwab *et al.*1990). Plants accumulate some ions and some metabolites in their vacuoles mainly proline, that will decrease osmotic potential of the plant cells and maintain turgor pressure at high level and help these plants to maintain their metabolic and physiological process (Hossein Ansary *et al.* 2012). Inoculation of plant seedlings with ***Psedomonas*** spp. increased the proline contents under drought stress; it may be due to up regulation of biosynthesis pathway of proline to keep the proline in higher concentration, that helped in maintaining the water status of cell and protect the membranes under drought stress (Yoshiba *et al.*1997). In maize plants proline level increased as much as 100 folds under low water potential (Voetberg and sharp 1991).

2.11. Relative water content of leaves under drought stress

Relative water content was thought to be best criteria for water status of plants. Relative water content was the relation between the water absorbed by the plants and water transpired through leaves(Ganji Arjenaki *et al.* 2012). It has been showed that wheat varieties with high relative water contents were more resistant to drought stress (Schonfeld *et al.*. 1988). In plants osmoregulation is the main mechanism that maintains the turgor pressure under water loss, that enables the plants continuously absorb the water and maintain metabolic activities (Gunasekera *et al.* 1992). In non-inoculated plants the value of relative water content decreases than bacterial inoculated plants during drought period.

2.12. Effect of EPS producing bacteria on Soil moisture contents

Bacterial exopolysaccharides have an ability of water holding and cementing due to which exopolysaccharides producing bacteria helped to maintain the moisture content of soil and flow of water across the plant roots due to formation of soil aggregates (Roberson *et al.*. 1992; Tisdall *et al.*1982). Increased moisture and carbon contents helped to increase the soil aggregation around the plant roots and also moisture holding capacity of the soil due to presence of exopolysaccharides (Amellal *et al.* 1998).

MATERIALS AND METHODS

3.1. Collection of soil samples

Soil samples for the isolation of bacteria were collected from depth of 6 inches from rhizosphere soil of two different rainfed areas of Pakistan, including Ghotki and Kallar Sayedan. Ghotki included in the Nara dessert, an extension of Great Indian Desert, located in Sindh that is southern east province of Pakistan. This desert lies between 26°-28° North and 68°-70° East (Bhatti et al. 2001; Qureshi et al. 2004). It is about 23000 Km^2. It is arid area with extreme temperature range, severe drought with high wind and scanty rainfall, average rainfall of 100-250 mm mostly during July and September. Temperature range from 40-52°C in summer and below 0°C in winter (Qureshi et al. 2005).

Kallar Saydan is located in district Rawalpindi. It lies at altitude of 27°-33° north and longitude of 16°-73° east, with 8% of soil moisture. It belongs to semiarid area.

3.1.1. PH and electrical conductivity of soil samples used for isolation

The pH and electrical conductivity of soil samples were measured by the method of McKeague, (1978) and McLean (1982). Air dried sample (1g) was mixed with (10ml) of dist. water, stirred on magnetic stirrer for 1 hour to make homogeneous mixture of soil and water. Mixture was filtered with Whatman No. 42 filter paper. Filtrate obtained after filtration of homogeneous mixture of soil was used for the determination of pH and electrical conductivity.

3.1.2. Isolation of bacteria from soil

Isolation of bacteria from soil samples collected from Ghotki and Kallar Sayedan areas of Pakistan was done by serial dilution method. During this method (10g) of each soil sample was suspended in (9ml) of distilled water. Soil suspension was centrifuged at 3000rpm for 10-15 min. Decimal dilutions were made from the aliquot of each soil solution after centrifugation. (100ul) of Aliquot from three dilutions 10^{-3}, 10^{-5} and 10^{-7}

were spread on LB agar plates, incubated for 48 hrs at 28-35°C. After 48 hours distinct bacterial colonies were streaked on LB agar plates 3-4 times until single colony was obtained.

3.2. Identification and characterization of EPS producing bacterial isolates

3.2.1. Colony morphology

For bacterial identification bacterial colony diameter, color, shape, margins and appearance were examined.

3.2.2. Gram staining and motility test

Vincent method (1970) was used for the preparation of slides of isolated bacterial cultures for Gram staining. A drop of saline solution was placed on slide, picked a single bacterial colony from plate smeared on saline solution, air dried; heat fixed; stained with crystal violet for 1 min and slightly washed with sterile water. The smear was than washed with iodine solution for 1 min and decolorized with absolute alcohol for 1min, and finally washed with dist. water, air dried and observed under light microscope (Nikon, Japan) at 100X magnification using oil emulsion.

Motility test is used to check the ability of bacteria to migrate away from the line of inoculation. The bacterial culture was inoculated into motility media, i.e, mannitol agar with a needle by streaking the culture in a straight line and was observed after 24-48 hours incubation. If the test organism migrates away from the line of inoculation, the bacteria are motile.

3.2.3. Catalase test

(24 hrs old) Bacterial cultures were used for catalase test. Single bacterial colony was placed on glass slide and a drop of 30% hydrogen peroxide H_2O_2 was added. Appearance of gas bubbles indicates the presence of catalase enzymes in the bacteria (McFadden, 1980).

3.2.4. Oxidase test

For the determination of oxidase in the bacterial strains (1% N, N, N, N-tetramethyle-phenylene diamine) Kovacks's reagent was used (Kovacks, 1956). The reagent was mixed with hot water and kept in dark. Filter paper strip was dipped in Kovack's solution and then air dried. 24 hrs old cultures were placed on this paper strip. Bacterial strains positive for oxidase will show lavender color that will slowly change into dark purple and then black (Steel, 1961).

3.3. Phosphorus solubilization index

For the determination of phosphates solubilization specifically Pikoviskaya's agar media was used. For determining phosphates solubilizing index autoclaved Pikoviskaya's media was poured in Petri-plates under sterilized conditions and wait for solidification of media. With the help of sterilized tooth picks, a pin point inoculation was done on these agar plates under same sterilized conditions. It was allowed to grow the plates in incubator at 28°C for 7 days.Formation of clear halo-zone was the indication of phosphorus solubalization on plates. Index of phosphorus solubalization was calculated by colony diameter and halo-zone diameter (Edi-Premono, 1996).

3.3.1. Quantification of available phosphates solubilized by EPS producing bacteria

Quantification of available phosphates solubilized by bacteria was done on the basis of phospho-molybdateblue color method (Murphy and Riby, 1962). Pikoviskaya's broth of pH 7 with sucrose was used for this purpose and solution of tri-calcium phosphate (0.3g/100ml) was made separately. Pikoviskaya's broth and tri-calcium phosphate was autoclaved separately. Mix the two solutions and 2 loops full of phosphates solubalizing strains were inoculated in each of flask under aseptic conditions and placed on the rotary shaker at 12000 rpm and wait for 12 days. After 12 days the suspension was centrifuged at 10000 rpm for 15mints. The supernatant obtained was used

for determining the available phosphorus by using spectrophotometer at 882nm and calibrated against standard curve of phosphorus.

3.4. DNA Extraction

Extraction of genomic DNA of bacterial strain was carried out by using following method. The bacterial strains were grown in nutrient broth and were kept overnight for the extraction of genomic DNA. About 1.5ml of saturated bacterial culture was transferred to eppendroff tube and was harvested through centrifugation at 10000rpm for 2 minutes. The pellets were collected and were harvested again with 1.5ml of cell culture. Thereafter the pellets were drained on paper towel. The cell pellet was re-suspended and lysed in the 450ul of Transcription buffer (TF) by vigorous pipetting. To remove protein and cell debris 45ul of 10% Sodium Dodecyl Sulfate (SDS) solution and 5ul of 20mg/ml proteinase K mix was added and mixed well and was incubated for an hour at 37°C. Thereafter the clear supernatants was transferred to clean vial and about 500ul phenol-chloroform was added and mixed vigorously well by inverting the tube until the phases were completely mixed. Thereafter the vial was centrifuged at10000rpm for 2minutes. The upper aqueous phase was transferred to a new eppendroff and was mixed with phenol-chloroform mixture again and centrifuged at 10000rpm for 5 minutes. The aqueous phase obtained was transferred to new tube and 50ul of sodium acetate was added and mixed well by hand. Thereafter, 300ul of isopropanol was added and mixed gently to precipitate the DNA and was centrifuged at 10000rpm for 2-5 minutes. The DNA was washed by 1ml of 70% ethanol for 30sec and centrifuged. The DNA was drained with ethanol, dried and re-suspended in 50-100ul T.E buffer and DNA was stored at -20°C for a long period of time.

3.5. PCR (Polymerase Chain reaction)

The genomic DNA of PGPR isolates was amplified by the method as described by Weisburg (1991). The Polymerase chain reaction was performed by using two universal primers fd1fD1 = (AGAGTTTGATCCTGGCTCAG) and rd1= (AAGGAGGTGATCCAGCC). The chain reaction was carried out in Thermocycler.

Each 50ul reaction volume (master mix) contained 1ul Template DNA, 1ulfd1 and 1ul rd1 for each reaction and 25ul of prepared master mix were used and the volume is raised to 50 ul by adding autoclaved cold water. The thermocycler was programed as follow; after incubation at 95°C for 5 min sample were cycled for 94°C for 40 sec annealing at 57°C for 30sec, extension was carried out at 72°C for 2 min and one additional cycle for chain elongation. Then 5 ul of amplified sample was electrophoresed on 1% (w/v) agarose gel in 1X TBE buffer at 80 V and then stained with ethidiumbromide (0.01g/ml).Gel was visualized under UV transilluminator lamp. 1Kb DNA gene ladder was used as molecular marker.

3.6. Culturing of EPS producing bacteria

EPS producing bacteria were cultured in optimized mineral salts medium with 12.6% K_2HPO_4, 18.2% KH_2PO_4, 10% NH_4NO_3, 1% $MgSO_4.7H_2O$, 0.6% $MnSO_4$, 1% $CaCl_2.2H_2O$, 0.06% $FeSO_4.2H_2O$, 1% sodium molybdate, 1.5% NaCl and 0.2% of glucose in 1l of distilled water for 10 days (Bramchari and S.K. Dubey, 2006). 250ml mineral salts media was poured in 500ml flasks for each bacterial culture and autoclaved. Bacterial colony of each strain with the help of loop were inoculated in each flask with mineral salts medium and placed on rotary shaker for 10 days at 37°C.

3.7. Extraction of EPS

After incubation of 10 days the bacterial cultures of 250ml were centrifuged at 15,000 rpm for 20min at 4°C. The exopolysaccharides were extracted from the supernatant by the addition of two folds ice cold ethanol (95%), the solution was chilled at 4°C for complete precipitation.Exopolysaccharides were collected from above solution.

3.8. Purification of EPS

Exopolysaccharides extracted were washed with 70-100% ethanol-water mixture. After washing exopolysaccharides were redissolved in distilled water and dialyzed with dialysis tubing (molecular weight cut-off of 13 kDa; Sigma-Aldrich Chemie GmbH, Seelze, Germany) against distilled water at 4°C for 24hrs to remove excess salts from

exopolysaccharides. Extracted exopolysaccharides were lyophilized with Laboncolyophilizer at 3000 psi and stored at room temperature.

3.9. Solubility of EPS

To check the solubility of exopolysaccharides in different solvents, small quantities of lyophilized exopolysaccharides were taken in eppendroffs. Then 2ml of each solvent was added such as benzene, water, chloroform, acetone, ethanol and methanol. The mixture was vortexed and allowed to stabilize for some time and the pellet formation was observed.

3.10. Chemical characterization of EPS

Chemical characterization of exopolysaccharides was done by evaluating total sugar, protein and uronic acid. Suspension of exopolysaccharides was prepared by resuspended 2g of lyophilized exopolysaccharides in 10ml of distilled water for evaluation.

3.10.1. Quantification of sugar

Quantification of sugar was done by Phenol-Sulfuric acid (PSA) method (Dubois et al, 1956). 2ml of resuspended EPS solutions were taken in test tubes, and 50µl of 80% (w/w) phenol solution was added, that followed by the addition of 5ml of 95.5% concentrated H_2SO_4. The mixture of phenol, sulfuric acid and EPS was incubated at room temperature (22-25°C) for 10mints, that followed by the incubation at (25-30°C) in water bath for 20mints. After incubation the solution required the additional stabilization for 4hrs at room temperature. For final measurements absorbance was taken at spectrophotometer at 480 nm by using glucose as a standard.

Control was run as a mixture of 50µl of 80% (w/w) phenol solution and 5ml of 95.5% concentrated H_2SO_4, without EPS solution.

3.10.2. Quantification of protein

Quantification of protein was done by Lowery method (Lowery *et al.* 1951) by using bovine serum albumin (BSA) as a standard, and absorbance was measured by spectrophotometer at 500 nm wavelength. 0.3ml of resuspended EPS was poured in glass test tubes, and 1.5ml of alkaline copper reagent was added to it that followed by the addition of 75µl of folin phenol reagent in the mixture in test tubes. The mixture was incubated at room temperature for 30min and absorbance was measured on spectrophotometer. The absorbance of solutions was compared with the BSA standard curve to calculate the amount of protein in EPS.

Control was run as test tube with 1.5ml of alkaline copper reagent and 75µl of folin phenol reagent, without sample solution.

3.10.3. Estimation of uronic acid

Quantification of uronic acid was carried out by carbazole assay (Taylor *et al.* 2001)

Reagents

1. Dissolve 0.9gm sodium tetra-borate decahydrate in 10ml of distilled water. Add 90ml of ice cold 98% conc. H_2SO_4 to form a layer and incubate overnight without disturbing.
2. 0.1% (w/v) carbazole in ethanol.

Assay

Set up tubes containing 0.2-20µg of D-galacto-uronic acid in 250µl of dist water. Set up tubes for unknown samples. Add 1.5ml of ice cold reagent (1) in each test tube. Mix well and incubate for 15min at 100°C. Cool the mixture in ice bath and add 50µl of reagent (2). Mix well and incubate for 15min at 100°C. Cool in ice bath and read absorbance at 525nm on spectrophotometer. Compare the absorbance of samples with standard curve of D-galacto-uronic acid.

Control was run as test tube with 1.5ml of ice cold reagent (1) and 50µl of reagent (2).

3.10.4. Emulsification activity of EPS

To determine emulsification activity a modified method of Rosenberg *et al.* (1979) was used. Lyophilized EPS (0.5mg) was dissolved in 0.5ml of distilled water by heating for 15-20min then cooled at room temperature. Phosphate buffer saline (PBS) was added to the EPS solution to make a total volume of 2ml. 0.5ml of hexadecane was added in the mixture and was vortexed for 1 mint. The absorbance was taken immediately after vortex (A_o) at 540 nm on spectrophotometer. The mixturewas incubated at room temperature for 30 and 60mints, and absorbance was measured after 30min and 60min (At). After incubation at room temperature for 30 and 60min, fall in absorbance was recorded. Emulsifying activity of EPS was calculated in % as percentage retention after incubation time $t = At/A_o \times 100$.

Control was run as a mixture of 2ml (PBS) and 0.5ml hexadecane.

Preparation of phosphate-buffer saline (PBS)-1L

1X phosphate-buffer saline was prepared by (Dulbecco, R. *et al.* 1954)

1. 8gNaCl
2. 0.2g KCl
3. 1.44g Na_2HPO_4
4. 0.24g KH_2PO_4
5. 800ml dist water

- Dissolve all dry reagents in 800ml dist water.
- Adjust pH 7.4.
- Add dist water to a final volume of1L.
- Sterilized by autoclaving

3.11. FTIR spectroscopy of EPS

To analyze the functional groups of EPS, Fourier Transformed Infra-Red (FTIR) spectroscopy of lyophilized EPS was done by single reflection ATR accessories; diamond being the preferred choice for most operations and applications because of its robustness and durability. Very small amount of pellets or dry pellets of lyophilized exopolysaccharides were placed onto the Universal diamond ATR plate. The pressure arm positioned on the sample/crystal area and locked onto a precise position above the diamond crystal. Force was applied on the sample by pushing it onto a diamond surface. PerkinElmer's revolutionary Spectrum™ FT-IR software utilizes a 'Preview Mode' which allows the quality of the spectrum to be monitored in real-time while fine tuning the exerted force.

3.12. Inoculation of maize (*Zea mays L.*) with EPS producing bacteria

Effect of exopolysaccharides producing bacteria on the growth of maize was studied by the inoculation of maize seeds with EPS bacterial cells only and in combination to their respective exopolysaccharides. Seeds of maize variety (agaiti-2002) were collected from NARC (National Agricultural Research Centre) Islamabad. Seeds were surface sterilized by the following chemicals, 3 mints with 10% Clorox, and 3 mints with 95% ethanol and followed by washing with sterilized water. For seeds inoculation with only bacterial cells (48 hrs old cultures) were prepared in L.B broth media on shaker and for inoculation of seeds with cells + their respective exopolysaccharides 10 days old cultures were used. Sterilized seeds were soaked in both 48 hrs old cultures and 10 days old cultures of bacteria for 3-4 hrs. Seeds were sown in pots containing autoclaved mixture of soil and sand with the ratio of 3:1 respectively. Soil and sand mixture was autoclaved three times for complete sterilization. Pots were placed in growth chamber with average day and night temperature of 25-18°C respectively. After 1 weak of seed germination plants seedlings were subjected to the drought stress by withholding water supply for 10 days and non-stressed plants were kept well watered. After 10 days of drought stress plants were harvested for further analysis of both growth parameters and physiological parameters.

Physiological and biochemical analysis of plants inoculated with EPS producing bacteria.

3.13. Soil moisture content (%)

After harvesting the plants, moisture content of soil was measured of both stressed plants and non-stressed plants. Fresh weight of soil samples (20g) from depth of 6 inches from rhizosphere of plants were dried in oven for 72 hr at 70°C. Dry weight of soil was recorded and moisture content was measured

$$\text{Soil moisture (\%)} = \frac{\text{weight of wet soil (g)- weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

3.14. Relative water content of leaves (%)

After harvesting the plants, relative water content of leaves was measured by the method of (Weatherly, 1950). Fresh weight of leaves was recorded and then dipped the plants in water for 24 hrs. After dipping in water for 24 hrs fully turgid weight of leaves was recorded. The leaves were dried in oven for 72 hr at 70°C. The dry weight of leaves was recorded, and the relative water content of leaveswas measured

$$\text{RWC \%} = \frac{\text{FW (g)} - \text{DW (g)}}{\text{FTW (g)} - \text{DW (g)}} \times 100$$

- FW= fresh weight
- DW = dry weight
- FTW = fully turgid weight

3.15. Leaf area (cm^2)

Leaf area of randomly collected plants from each treatment was calculated as the product of length and width. Total leaf area was calculated by formula used by (Mckee, 1964).

Leaf area = length of leaf (cm) × width of leaf (cm) × 0.74

3.16. Shoot length (cm)

By using measuring tape, length of freshly harvested plant's shoots was taken in (cm) from each treatment.

3.17. Fresh weight of shoots (cm)

Plants were selected randomly from each treatment and fresh weight of shoots was recorded.

3.18. Dry weight of shoots (cm)

Plants which are selected for fresh weight measurements from each treatment were dried in oven for 72 hrs at 70°C. Record the dry weight of shoots for each plant.

3.19. Root length (cm)

Length of freshly harvested plant's roots was taken in (cm) from each treatment by using measuring tape.

3.20. Fresh weight of roots (cm)

Plants were selected randomly from each treatment and fresh weight of roots was recorded in (g).

3.21. Dry weight of roots (cm)

Plants which are selected for fresh weight measurements of roots from each treatment were dried in oven for 72 hrs at 70°C. Record the dry weight of roots for each plant.

3.22. Protein content of leaves (µg/g)

By using method of (Lowery et al. 1951) leaf protein content was calculated.

Preparation of chemicals

Phosphate buffer stock

1. Monobasic sodium phosphate was prepared by:
dissolving (27.6g) of Na_2HPO_4 in 1L of distilled water.
2. Dibasic sodium phosphate was prepared by:
dissolving (53.6g) of NaH_2PO_4 in 1L of distilled water.

Phosphate buffer of pH (7.5)

16ml of monobasic sodium phosphate and (84ml) of dibasic sodium phosphate were mixed to get the phosphate buffer of pH (7.5).

Reagents preparation

A. Reagent A was prepared by dissolving (2g) of Na_2CO_3, (0.4 g) of NaOH and (1g) of Na-K tartrate in (100ml) distilled water.

B. Reagent B was prepared by dissolving (0.5g) of $CuSO_4$in (100ml) distilled water.

C. Reagent C was prepared by mixing (50ml) of reagent A and (1ml) reagent B.

D. Reagent D was prepared by mixing folin-phenol and distilled water in 1:1 ratio.

Procedure for protein estimation

Weigh 0.1g of fresh leaves and ground in 1 ml of phosphate buffer with pH of 7.4 by using mortar and pestle, centrifuged the mixture at 3000 rpm for 10mints. 0.1ml of aliquot pipette out from the mixture and poured in the test tubes. Add distilled water up to final volume of 1ml. Shake the test tubes after adding 1ml of reagent C, then add 0.1 ml of reagent D, allowed to incubate at room temperature for 30min and recorded the absorbance of each sample at 650 nm on spectrophotometer. Compare the readings of

sample solutions with (BSA) standard curve. BSA was used with different concentrations of 20, 40, 60, 80, 320 and 640mg. Phosphate buffer with pH of 7.4 was a sample for blank reading.

Calculations

$$\text{Protein } \mu g/g = \frac{\text{K value} \times \text{dilution factor} \times \text{absorbance of sample}}{\text{Weight of sample (g)}}$$

- K value for protein = 17.52

3.23. Sugar content of leaves (µg/g)

Sugar content of leaves was calculated by using a phenol-sulfuric acid (PSA) method of (Dubois *et al.*1 956). 0.5g of fresh plant leaves were homogeneously grinded with by using mortar and pestle in 10ml of distilled water. Mixture was centrifuged for 5min at 3000 rpm. Took 0.1ml supernatant in test tubes and add 1ml 80% phenol solution with water. Allowed to incubate at room temperature for 1hr and then 5ml of conc. $H_2SO_4$was added. Incubate for 4hrs at room temperature and absorbance was read on spectrophotometer at 420 nm. Glucose solution of different concentrations was used as standard. Distilled water was used as a blank.

Calculations

$$\text{Protein } \mu g/g = \frac{\text{K value} \times \text{dilution factor} \times \text{absorbance of sample}}{\text{Weight of sample (g)}}$$

- K value for sugar = 80

3.24. Proline content of leaves (mg/g)

Proline content of leaves was measured by using a method of (Bates *et al.*1973). For proline estimation 0.1g of leaf samples were grinded with 4ml of (3%)

sulphosalicyclic acid and incubate overnight at 5°C. Mixture was centrifuged for 5min at 3000 rpm. Mixed 4ml of freshly prepared acidic ninhydrin in the supernatant and incubate in water bath at 100°C for 1hr. cooled the mixture in ice bath and proline contents extracted in separating funnel by using toluene. The upper layer obtained has the proline contents in it. The absorbance was recorded at 520nm. Blank was used as toluene.

Calculations

$$\text{Protein } \mu g/g = \frac{\text{K value} \times \text{dilution factor} \times \text{absorbance of sample}}{\text{Weight of sample (g)}}$$

- K value for proline = 19.6

3.25. Extraction for Antioxidant Enzymes (g^{-1}f.wt.)

The activity of antioxidant enzymes (SOD, POD and CAT) superoxide dismutase, peroxidase and catalase was determined by extraction in following way. 0.2g of fresh leaves was ground in 4ml of phosphate buffer with pH of 7.8 + 1% PVP (polyvinyl pyrolidone). The mixture was centrifuged at 15000 rpm for 15min. The supernatant was used for antioxidant enzymes estimation.

3.25.1. Superoxide dismutase enzyme (SOD)

The method of (Beauchamp and Frodovich, 1971) was used for Superoxide dismutase (SOD) estimation. 0.5ml of enzyme extract was collected in test tubes and final volume rose up to 0.8ml. Three solutions were prepared for dark, light and blank.

Reference mixture

- 0.5ml of enzyme extract obtained from leaves
- 2.5ml of solution from step 5
- 0.5ml of solution from step 8

Reaction mixture

- 0.5ml of enzyme extract obtained from leaves
- 2.5ml of solution from step 5
- 0.5ml of solution from step 8

Blank

- 0.5ml of phosphate buffer with pH of 7.0
- 2.5ml of solution from step 5
- 0.5ml of solution from step 8

Reaction mixture was kept in light and reference mixture in dark for 20min. Absorbance of SOD enzyme's samples was recorded at 560 nm.

Calculations

$$SOD\ (g^{-1}f.wt.) = \frac{R_4}{A}$$

- $R_4 = R3 - R2$
- $A = R1 \times 0.5$
- R_1 = dark
- R_2 = blank
- R_3 = light

3.25.2. Peroxidase enzyme (POD)

Estimation of peroxideenzyme was done by method of (Vetter *et al.*1958) and modified by (Gorin and Heidema, 1976). Poured 0.1ml of enzyme extract in test tubes and add 1.35ml of 100mM of MES buffer with pH of 5.5. Then added 3µl of 0.1% phenylenediamine in the mixture and at the end 5µl of 0.05% H_2O_2 was added in it and

took the readings at 485nm immediately after the addition of H_2O_2 that was 1st reading and 2nd reading was took 3min after the addition of H_2O_2 in the mixture.

Calculations

$$POD\ (g^{-1}f.wt.) = \frac{\text{final reading} - \text{initial reading}}{3}$$

- Final reading = 2nd reading (3 mints after the addition of H_2O_2)
- Initial reading = 1st reading (immediately after the addition of H_2O_2)

3.25.3. Catalase enzyme (CAT)

Catalase activity was estimated by the method of (chandlee and scandalios, 1984). 0.2ml of enzyme extract was poured in test tubes and 0.5ml of 0.05M phosphate buffer was added. At the end 0.1ml of 3% H_2O_2 was added in the mixture. The reading of mixture containing enzyme extract was recorded at 240 nm 485nm immediately after the addition of H_2O_2 that was 1st reading and 2nd reading was took 3min after the addition of H_2O_2 in the mixture.

Calculations

$$CAT\ (g^{-1}f.wt.) = \frac{\text{initial reading} - \text{final reading}}{3}$$

- Initial reading = 1st reading (immediately after the addition of H_2O_2)
- Final reading = 2nd reading (3 mints after the addition of H_2O_2)

3.26. Statistical Analysis of Data

The data recorded in laboratory and pot experiments were subjected to statistical analysis (Steel and Torrie, 1980) with statistix version 8.1. Means and standard errors of the means were calculated. Results were evaluated by analysis of variance (ANOVA).

The differences between the means of inoculated and control treatments were tested using the Least Significant Differences test (at $p < 0.05$).

RESULTS

For the isolation of EPS producing bacteria soil samples were collected from two different areas.These soil samples were collected from rainfed areas like

- Sindh (Ghotki) (located in Nara dessert of Sindh), lies between 26°-28° north and 68°-70° east, it is arid, with severe drought, 40-52°C temperature in summer and freezing in winter, average annual rainfall 100-250mm.
- Kallar Saydan is located in district Rawalpindi and semiarid area. It lies at altitude of 27°-33° north and longitude of 16°-73° east, with 8% of soil moisture.

4.1. pH and EC of soil samples

Electrical conductivity and pH both were higher for the soil from Sindh. pH of soil from Ghotki Sindh was 8.72 and from Kallar Saydan was 8.10. Electrical conductivity of soil sampled from Ghotki Sindh was 210 and from Kallar Saydan were 160.

4.2. Isolation and Identification of EPS producing bacteria

Ten bacterial strains were isolated from three soil samples. All bacterial colonies were streaked on LB agar plates but three bacterial strains (S1, S2 and S3) were selected for EPS production on the basis of mucoid colony morphology and other biochemical tests.The bacterial strains S1produced green mucoid colony on LB agar plate and S2 produced yellow green mucoid colony and S3 gave non-pigmented colonies on LB agar plate. S1 has soapy odor, S2 has grape like odor and S3 has pleasant fruity odor. All colonies were circular and their size range from 0.3 to 0.5cm, margins entire as in (table 1). S1 isolate was positive for catalase and negative for oxidase, while S2 and S3 were positive for both oxidase and catalase tests.

4.3. Gram staining and motility

All bacterial isolate were gram negative, S1 was rod shaped, S2 was bacilli and S3 was cocci. All bacterial strains were motile and swarm on agar plates (table 2).

4.4. Phosphorus solubilization index and quantification of available phosphates solubilized by bacterial isolates

Only one bacterial strain (S1) was phosphorus solubilizing that form a clear circular and regular halo-zone around the bacterial colony. Index of phosphorus solubilization was 2.11cm and the total phosphates solubilized by thestrain(S1) were 0.49ug/ml.

4.5. Identification of bacterial isolates by 16s rRNA sequence analysis

4.5.1. Alignment of 16S rRNA sequence of S1 strain

For the isolate S1 obtained from arid region of Ghotki Sindh the total length of sequence with 1537 nucleotide was obtained (Fig.1). The comparison of the nucleotide sequence with data nucleotide bank showed highest sequence similarity with 1464/1469 and 99% with that of ***Proteus penneri*** strain(AC No: JN092595.1) respectively.

2TCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAACAGGAGAAAGC61

21TCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGGGCGGTAACAGGAGAAAGC 80

62 TTGCTTTCTTGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCCCGATAGA 121

81 TTGCTTTCTTGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCCCGATAGA140

122GGGGGATAACTACTGGAAACGGTGGCTAATACCGCATGACGTCTACGGACCAAAGCAGGG181

141GGGGGATAACTACTGGAAACGGTGGCTAATACCGCATGACGTCTACGGACCAAAGCAGGG200

182 GCTCTTCGGACCTTGCGCTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGGGGTAA 241

201 GCTCTTCGGACCTTGCGCTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGGGGTAA 260

242 AGGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACT 301

261 AGGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACT 320

302GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA361

321GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA380

362 GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGC 421

381 GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGC 440

422 GGGGAGGAAGGTGTTAAGATTAATACTCTTAGCAATTGACGTTACCCGCAGAAGAAGCAC481

441 GGGGAGGAAGGTGTTAAGATTAATACTCTTAGCAATTGACGTTACCCGCAGAAGAAGCAC500

482 CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA 541

501 CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA 560

542 CTGGGCGTAAAGCGCACGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCCGAGCTTAAC 601

561 CTGGGCGTAAAGCGCACGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCCGAGCTTAAC 620

602 TTGGGAATTGCATCTGAAACTGGTTGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCACG 661

621 TTGGGAATTGCATCTGAAACTGGTTGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCACG 680

662 TGTAGCGGTGAAATGCGTAGAGATGTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGA721

681 TGTAGCGGTGAAATGCGTAGAGATGTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGA740

722 CAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG781

741 CAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG800

782 TCCACGCTGTAAACGATGTCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCT841

801 TCCACGCTGTAAACGATGTCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCT 860

842 AACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGA901

861 AACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGA920

902 CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA 961

921 CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA 980

962 CCTACTCTTGACATCCAGCGAATCCTTTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAG 1021

981 CCTACTCTTGACATCCAGCGAATCCTTTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAG 1040

1022 ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAAC 1081

1041 ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAAC1100

1082 GAGCGCAACCCTTATCCTTTGTTGCCAGCGCGTAATGGTGGGAACTCAAAGGAGACTGCC1141

1101 GAGCGCAACCCTTATCCTTTGTTGCCAGCGCGTGATGGCGGGAACTCAAAGGAGACTGCC 1160

1142 GGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGG 1201

1161 GGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGG 1220

1202 CTACACACGTGCTACAATGGCAGATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAAC1261

1221 CTACACACGTGCTACAATGGCAGATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAAC1280

162 TCATAAAGTCTGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATC 1321

1281 TCATAAAGTCTGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATC 1340

1322 GCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGC 1381

1341 GCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGC 1400

1382 CCGTCACACCATGGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCCGGGAGGGC 1441

1401 CCGTCACACCATGGG-AGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTC-GGGAGGGC 1458

1442 GCTTACCACTTTGTGATTCATGACTGGGG 1470

1459 GCTTACCACTTTGTGATTCATGACTGGGG 1487

Fig 1: Alignment of 16S rRNA sequence of ***Proteus penneri*** strain (isolated from arid region of Ghotki Sindh).

4.5.2. Alignment of 16S rRNA sequence of S2 strain

For the isolate S2 obtained from semi-arid region of Kallar Sayedan the total length of sequence with 1498 nucleotide was obtained (Fig. 2). The comparison of the nucleotide sequence with data nucleotide bank showed highest sequence similarity with 1465/1465 and 100% with that of ***Pseudomonas aeruginosa*** strain (AC No: AY792969.1) respectively.

1 CTGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAAGGGAG 60

14 CTGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAAGGGAG 73

61 CTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGG 120

74 CTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGG 133

121 GGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGA180

134 GGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGA193

181 TCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAG 240

194 TCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAG 253

241GCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGA 300

254GCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGA 313

301 GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGC360

314 GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGC373

361 CTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTG 420

374 CTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTG 433

421 GGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCG 480

434 GGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCG 493

481 GCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACT 540

494 GCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACT 553

541 GGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCT 600

554 GGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCT 613

601 GGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTG 660

614 GGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTG 673

661 TAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACT720

674 TAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACT733

721 GATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC 780

734 GATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC 793

781 CACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAA 840

794 CACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAA 853

841 CGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG 900

854 CGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG 913

901 GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC 960

914 GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC 973

961 TGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACAC 1020

974 TGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACAC 1033

1021 AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGA1080

1034 AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGA1093

1081 GCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGT1140

1094 GCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGT1153

1141 GACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTA1200

1154 GACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTA1213

1201 CACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCA1260

1214 CACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCA1273

1261 TAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCT1320

1274 TAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCT1333

1321 AGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG1380

1334 AGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG1393

1381 TCACACCATGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTTA 1440

1394 TCACACCATGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTTA 1453

1441 CCACGGAGTGATTCATGACTGGGGT 1465

1454 CCACGGAGTGATTCATGACTGGGGT1478

Fig 2: Alignment of 16S rRNA sequence of ***Pseudomonas aeruginosa*** strain (isolated from semi-arid region of Kallar Sayedan)

4.5.3. Alignment of 16S rRNA sequence of S3 strain

For the isolate S3 obtained from semi-arid region of Kallar Sayedan the total length of sequence with 1462 nucleotide was obtained (Fig. 3). The comparison of the nucleotide sequence with data nucleotide bank showed highest sequence similarity with 1460/1461 and 99% with that of ***Alcaligenes faecalis*** strain (AC No: AB680626.1) respectively.

3 ATTGAACGCTAGCGGGATGCTTTACACATGCAAGTCGAACGGCAGCGCGAGAGAGCTTGC 62

1 ATTGAACGCTAGCGGGATGCTTTACACATGCAAGTCGAACGGCAGCGCGAGAGAGCTTGC 60

63 TCTCTTGGCGGCGAGTGGCGGACGGGTGAGTAATATATCGGAACGTGCCCAGTAGCGGGG 122

61 TCTCTTGGCGGCGAGTGGCGGACGGGTGAGTAATATATCGGAACGTGCCCAGTAGCGGGG 120

123 GATAACTACTCGAAAGAGTGGCTAATACCGCATACGCCCTACGGGGGAAAGGGGGGGATC 182

121 GATAACTACTCGAAAGAGTGGCTAATACCGCATACGCCCTACGGGGGAAAGGGGGGGATC 180

183 GCAAGACCTCTCACTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGTAAAGGC 242

181 GCAAGACCTCTCACTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGTAAAGGC 240

243 TCACCAAGGCAACGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGAGA 302

241 TCACCAAGGCAACGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGAGA 300

303 CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCT 362

301 CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCT 360

363 GATCCAGCCATCCCGCGTGTATGATGAAGGCCTTCGGGTTGTAAAGTACTTTTGGCAGAG 422

361 GATCCAGCCATCCCGCGTGTATGATGAAGGCCTTCGGGTTGTAAAGTACTTTTGGCAGAG 420

423 AAGAAAAGGTATCCCCTAATACGGGATACTGCTGACGGTATCTGCAGAATAAGCACCGGC 482

421 AAGAAAAGGTATCCCCTAATACGGGATACTGCTGACGGTATCTGCAGAATAAGCACCGGC 480

483 TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGG 542

481 TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGG 540

543 GCGTAAAGCGTGTGTAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGGGCTCAACCTTG 602

541 GCGTAAAGCGTGTGTAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGGGCTCAACCTTG 600

603 GAACTGCATTTTTAACTGCCGAGCTAGAGTATGTCAGAGGGGGGTAGAATTCCACGTGTA 662

601 GAACTGCATTTTTAACTGCCGAGCTAGAGTATGTCAGAGGGGGGTAGAATTCCACGTGTA 660

663 GCAGTGAAATGCGTAGATATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGATAA 722

661 GCAGTGAAATGCGTAGATATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGATAA 720

723 TACTGACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA 782

721 TACTGACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA 780

783 CGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGC 842

781 CGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGC 840

843 GTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGG 902

841 GTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGG 900

903 ACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTAC 962

901 ACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTAC 960

963 CCTTGACATGTCTGGAAAGCCGAAGAGATTTGGCCGTGCTCGCAAGAGAACCGGAACACA 1022

961 CCTTGACATGTCTGGAAAGCCGAAGAGATTTGGCCGTGCTCGCAAGAGAACCGGAACACA 1020

1023 GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG1082

1021 GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG 1080

1083 CGCAACCCTTGTCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAAC 1142

1081 CGCAACCCTTGTCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAAC1140

143 CGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGT1202

1141 CGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGT 1200

1203 CATACAATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGGGAGCCAATCTCAGAAACCC1262

1201 CATACAATGGTCGGGACAGAGGGTCGCCAACCCGCGAGGGGGAGCCAATCTCAGAAACCC1260

1263 GATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATC1322

1261 GATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATC1320

1323 GCGGATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACC1382

1321 GCGGATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACC1380

1383 ATGGGGAGTGGGTTTCACCAGAAGTAGGTAGCCTAACCGTAAGGAGGGCGCTTACCACGG1442

1381 ATGGG-AGTGGGTTTCACCAGAAGTAGGTAGCCTAACCGTAAGGAGGGCGCTTACCACGG 1439

1443 TGGGATTCATGACTGGGGTGA1463

1440 TGGGATTCATGACTGGGGTGA1460

Fig 3: Alignment of 16S rRNA sequence of ***Alcaligenes faecalis*** strain (isolated from semi-arid region of Kallar Sayedan)

4.6. Exopolysaccharides Solubility

The lyophilized EPS were only soluble in water and insoluble in other solvents like benzene, acetone, chloroform and ethanol. After vortex when left undisturbed pellet formation in all solvents was observed except water. Similar results were also reported by (Vimala and Lalithakumari, 2003).

4.7. Chemical analysis of exopolysaccharides

The chemical composition of exopolysaccharideswas shown in (Table: 3). Sugar contents were 98% in S2 and S3 strains and 97% in S1 strain and. There were no significant differences in sugar and uronic acid contents of the three isolates. Uronic acid was found in very small quantity than sugar in exopolysaccharides of all strains.

4.8. Emulsification activity of exopolysaccharides

Emulsification activity gives the strength of EPS in retaining the emulsion breaks. Dialysed-exopolysaccharides have greater activity of emulsification than non-dialysed exopolysaccharides. The non-dialysed exopolysaccharides of strain S-1retained 42.11% and 10.52% after 30 and 60 min while dialysed exopolysaccharides 46.88% and 34.38% respectively. Non-dialysed exopolysaccharides of S-2 retained 42.85% and 4.76% after 30 and 60 min while dialysed 44.73% and 34.21% respectively. Similarly in case of S-3 strain non-dialysed exopolysaccharides retained 37.5% and 18.75% after 30 and 60 min while dialysed exopolysaccharides 46.15% and 33.33% (table 4). These show that exopolysaccharides produced by S-1 show a good stability for emulsion. In all cases dialysed lyophilized exopolysaccharides show greater stability than non-dialysed.

4.9. Fourier-transformed infrared spectroscopy of exopolysaccharides

FTIR spectrum of EPS of all bacterial isolates show slight differencein peaks, most of the peaks are similar in all. Several characteristic bands of FTIR spectrum represent different functional groups (fig 4, 5, 6). Peaks that are common to all EPS samples are (1) 3275.12 cm^{-1} in S1, 3273.01 cm^{-1} in EPS of S2, and 3278.91 cm^{-1} represents O-H stretching and hydrogen bonding; (2) 2923.08 cm^{-1} in case of S1, 3016.74 cm^{-1}, 2926.98 cm^{-1}in case of S2, and 3016.78 cm^{-1}, 2908.98 cm^{-1} in S3 represents (aliphatic CH symmetric and asymmetric stretching in CH_3 and CH_2) that is fatty acid region; (3) 1633.61 cm^{-1} of S1, 1634.50 cm^{-1} of S2, and 1634.33 cm^{-1} of S3 shows (C=O asymmetric stretching of –NH-CO-R and/or N-H bending of H_2N-CO-R (Amide I); (4) 1540.0 cm-1in S1, 4 1539.58 cm^{-1} in S2, 1539.17 cm^{-1} in S3 are the peaks of (N-H bending of –NH- (Amide II), as protein region; (5) 1379.02 cm^{-1} range in S1, 1304.84 cm^{-1} range in S2, 1377.97 cm^{-1} range in S3 is of (P=O stretching of phosphate $PO4^{3-}$) as nucleic acid region and peaks which are different are (6) 1379.02 cm^{-1} in S1, and 1377.97 cm^{-1} in S3 shows C=O symmetric stretching of carboxylate and/or C-OH stretching of phenolic OH represent esters but absent in S2; (7) 1304.84 cm^{-1} in both S2 and S3 show O-H bonding in carboxylic acid but absent in S1; (8) 1227.97 cm^{-1}, 1221.38 cm^{-1} in EPS of S1 and S3 respectively but absent in EPS of S2 show absorbance of P=O stretching of phosphate $PO4^{3-}$ and/or C-O stretching of –O-COR that is phosphate group;

(9) 1070.29 cm^{-1} in case of EPS of S1 strain, 1029.43 cm^{-1} in case of EPS of S2 strain, 1023.33 cm^{-1} and 807.70 cm^{-1} in case of EPS of S3 strain are of C-O-C group vibrations in the cyclic structures of carbohydrates that represent polysaccharide region (table 5, 6, 7).

Effect of EPS producing bacterial inoculation on growth and physiology of maize (*Zea mays L.*)

Plant growth was significantly enhanced by inoculation of EPS producing bacterial strains. After exposure to drought stress un-inoculated plants started wilting after 3 days but inoculated plants with (bacterial cellsalone) resist to stress up to 7 days then started wilting. While seeds inoculated with (bacterial cells and their EPS in combination) resist stressing up to 10 days.

4.10. Soil moisture content (%)

All treatments have increasedthe moisture content of soil than un-inoculatedcontrol. Soil moisture content was calculated that was increased in case of soil inoculated with all strains in combination with their respective exopolysaccharides that is S-1+EPS, S2+EPS and S3+EPS by 68%, 67% and 65% respectivelyin non-stressand 74%, 51% and 66% respectively in stress. Soil inoculated with S-1+EPS (strain1 in combination with its exopolysaccharides) has maximum moisture content that was 68% in non-stress and 74% in stressover control (fig: 7). All strains with their respective exopolysaccharides showed greater moisture content than soil inoculated with only bacterial cells and un-inoculated control.

4.11. Relative water content of leaves (%)

Relative water contents as in (fig: 8) showed maximum increase in treatment S2+EPS in non-stress that was 45% over control, while underdrought stress condition relative water content was higher in (S3+EPS) by 47%. The treatments with only bacterial cells S2 and S3 have almost equal amount of RWC in both stress and non-stress conditions that was 39% and 26% in drought stress while 22%and 13% in non-stress

condition respectively as compared to control. Seeds inoculated with all treatments show increased RWC in both stress and non-stress than un-inoculated control.

4.12. Leaf area (cm^2)

Fig: 9 showed leaf area of plants that was increased in plants inoculated with EPS producing bacteria alone and also in combination to their respective exopolysaccharides. In case of seeds inoculated with S2+EPS showed increase maximum in leaf area as compared to all other treatments both in stress and non-stress that was 130% in non-stress and 127% in drought stress. Also in case of seeds inoculated with only bacterial strains, like S2 has maximum leaf area 84% in stress and 78% non-stress than other inoculation with only bacterial strains and control.Seeds inoculated with all treatments show increased RWC in both stress and non-stress than un-inoculated control.

4.13. Shoot length (cm)

All treatments have increased effect on shoots length in both drought stress and no- stress than non-inoculated control. Treatment S2+EPS showed maximum increase in shoot length as 76% in stress and 72% in non-stress over non-inoculated control. S2, S3 and S1+EPS treatments show same effect on shoot length in both stress and non-stress that was 50%, 33% and 42% respectively,while in other treatments S2+EPS, S3+EPS, S1 and non-inoculated control showed increased effect on shoot length in non-stress condition (Fig: 10).

4.14. Fresh weight of shoot (g)

All treatments increased the fresh weight of shoots as compared to un-inoculated control plants in both stress and non-stress. Seeds treated with S3 have maximum increase in fresh weight in non-stress conditions by 83% than control. Seeds inoculated with S1+EPS and S3+EPS have almost same value of fresh weight in both stress and non-stress conditions that was 17% in case of S1+EPS and 31% in S3+EPS as compared to control (fig: 11).

4.15. Dry weight of shoot (g)

All treatmentshave increased effect on dry weight of shoots than control plants in both drought stress and non-stress condition.S3 has maximum increase in dry weight 44% in non-stress condition and 61% in stressthan control plants. After S3, treatment S2+EPS has maximum value of dry weight that was 41% than control in non-stress. In case of seeds inoculated with S1, S1+EPS and S3+EPS have equal values of dry weight in both stress and non-stress. While in other cases it was greater in non-stress than drought stress (Fig: 12).

4.16. Root length (cm)

All treatments have increased effect on roots length in both drought stress and non-stress than non-inoculated control. Treatment S3+EPS showed maximum increase in root length as 50% in non-stress and 42% in stress over non-inoculated control. S2 and S1+EPS treatments show same effect on root length in both stress and non-stress that was 27% and 33% respectively over control, while in other treatments S2+EPS, S3+EPS, S1, S3and non-inoculated control showed increased effect on shoot length in non-stress condition (Fig: 13).

4.17. Fresh weight of root (g)

It was observed that all treatments have increased effect on fresh weight of roots than non-inoculated control. The treatment S2+EPS have maximum increase of root length in both stress and non-stress condition, that was 57% in non-stress and 37% in stress condition over control. Treatment S3 has equal effect in both stress and non-stress condition. and S3 has maximum increase in stress condition than all other treatments and control. All treatments of bacterial cells with their respective exopolysaccharides have greater effect on the fresh weight of roots on both stress and non-stress conditions (fig: 14).

4.18. Dry weight of root (g)

It was observed that all treatments have increased effect on dry weight of roots than non-inoculated control. The treatment S2+EPS have maximum increase of root length in both stress and non-stress condition, that was 65% in non-stress and 43% in stress condition over control. All treatments of bacterial cells with their respective exopolysaccharides have greater effect on the dry weight of roots on both stress and non-stress condition (fig: 15).

4.19. Protein contents of leaves (µg/g)

A protein content of leaves was increased in all treatments as compared to control. Treatment S2 has maximum increase in protein content of leaves in stress condition that was 58% and S1 has maximum increase in protein content of leaves in non-stress condition that was 93%. Protein content was higher in drought stress than in non-stress including all treatments and control, but in case of S1+EPS and S3+EPS show different behavior to amount of leaves protein, both have equal effect on protein contents of leaves in both stress and non-stress that was 77% in non-stress and 15% in stress forS1+EPS, 87% in non-stress and 23% in case of S3+EPS (fig: 16).

4.20. Sugar content of leaves (µg/g)

All treatments showed increased amount of sugar content of leaves in both inoculation with only EPS producing bacterial strains and in combination with their respective exopolysaccharides than un-inoculated control (fig: 17). Seed inoculated with S1+EPS and S2+EPS have highest sugar level in both drought stress and non-stress conditions that was 50% in non-stress and 33% in stress for S1+EPS and S2+EPS. Sugar values increases in stress than non-stress conditions in plants inoculated with S1 and S2 as in control plants. But seed inoculated with S1+EPS and S2+EPS andS3 show different results, have greater value of sugar content in non-stress.

4.21. Proline content of leaves (mg/g)

All treatments have decreased effect on proline content of leaves than non-inoculated control in both stress and non-stress condition. Treatment S2+EPShas maximum decrease in proline content of leaves than un-inoculated control (Fig: 18).

4.22. Superoxide dismutase (g^{-1}f.wt.)

Fig: 19 show that all treatments have decreased effect on activity of superoxide dismutase enzyme than non-inoculated control. Treatment S2+EPS show maximum decrease in the value of superoxide dismutase enzyme of leaves in both drought stress and non-stress than control. All treatments including control have greater value of superoxide dismutase enzyme in drought stress than non-stress condition.

4.23. Peroxidase (g^{-1}f.wt.)

All treatments have decreased activity of peroxidase enzyme than non-inoculated control both in stress and non-stressed condition. Maximum decrease in the value of peroxidase enzyme of leaves was found S2+EPS in both drought stress and non-stress. All treatments including control have greater value of peroxidase in drought stress than non-stress condition (fig: 20).

4.24. Catalase (g^{-1}f.wt.)

In (fig: 21) all treatments have decreased activity of catalase enzyme than non-inoculated control both in stress and non-stressed condition. Maximum decrease in the value of catalase enzyme of leaves was found S2+EPS in both drought stress and non-stress. All treatments including control have greater value of catalase in drought stress than non-stress condition.

Table 1: Morphological characteristics of Bacterial Isolates

Bacterial Isolates	Color	odor	Size (cm)	Elevation	Margins	Colony appearance
AS-1	Off white	Soapy	0.2	Pulvinate	Entire	Smooth shiny
AS-2	Creamy	Bitter	0.45	convex	Irregular	Rough opaque
BS-1	Creamy	Bitter	0.2	Raised	Irregular	Rough opaque
BS-2	Off white	Bitter	0.6	Convex	Irregular	Smooth shiny
7H	Light yellow	Pungent	0.4	Convex	Regular	Smooth shiny
7C	Light brown	Pungent	0.3	Convex	Erose	Smooth translucent
SP	Creamy	Sulpher like	0.3	Raised	Erose	Rough opaque
S1	Green	Fruity	0.3	Convex	Entire	mucous
S2	Yellowish green	Grapes like	0.5	Raised	Entire	mucous
S3	Off white	Fruity	0.3	Raised	Entire	mucous

Note: Three bacterial isolates (S1, S2 and S3) were selected on the basis of mucus colony formation

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and**S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

Table: 2 Gram staining and motility

Bacterial isolates	Gram stain	Shape	Motility
S1	Negative	Rod	+
S2	Negative	Bacilli	+
S3	Negative	cocci	+

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

+ stands for positive in test

Table 3: chemical characterization, sugar, protein, uronic acid contents of EPS

Bacterial isolates	Sugar (µg/mg)	Protein (µg/mg)	Uronic acid (µg/mg)
control	309.9 B	20.2 C	0.8500 A
S1	6981.9 A	703.0 B	1.1193 A
S2	6948.7 A	1121.2 A	0.9250 A
S3	7066.7 A	1074.7 A	1.1337 A

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

Table 4: Emulsifying activity of EPS

Bacterial strains	EPS	Incubation time (min)	Sample OD at 540nm	Emulsifying Activity (%)
S1	Non-dialysed	0	0.19	100
		30	0.08	42.105
		60	0.02	10.52
	Dialysed, lyophilized	0	0.32	100
		30	0.15	46.88
		60	0.11	34.38
S2	Non-dialysed	0	0.21	100
		30	0.09	42.86
		60	0.01	4.76
	Dialysed, lyophilized	0	0.38	100
		30	0.17	44.73
		60	0.13	34.21
S3	Non-dialysed	0	0	100
		30	30	37.5
		60	60	18.75
	Dialysed, lyophilized	0	0	100
		30	30	46.15
		60	60	33.33

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

Table 5: Potential functional groups in EPS of S1 bacterial strain

Potential functional groups	EPS of S1	
	Wave number (cm^{-1})	**Characteristics of peak**
O-H stretching and hydrogen bonding	3275.12	broad
Aliphatic CH stretching (symmetric and asymmetric stretching of CH3 and CH2)	2923.08	strong
C=O asymmetric stretching of –NH-CO-R and/or N-H bending of H2N-CO-R (Amide I)	1633.61	Very strong
N-H bending of –NH- (Amide II) and/or C=C stretching of aromatic ring	1540.04	strong
C=O symmetric stretching of carboxylate and/or C-OH stretching of phenolic OH	1379.02	Weak, a little broad
P=O stretching of phosphate PO4 3- and/or C-O stretching of –O-COR	1227.97	strong
C-O-C group vibrations in the cyclic structures of carbohydrates	1070.29	strong

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

Table 6: Potential functional groups in EPS of S2 bacterial strain

Potential functional groups	EPS of S2	
	Wave number (cm^{-1})	**Characteristics of peak**
O-H stretching and hydrogen bonding	3273.01	Very broad
Aliphatic CH stretching (symmetric and asymmetric stretching of CH3 and CH2)	3016.74, 2926.98	Strong, little broad
C=O asymmetric stretching of –NH-CO-R and/or N-H bending of H2N-CO-R (Amide I)	1634.50	Sharp and strong
N-H bending of –NH- (Amide II) and/or C=C stretching of aromatic ring	1539.58	strong
O-H bending in carboxylic acid	1304.84	Doublet, fairly strong
C-O-C group vibrations in the cyclic structures of carbohydrates	1029.43	Very sharp and strong

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

Table 7: Potential functional groups in EPS of S3 bacterial strain

Potential functional groups	EPS of S3	
	Wavenumber (cm^{-1})	**Characteristics of peak**
O-H stretching and hydrogen bonding	3278.91	Very broad band
Aliphatic CH stretching (symmetric and asymmetric stretching of CH3 and CH2)	3016.78, 2908.98	Doublet fairly strong
C=O asymmetric stretching of –NH-CO-R and/or N-H bending of H2N-CO-R (Amide I)	1634.33	Strong and sharp
N-H bending of –NH- (Amide II) and/or C=C stretching of aromatic ring	1539.17	Strong
C=O symmetric stretching of carboxylate and/or C-OH stretching of phenolic OH	1377.97	Strong
O-H bending in carboxylic acid	1304.82	Doublet, fairly strong
P=O stretching of phosphate PO4 3- and/or C-O stretching of –O-COR	1221.38	Strong but little broad
C-O-C group vibrations in the cyclic structures of carbohydrates	1023.33	Strong, very sharp
C-O-S stretching of –O-SO4-	807.70	Strong

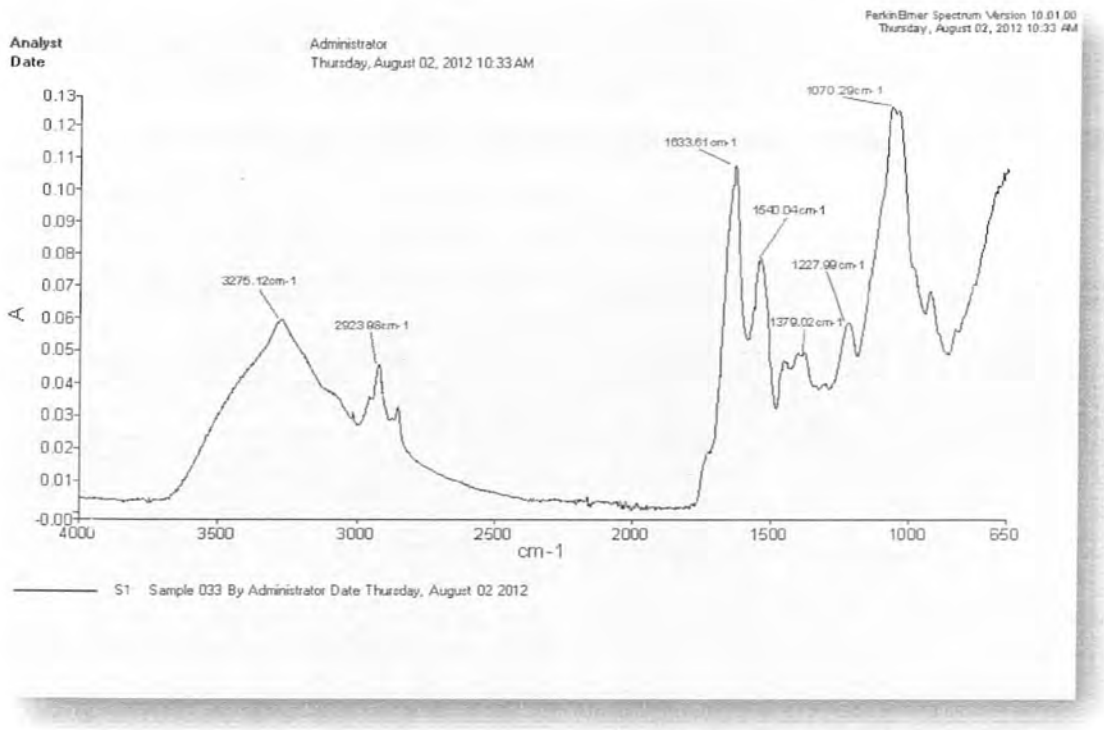


Figure 4: ATR-FTIR spectra of EPS of S1 strain

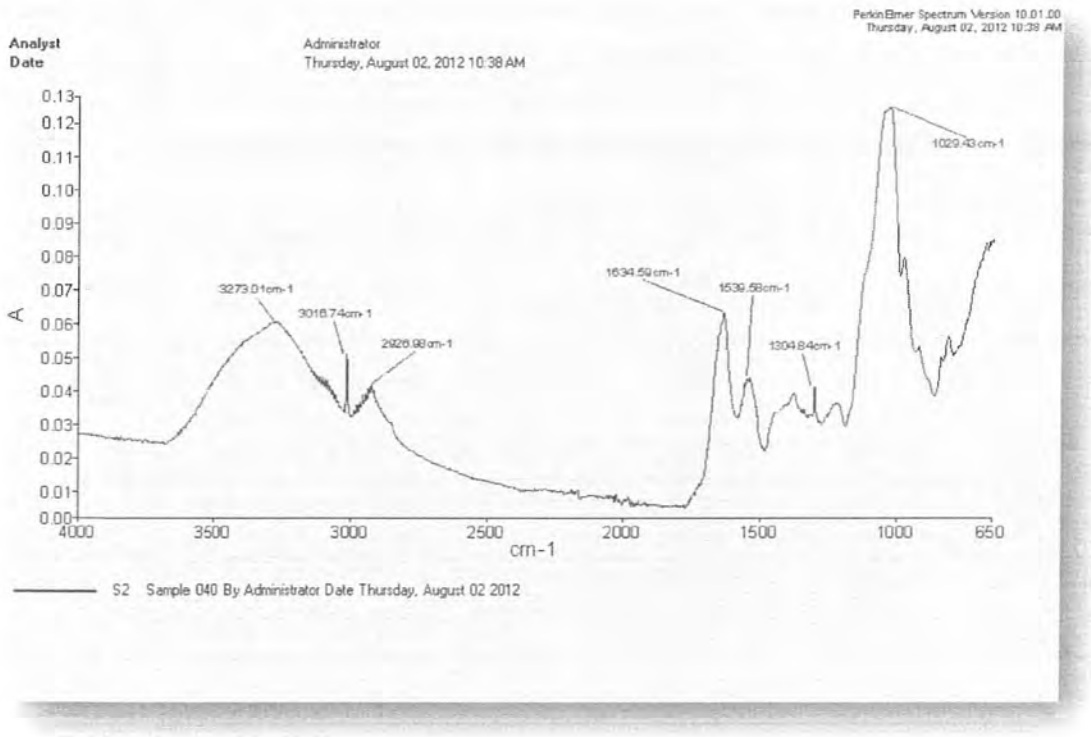


Figure 5: ATR-FTIR spectra of EPS of S2 strain

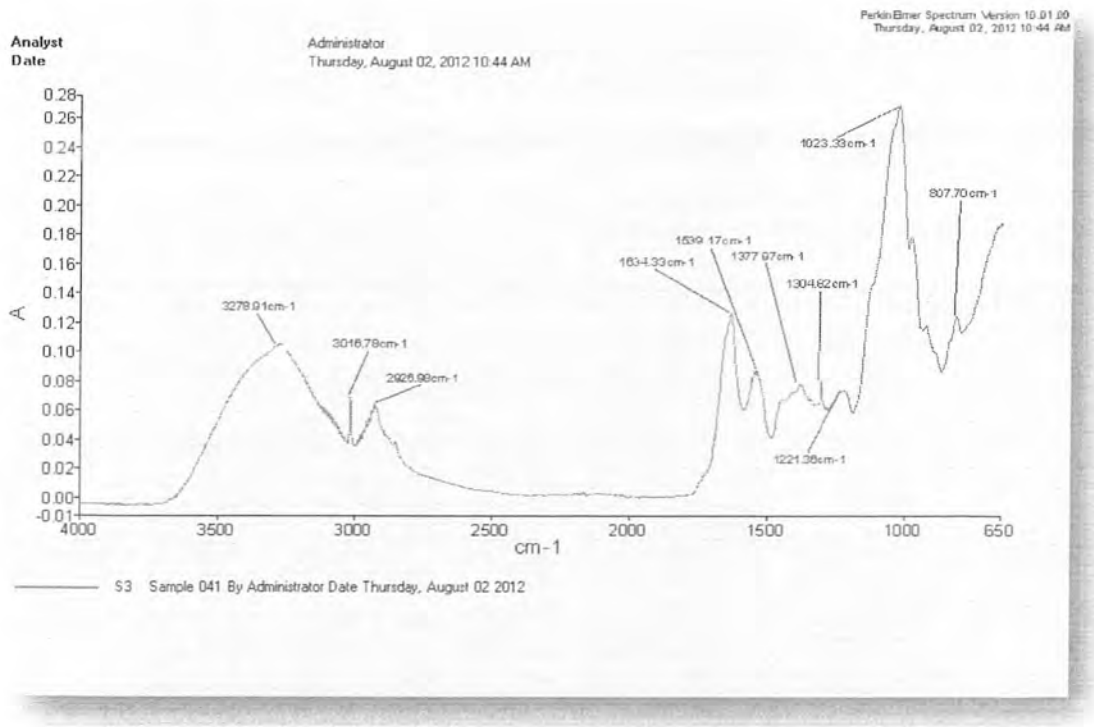
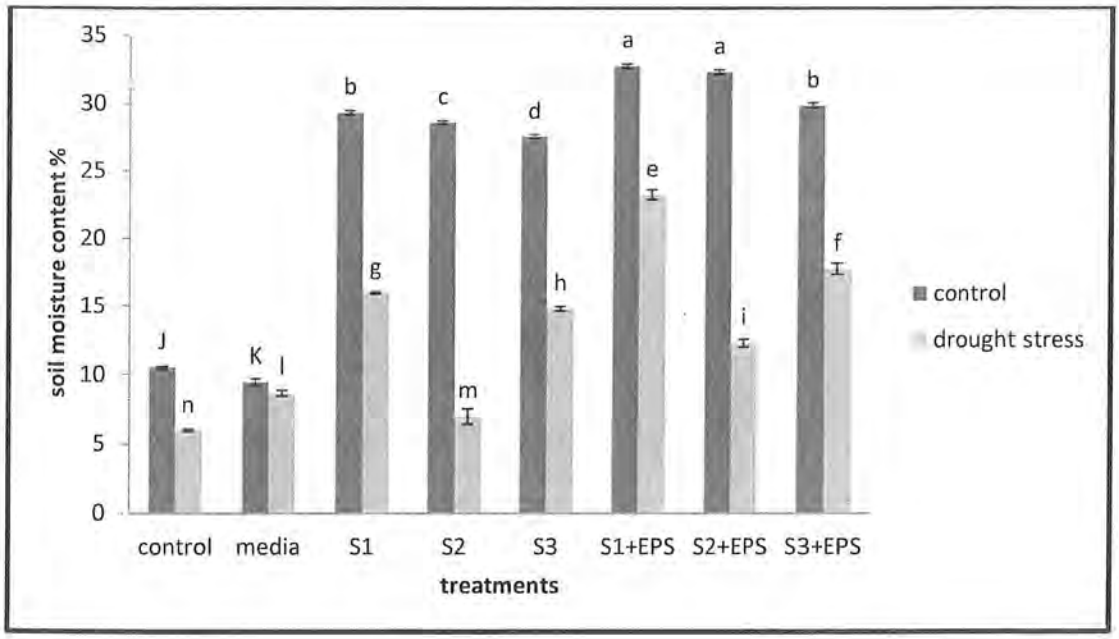


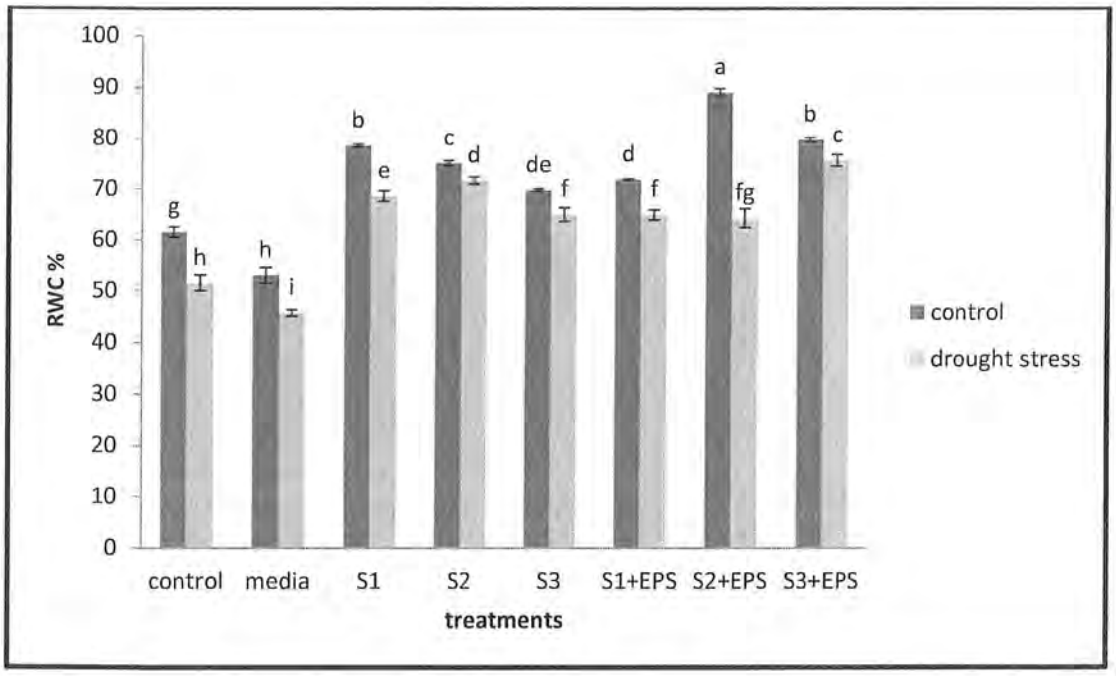
Figure 6: ATR-FTIR spectra of EPS of S3 strain



LSD value = 0.6063
P value = 0.0000

Figure 7: Effect of EPS producing bacterial strains on soil moisture contents in both drought stress and non-stress. S1 (strain 1 alone), S2 (strain 2 alone), S3 (Strain 3 alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

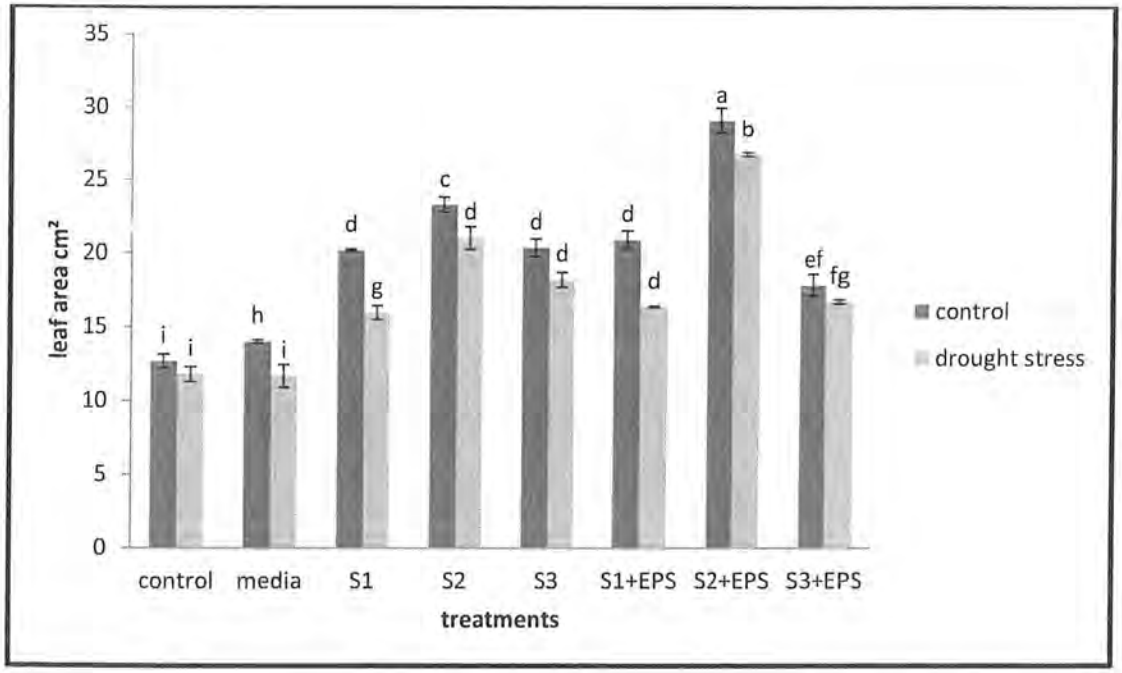
S1 (isolated from soil sample collected from arid region of Ghotki Sindh) **S2** and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 2.8720
P value = 0.0000

Figure 8: Effect of EPS producing bacterial strains on relative water contents in both drought stress and non-stress. S1 (strain 1 alone), S2 (strain 2 alone), S3 (Strain 3 alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

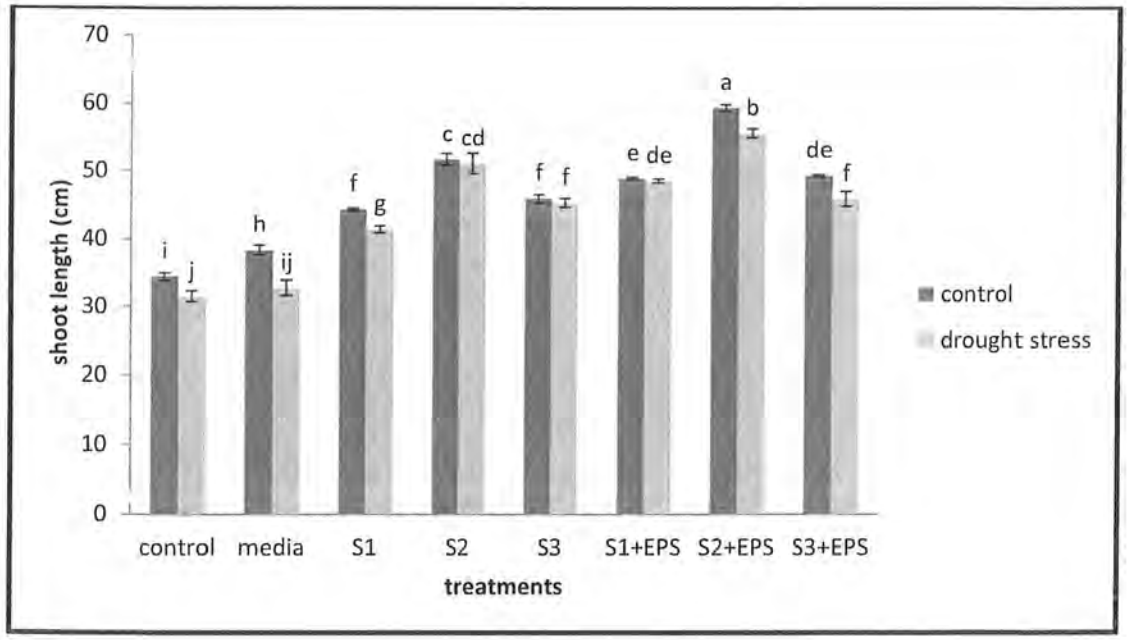
S1 (isolated from soil sample collected from arid region of Ghotki Sindh) **S2** and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 1.2641
P value = 0.0000

Figure 9: Effect of EPS producing bacterial strains on leaf area in both drought stress and non-stress. S1 (strain 1alone), S2 (strain 2alone), S3 (Strain 3alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

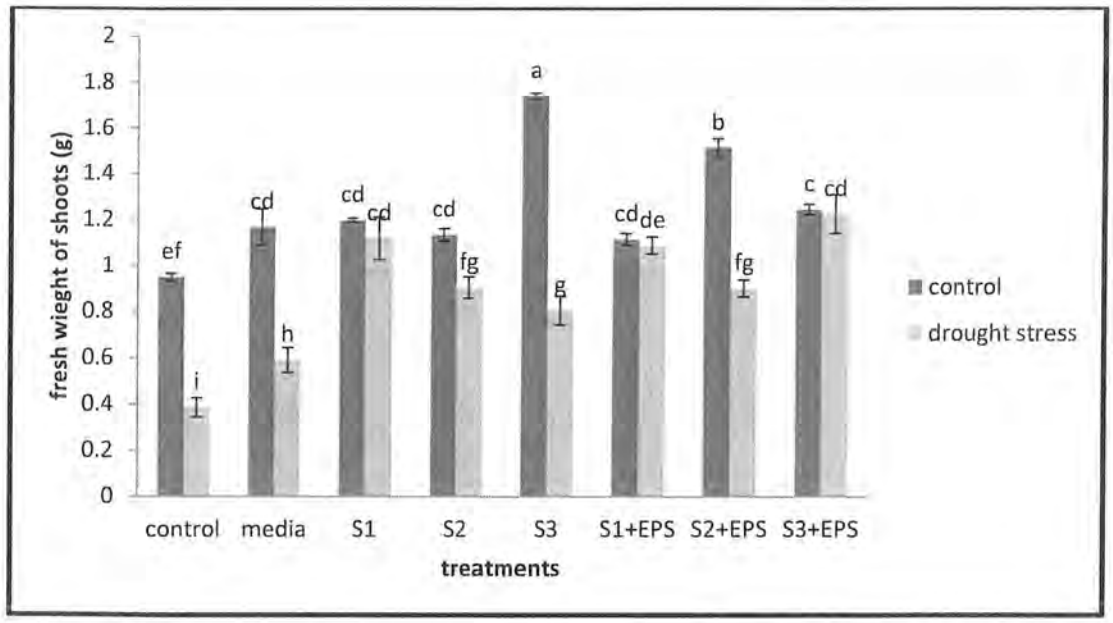
S1 (isolated from soil sample collected from arid region of Ghotki Sindh) **S2** and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 1.9913
P value = 0.0000

Figure 10: Effect of EPS producing bacterial strains on shoot length in both drought stress and non-stress. S1 (strain1 alone); S2 (strain2 alone); S3 (Strain3 alone); S1+EPS (strain1 in combination of its exopolysaccharides); S2 (strain2 in combination of its exopolysaccharides); S3 (strain3 in combination of its exopolysaccharides).

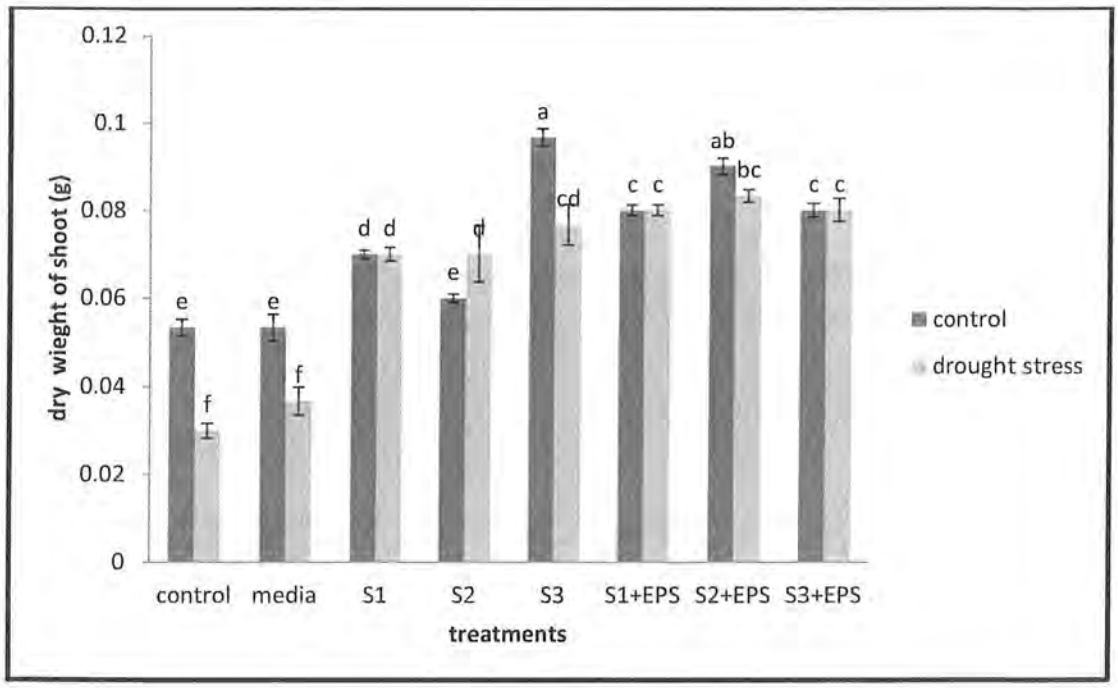
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 0.1448
P value = 0.0000

Figure 11: Effect of EPS producing bacterial strains on fresh weight of shoots in both drought stress and non-stress. S1 (strain 1alone), S2 (strain 2alone), S3 (Strain 3alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

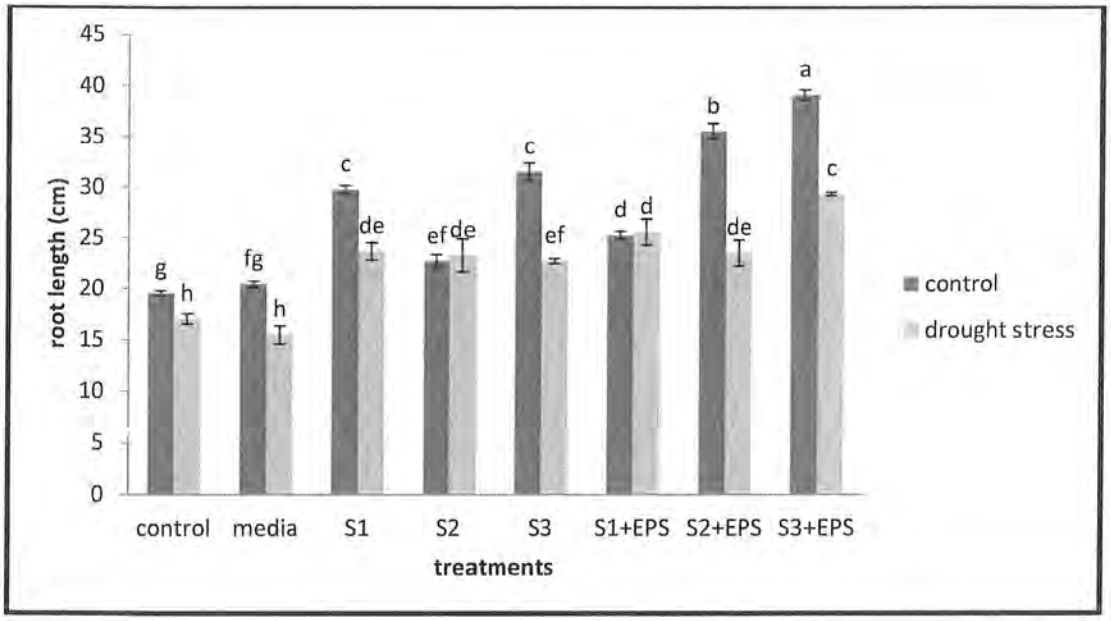
S1 (isolated from soil sample collected from arid region of Ghotki Sindh) **S2** and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 7.140
P value = 0.0000

Figure 12: Effect of EPS producing bacterial strains on dry weight of shoots in both drought stress and non-stress. S1 (strain1 alone), S2 (strain2 alone), S3 (Strain3alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

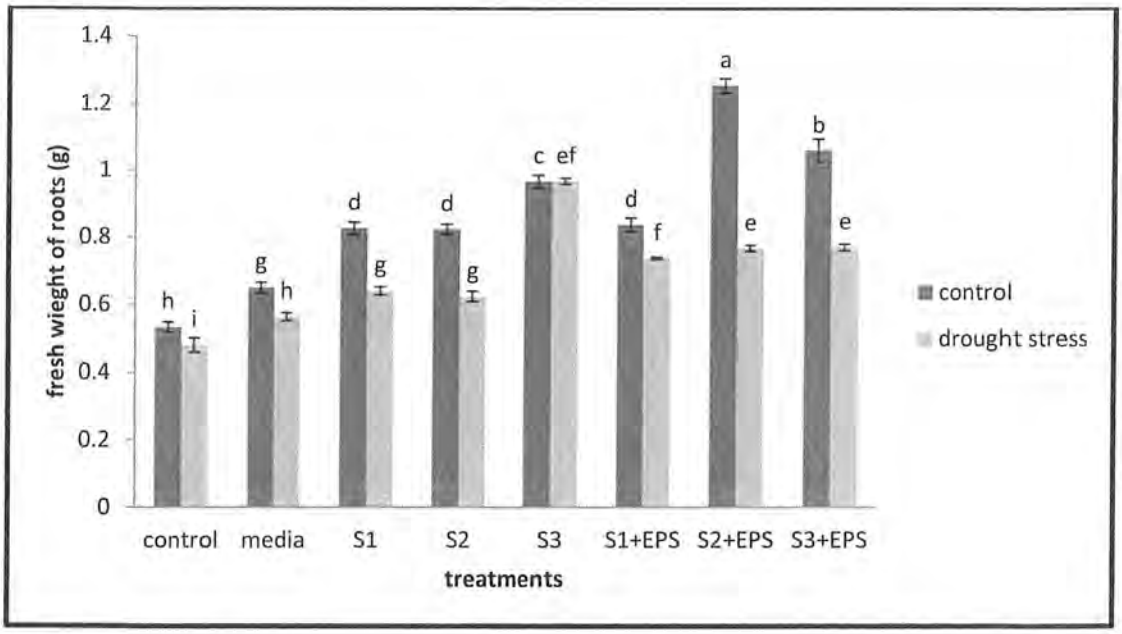
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 2.3110
P value = 0.0000

Figure 13: Effect of EPS producing bacterial strains on root length in both drought stress and non-stress. S1 (strain1 alone); S2 (strain2 alone); S3 (Strain3 alone); S1+EPS (strain1 in combination of its exopolysaccharides); S2 (strain2 in combination of its exopolysaccharides); S3 (strain3 in combination of its exopolysaccharides).

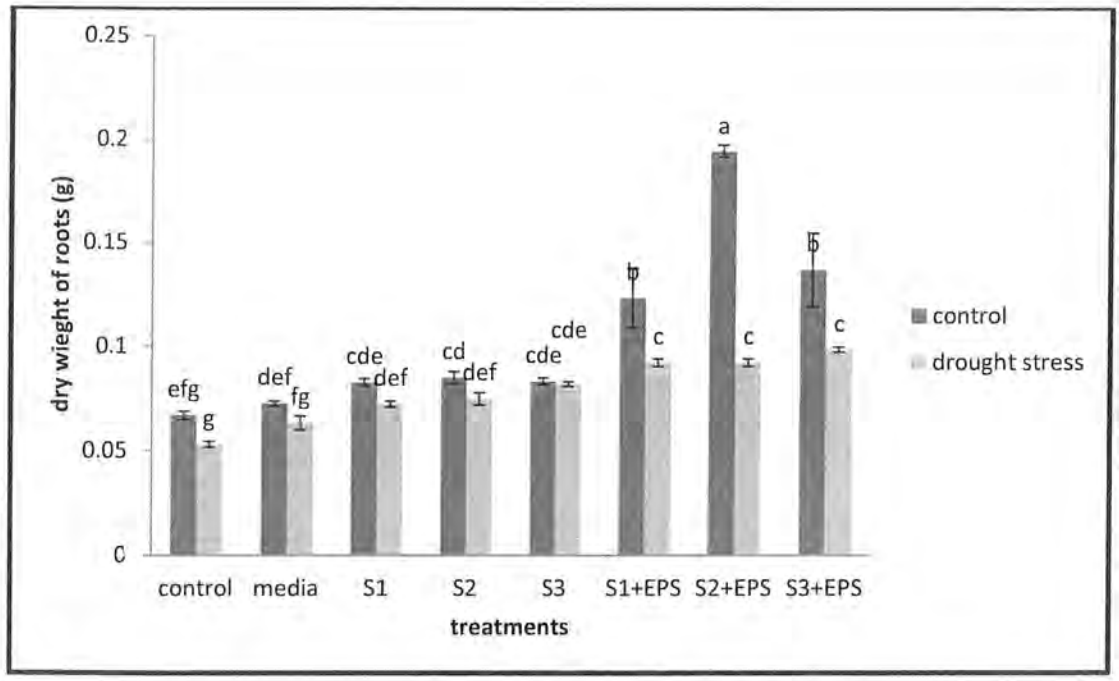
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 0.0431
P value = 0.0000

Figure 14: Effect of EPS producing bacterial strains on fresh weight of roots in both drought stress and non-stress. S1 (strain1alone),S2 (strain2 alone), S3 (Strain3alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

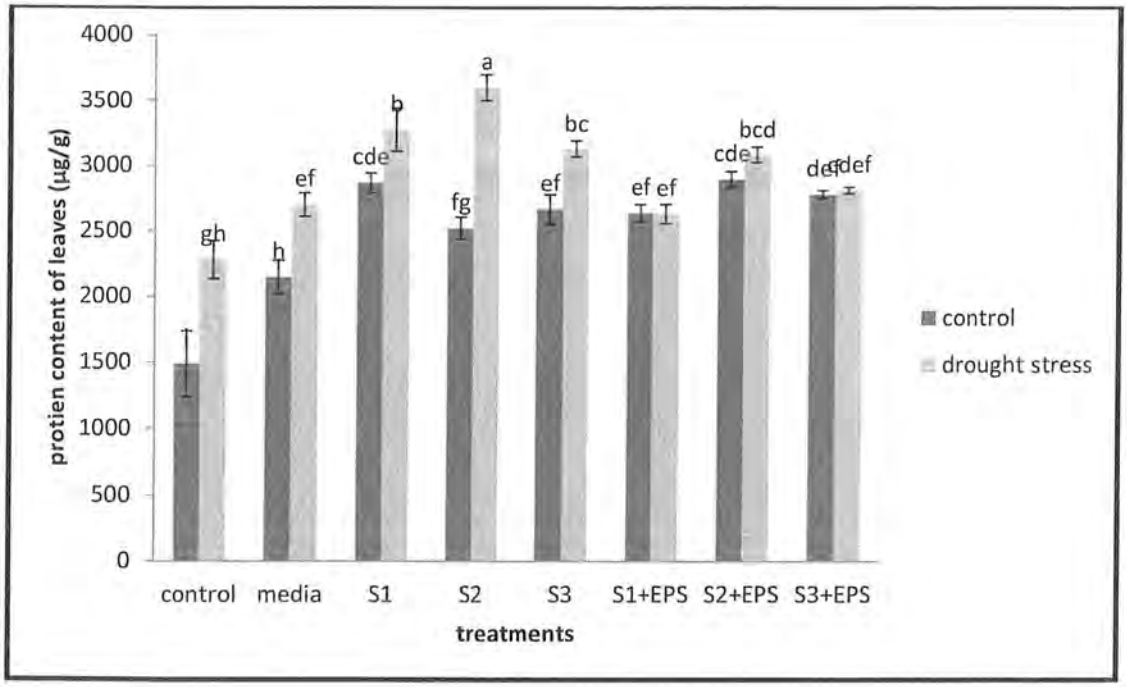
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 0.0171
P value = 0.0000

Figure 15: Effect of EPS producing bacterial strains on dry weight of roots in both drought stress and non-stress. S1 (strain1alone),S2 (strain2alone), S3 (Strain3alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

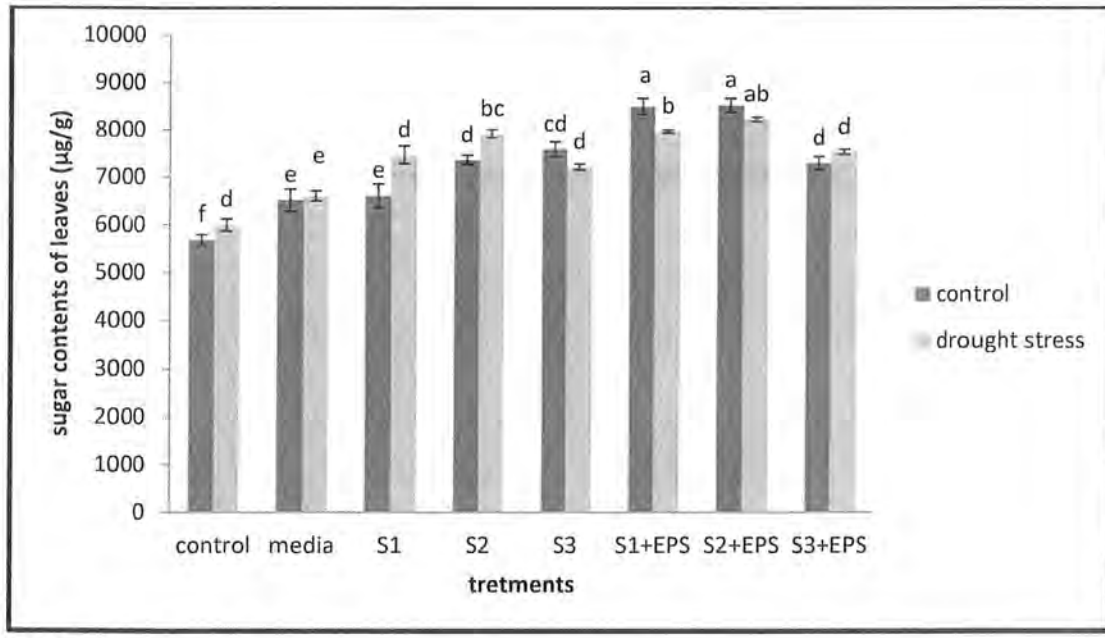
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 323.93
P value = 0.0000

Figure 16: Effect of EPS producing bacterial strains on protein content of leaves in both drought stress and non-stress. S1 (strain1 alone); S2 (strain2alone); S3 (Strain3 alone); S1+EPS (strain1 in combination of its exopolysaccharides); S2 (strain2 in combination of its exopolysaccharides); S3 (strain3 in combination of its exopolysaccharides).

S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)

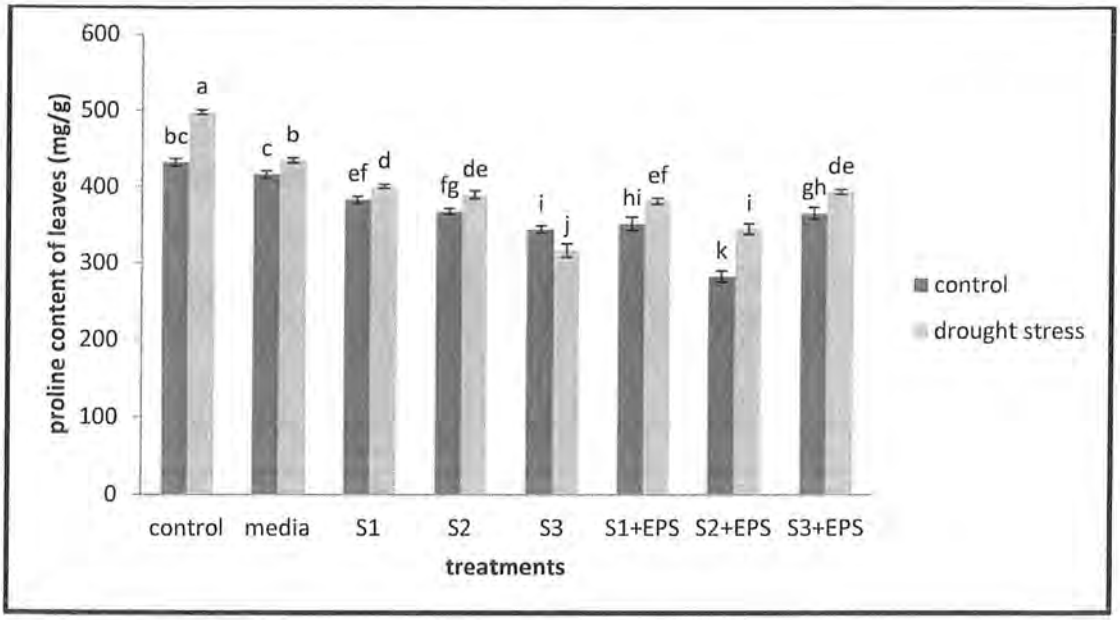


LSD value = 380.87

P value = 0.0000

Figure 17: Effect of EPS producing bacterial strains on sugar contents of leaves in both drought stress and non-stress. S1 (strain1 alone), S2 (strain2 alone), S3 (Strain3alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

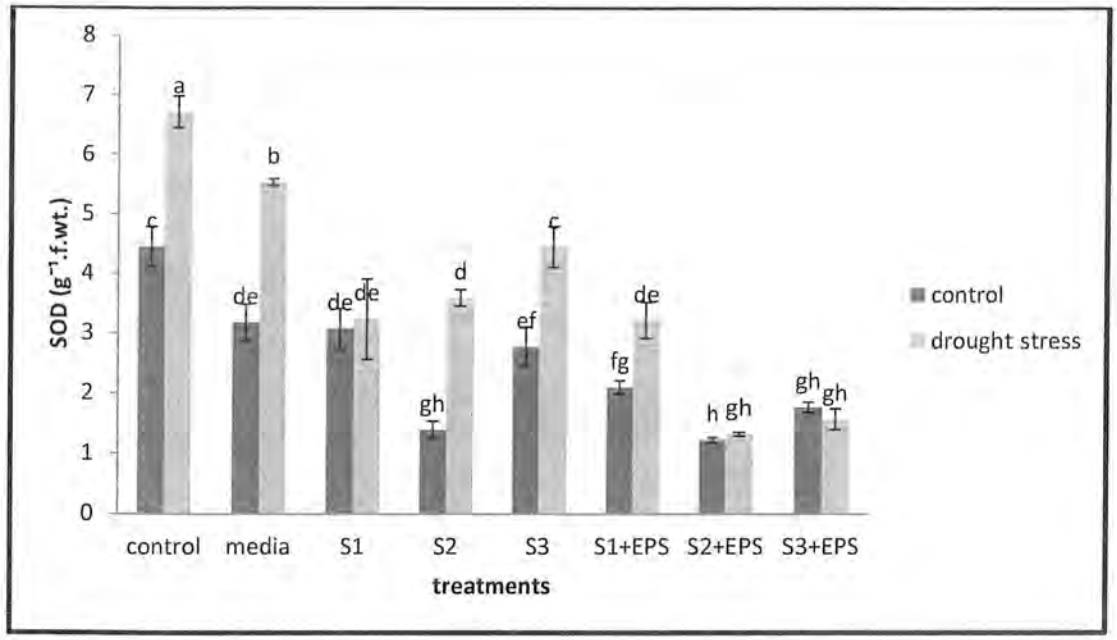
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 15.722
P value = 0.0000

Figure 18: Effect of EPS producing bacterial strains on proline contents of leaves in both drought stress and non-stress. S1 (strain1 alone), S2 (strain2 alone), S3 (Strain3 alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

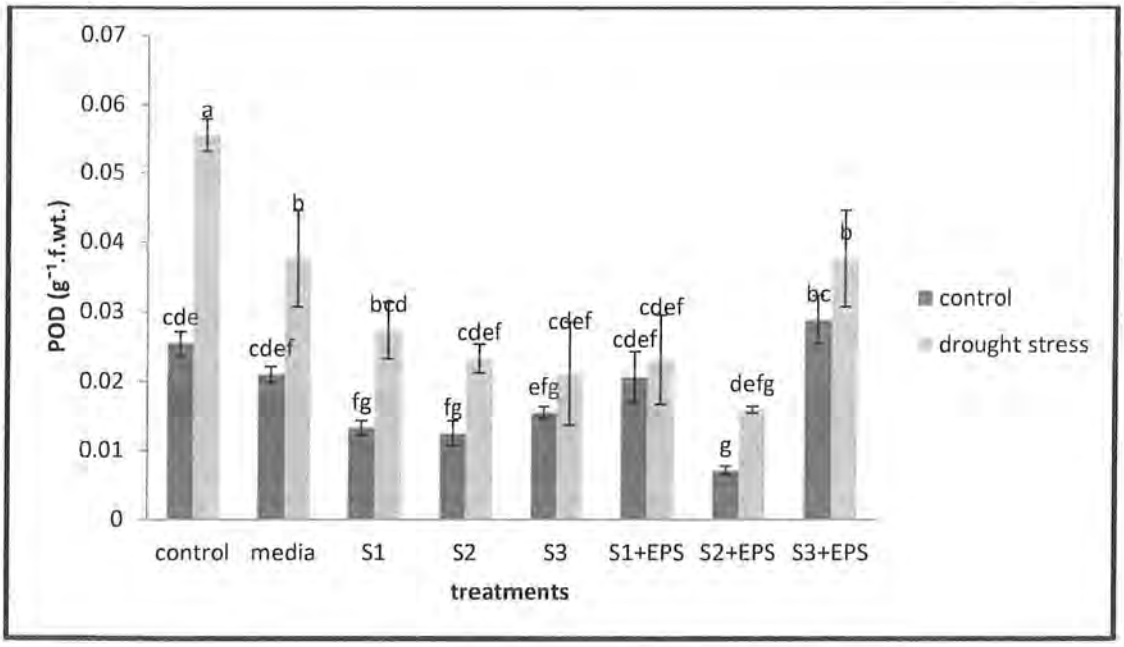
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 0.8118
P value = 0.0000

Figure 19: Effect of EPS producing bacterial strains on superoxide dismutase activity (SOD) of leaves in both drought stress and non-stress. S1 (strain1alone), S2 (strain2 alone), S3 (Strain3 alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

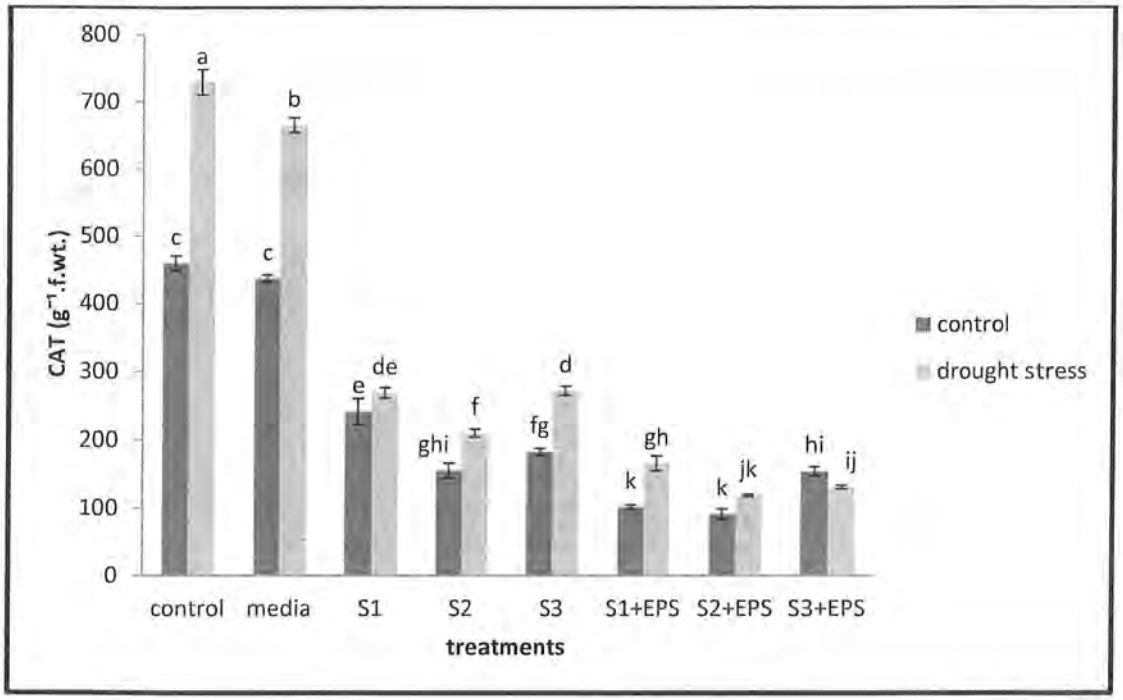
S1 (isolated from soil sample collected from arid region of Ghotki Sindh)
S2 and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



LSD value = 0.0115
P value = 0.0000

Figure 20: Effect of EPS producing bacterial strains on Peroxidase activity (POD) of leaves in both drought stress and non-stress. S1 (strain1 alone), S2 (strain2 alone), S3 (Strain3 alone), S1+EPS (strain1 in combination of its exopolysaccharides), S2 (strain2 in combination of its exopolysaccharides), S3 (strain3 in combination of its exopolysaccharides).

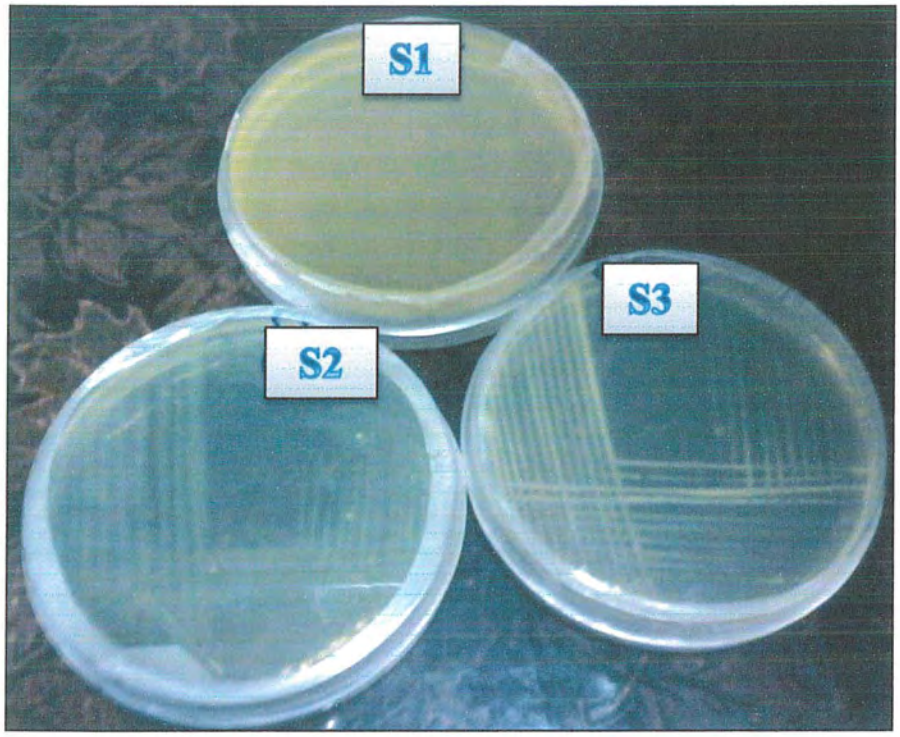
S1 (isolated from soil sample collected from arid region of Ghotki Sindh) **S2** and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



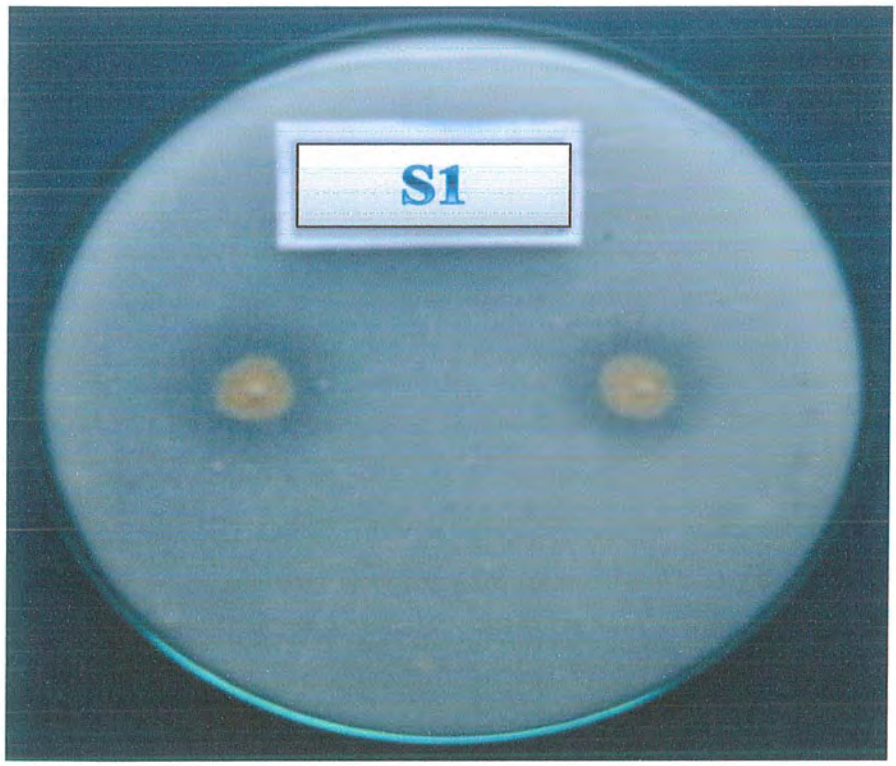
LSD value = 28.140
P value = 0.0000

Figure 21: Effect of EPS producing bacterial strains on catalase (CAT) activity of leaves in both drought stress and non stress. S1 (strain 1 alone), S2 (strain 2 alone), S3 (strain 3 alone), S1+EPS (strain 1 in combination of its exopolysaccharides), S2 (strain 2 in combination of its exopolysaccharides), S3 (strain 3 in combination of its exopolysaccharides).

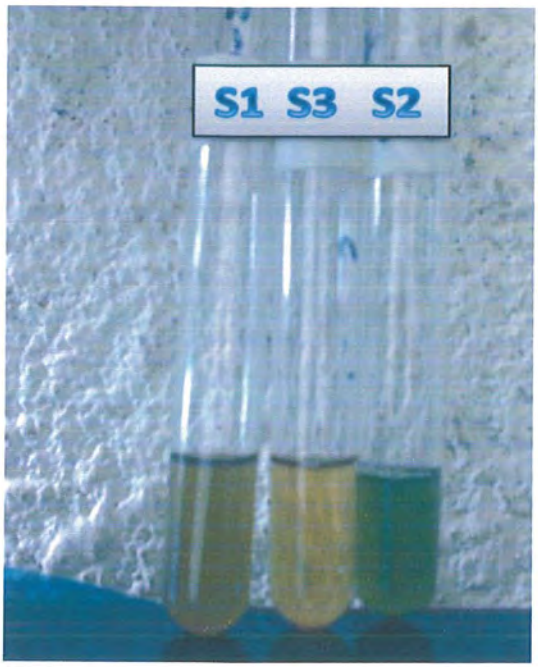
S1 (isolated from soil sample collected from arid region of Ghotki Sindh) **S2** and **S3** (isolated from soil sample collected from semi-arid region of Kallar Sayedan)



48h old colonies of EPS producing bacteria

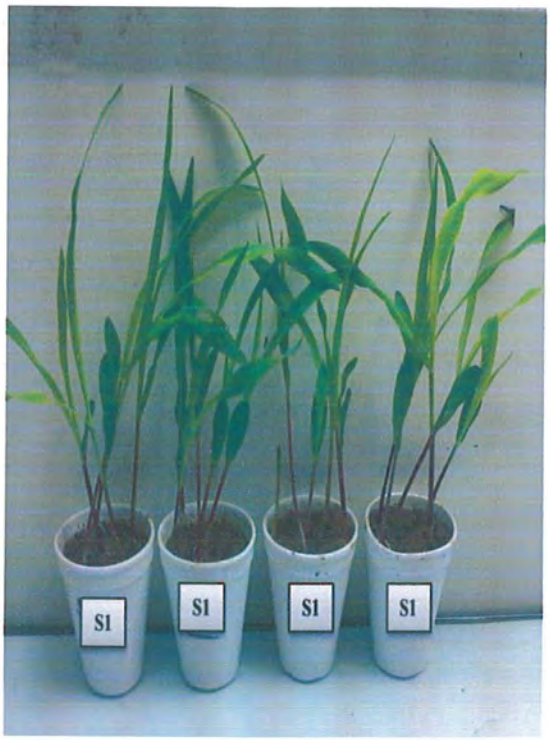


Phosphate solubilization by strain S1

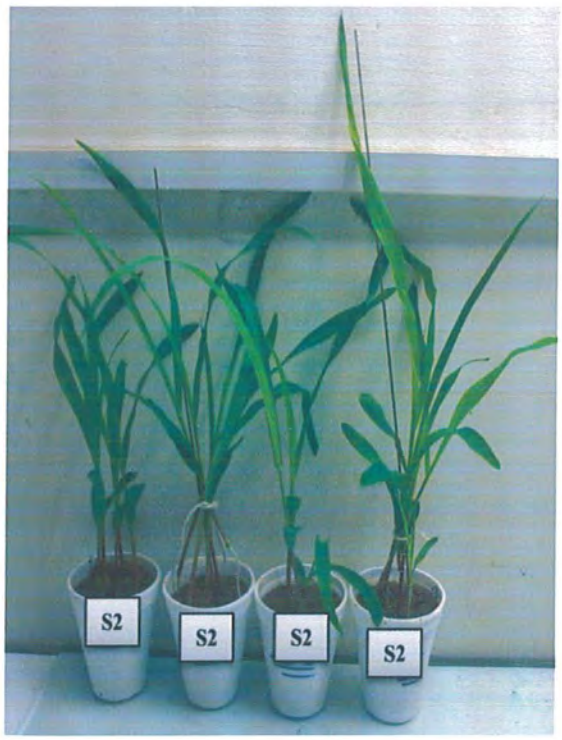


Bacterial culture after centrifugation

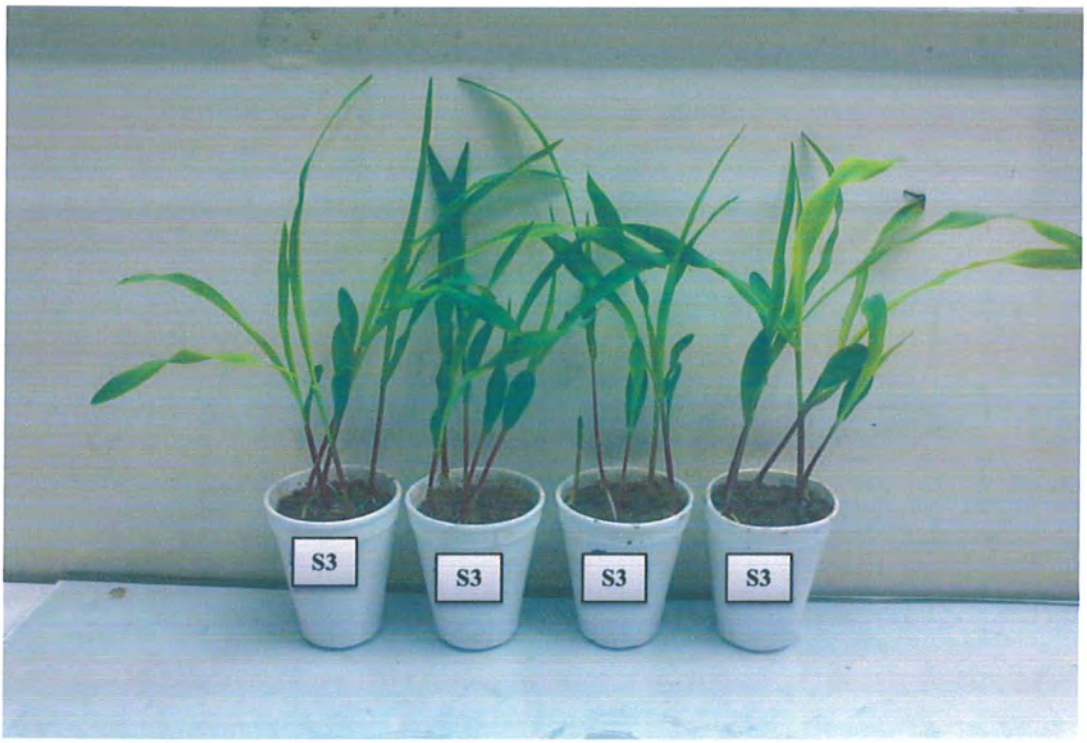
Bacterial culture after the addition of Ethanol



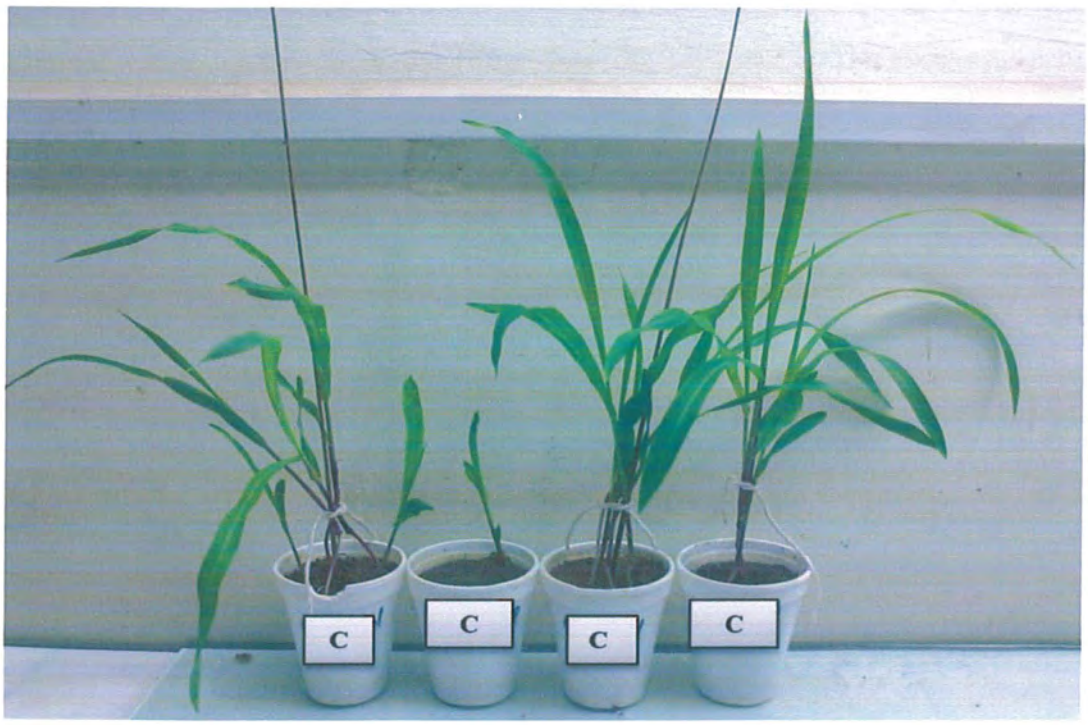
Maize inoculated with S1 strain



Maize inoculated with S2 strain



Maize inoculated with S3 strain



Un-inoculated maize

DISCUSSION

In the present study maize plants were inoculated with the (EPS) exopolysaccharides producing bacteria ***Proteus penneri***, ***Pseudomonas aeruginosa*** and ***Alcaligenes faecalis*** alone and in combination to their respective exopolysaccharides to alleviate the adverse effects of drought on the growth and physiology of maize.

The EPS producing bacterial strains were selected on the basis of formation of mucoid colony. All these bacterial strains ***Proteus penneri*** (S1), ***Pseudomonas aeruginosa*** (S2) and ***Alcaligenes faecalis*** (S3) produced mucoid colony on LB agar media. (Chan *et al.* 1984; Jankins *et al.* 1992) also studied with ***Pseudomonas aeruginosa*** and ***Escherichiacoli*** respectively and selected these microorganisms as EPS producing organisms on the basis of mucoid colony. According to Robertson and Firestone (1992) production of mucus related to the exopolysaccharides production in ***Pseudomonas*** bacteria.

Production of pigmentation in all bacterial strains was also detected during selection process both in L.B agar and L.B broth. (Nair *et al.* 1992; Arrage *et al.* 1993) demonstrated that production of pigmentation by exopolysaccharides producing bacteria was related to resistance in these bacteria against toxic substances in contaminated water.

The exopolysaccharides extracted contains total carbohydrates (neutral sugars), proteins and very small amount of uronic acids. According to Bramhachari and Dubey (2006) exopolysaccharides produced by ***Vibrio harveyi*** was acidic due to presence of neutral sugars, proteins, uronic acids.

Exopolysaccharides produced by ***Proteus penneri***, strain (S1) show a good stability for emulsion. In all cases dialysed lyophilized exopolysaccharides show greater stability than non-dialysed. Dialysed exopolysaccharides of ***Proteus penneri*** (S1) retained 46.88% and 34.38% after 30 and 60 min respectively. The emulsion's stability by exopolymer from ***Proteus penneri*** (S1) strain is comparable with ***Vibrioharveyi*** VB23 bacterial isolate reported earlier by (Bramhachari *et al.* 2006)

According to FTIR spectrum of EPS of present study may have cyclic carbohydrates of polysaccharides, proteins and uronic acid. Some authors like (Schmitt and Flemming, 1998; Sheng *et al.*2006) arbitrarily divided the spectrum obtained from

FTIR into several characteristic bands representing different compounds. According to them peaks within (1) 2956-2850 cm^{-1}(aliphatic CH symmetric and asymmetric stretching in CH3 and CH2) as fatty acid region; (2) 1652-1648 cm^{-1} (C=O asymmetric stretching of –NH-CO-R and/or N-H bending of H_2N-CO-R (Amide I)) and 1550-1548 cm^{-1} (N-H bending of –NH- (Amide II), as protein region; (3) 1300-1245 cm^{-1} (P=O stretching of phosphate PO_4^{3-}), as nucleic acid region; (4) 1085-800 cm^{-1} as polysaccharide region. According to them their EPS have cyclic carbohydrates of polysaccharides, uronic acid and proteins.

Absorption peak at 1,076 and 1,122 $cm^{-1}$are characterstic of uronic acid. It is well known that the presence of acidic functional groups (e.g. carboxyl) uronic acid (glucuronic acid) and of EPS involved in metal sequestration (Bramhachari and Dubey 2006; Bhaskar and Bhosle 2005; Pal and Paul 2008). Hydroxyl and amine groups of EPS were predominant in lead sequestration and thus protect ***E. cloacae*** strain P2B from lead toxicity (Pal and Paul 2008; Iyer *et al.* 2004). The presence of acidic sugars in the EPS may be important, helpful in the heavy metal-binding properties of this polymer. ***Vibrioharveyi*** excrete EPS that is highly surface active, which is probably due to polymer containing uronic acid-containing (Bramhachari *et al.* 2006). Chemical composition of EPS produced by ***Pseudomonas aeruginosa*** have analysed by Grobe *et al.* (1995).

Based on the charged groups EPS are classified into neutral polymers or acidic polymers but majority of the EPS are considered as polyanionic due to the presence of uronic acids, ketal linked pyruvate, phosphate and sulphated groups (Sutherland, 2001). FT-IR analysis of the three bacterial EPS also indicated the presence of many charged groups. The charged groups of the EPS are involved in biological and physiological functioning of the EPS (Sutherland, 2001) and the EPS producing rhizospheric bacteria, it can be expected that these EPS would also play important role in mechanical and structural stability of the salt-affected soils and soil aggregation around plant roots (Alami *et al.* 2000, Amellal *et al.* 1998).

Soil moisture content was highest in case of soil inoculated with all strains in combination with their respective exopolysaccharides both in drought stress and non-stress. Treatment of ***Proteus penneri*** (S1) and ***Pseudomonas aeruginosa*** (S2) with their respective EPS showed maximum increase in soil moisture. The high affinity of EPS for

water provides protection for bacteria under drought stress. An EPS matrix surrounding a bacterial colonymay slow down the drying process, thereby increasing the time available for metabolic adjustment. Exopolysaccharides holds the water in the soil surrounding the plant roots and soil dries more slowly with EPS; also protect the bacteria from desication and fluctuations in water potential (Hepper, 1975; Wilkinson, 1958). Roberson and Firestone (1992) suggested that the increase in EPS production by ***Pseudomonas*** during desiccation is required to ensure protection of this strain in sandy soil.

Development of optimal leaf area isimportant to photosynthesis and dry matter yield. Seeds inoculated with all strains in combinations with its exopolysaccharides showed maximum increase in leaf area as compared to control both in stress and non-stress. Inoculations of strain ***Pseudomonas aeruginosa*** (S2) with its EPS give maximum increase in leaf area under non-stress condition and also under drought condition.

Root length in all treatments was higher in both drought stress and non-stress conditions. The importance of root systems in acquiring water has long been recognized. The development of root system increases the water uptake and maintains requisite osmotic pressure in ***Phoenix dactylifera*** (Djibril *et al.* 2005). ***Pseudomonas spp.*** increased total microbial activity, shoot and root length, total dry weight (Ahn *et al.* 2007). The inoculation with EPS-producing bacteria could cause the development of much better root system; which subsequently increases the shoot growth (Nemat *et al.* 2012). All treatments have increased effect on shoots length in both drought stress and non-stress than non-inoculated control. Inoculation of ***Pseudomonas aeruginosa*** (S2) show maximum increase in shoot length Inoculation of ***Pseudomonas putida*** strain GAP-P45 increased total shoot length studied by (Sandhya, 2009).

Root and shoot fresh weight of all treated plants was significantly higher in comparison with un-inoculated control. Dry weight of roots and shoots was also higher in plants inoculated with EPS producing bacteria. Plants inoculated with strain ***Proteus penneri***, S1 have same value of fresh and dry weight of shoots in both stress and non-stress conditions show the resistance to drought effect. Plant growth promoting bacteria influence growth of plant through various mechanisms and several scientists have reported healthy effect of PGPR inoculation on various crops (Khalid *et al.* 2004).Plants inoculated with strain ***Pseudomonas aeruginosa*** (S2) show maximum increase in root

fresh and dry weight. ***Pseudomonas fluorescens*** biotype G (ACC-5), and ***Pseudomonas putida*** biotype A (Q-7) improved fresh and dry weight of plants (Zahir *et al.* 2008).

In this study, RWC of all the treatment was found to be significant as compared with the un-inoculated control. Treatment of ***Pseudomonas aeruginosa*** (S2) show maximum increase in RWC of plant leaves. Plant having higher yields under drought stressshould have high RWC and decrease in RWC in plants under drought stress may depend on plant vigor reduction and have been observed in many plants (Liu *et al.*2002). The plants inoculated with ***Pseudomonas putida*** strain GAP-P45 exopolysaccharides producing bacteria showed high RWC of leaves (Sandhya *et al.* 2009).

PGPR application significantly increased protein content when compared with the un-inoculated control and inoculated stressed control. Protein content was significantly greater in all treatments as compared to the un-inoculated control. Application of ***Pseudomonas aeruginosa*** (S2) show maximum increase in protein content of leaves.The increased protein content prevents denaturation and decomposition of the cellular molecules and components especially during a biotic stress conditions (Campell and Close, 1997).

Inoculation of EPS producing ***Pseudomonas*** sp. increased soluble sugar content in drought stress seedlings than un-inoculated seedlings indicate that PGP ***Pseudomonas*** sp. helps in degrading the starch content and biosynthesis of more sugar that is helpful for osmotic adjustment to alleviate that effect of drought stress. In case of un-inoculated seedlings under drought stress plant growth was affected due to the absence of PGP ***Pseudomonas*** sp. that in turn affected the biosynthesis and decreasing content of soluble sugar and starch (Sandhya *et al.* 2010).

The un-inoculated control plants, compared to the inoculated plants, under drought conditions had an increased activity of antioxidant enzymes SOD, POD and CAT and concentration of proline. Plants inoculated with ***Pseudomonas aeruginosa*** (S2) have maximum decrease in activity of antioxidant enzymes SOD, POD and CAT and concentration of proline. Plants Inoculated with PGPR strains under drought stress decreased activities of antioxidant enzymes. It is interesting to note that drought stress and antioxidant enzyme activity have significant interaction, but inoculation with plant

growth promoting rhizobacteria lessen the adverse effect of drought stress on the antioxidant enzymes activity (Hans and Lee, 2005).

CONCLUSION

It is concluded from present study that the bacterial isolates ***Proteus penneri*** (S1), ***Pseudomonas aeruginosa*** (S2) and ***Alcaligenes faecalis*** (S3) as exopolysaccharides producing bacteria proved to be best for drought tolerance in addition to their ability as plant growth promoting rhizobacteria. Exopolysaccharides producing bacteria has potential to impart drought tolerance in plants in combination to their potential as PGPR plant growth promoting rhizobacteria. A pronounced effect of inoculation of these exopolysaccharides EPS producing bacteria on growth of maize in a drought affected soil indicated their potential to be used as bio inoculums under drought-affected soil.

Since the chemical behavior of the exopolysaccharides with soil constituents is determined by their interaction with the inorganic ions (cations and anions), the interaction of exopolysaccharides obtained from EPS-producing bacteria native to the drought-affected soils with constituents of the soils in the presence of different cations should also be thoroughly studied.

References

Afzal A, Bano A (2008) *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *International Journal of Agriculture & Boilogy*, 1560-8530.

Aggarwal, P.K. and S.K. Sinha, (1983) Relationship between mother shoot and tillers as a criterion of selection for wide or specific adaptability to drought in wheat. *Zacker–Planzenb*, 152: 310–20

Ahn TS, Ka JO, Lee GH, Song HG (2007) Microcosm study for revegetation of barren land with wild plants by some plant growth-promoting rhizobacteria. *J Microbiol Biotechnol*, 17(1):52–57

Alami Y, Champolivier L, Merrien A, T Heulin T (2000) The role of *Rhizobium* sp.rhizobacterium that produces exopolysaccharide in the aggregation of the rhizospherical soil of the sunflower: Effects on plant growth and resistance to hydric constraint. *OCL - Oleagineux Corps Gras Lipides*, 6: 524-528.

Ali Sk Z, Sandhya V, Grover M, Kishore N, Rao LV, Venkateswarlu B (2009) *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. *Biology and Fertility of Soils*, 46:45–55.

Alvarez, M.I, R.J. Sueldo and C.A. Barassi (1996) Effect of *Azospirillum* on coleoptiles growth in wheat seedlings under water stress. *Cer. Res. Comm*, 24: 101–107.

Amellal N, Burtin G, Bartoli F and Heulin T (1998) Colonization of wheat rhizosphere by EPS producing *Pantoea agglomerans* and its effects on soil aggregation. *Appl Environ Microbiol*, 64: 37040-3747.

Ann Staudt (2009) IDENTIFICATION OF ENVIRONMENTAL FACTORS CRITICAL TO THE PRODUCTION OF EXOPOLYSACCHARIDES BY *RHIZOBIUM TROPICI* A Thesis Submitted to the Graduate School of the University of Notre Dame in Partial

Fulfillment of the Requirements for the Degree of Master of Science in Environmental Engineering.

Anonymous (**2000**) "Agricultural statistics of Pakistan", Government ofPakistan, Ministry of Food, Agriculture and Livestock, EconomicWing, Islamabad, p. 104.

Anselm Omoike and Jon Chorover (2004) Spectroscopic Study of Extracellular Polymeric Substances from Bacillus subtilis.*Biomacromolecules,*5:1219-1230

Anuar, A.R, Z.H. Shamsuddin, and O. Yaacob (1995) Contribution of legume- N bynodulated groundnut for growth of maize on an acid soil. *Soil Biol. Biochem,* 27:595–601.

Arnon, I (1972) Economic Importance of Maize, Crop production in dry regions.*Leonard Hill London,* 2:146

Arrage, A. A. Phelps, T. J., Benoit, R. E. & White, D. C. (1993) Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. *Appl. Environ. Microbio,* 59: 3545-3550.

Asada K (1992) Ascorbate peroxidase: a hydrogen peroxide-scavenging enzyme in plants. *Plant Physiology,* 85: 235–241.

Ashraf, M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ ExpBot,* 59:206–216.

Ashraf, M, S.H. Berge and O.T. Mahmood (2004) Inoculating wheat seedlings with exopolysaccharides producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fer. Soils,* 40: 157-162.

Bacilluspolymyxaon soil agregation in the wheat rhizosphere : preliminary examination. *Geoderma* 56: 479-490.

Bashan Y Holguin G, de-Bashan LE (2004) Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances. *Can J Microbiol* 50:521–577

Bashan, Y. (1991) Changes in membrane potential of intact soy- 115bean root elongation zone cells induced by *Azospirillum brasilense*. *Can J. Microbiol.* **37**: 958-963.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*, 39:205–207.

Beauchamp CO, Fridovich I (1971) Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44:276–287.

Bensalim S, Nowak J, Asiedu SK (1998) A plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato. *Am J Potato Res*, 75:145–152

Bezzate S, Aymerich S, Chambert R, Czarnes S, Berge O, Heulin T (2000) Disruption of the Paeni bacillus polymyxalevan sucrase gene impairs its ability to aggregate soil in thewheat rhizosphere. *Environ Microbiol*, 2:333–342

Bhaskar PV, Bhosle NB (2006) Bacterial extracellular polymeric substances (EPS) a carrier of heavy metals in the marine food-chain. *Environ Int*, 32:192–198

Bhaskar PV, Bhosle, NB (2005) Microbial extracellular polymeric substances in marine biogeochemical processes. *Curr. Sci.* 88(1):45-53.

Bhatti, G.R., R. Qureshi and M. Shah (2001) Ethnobotany of QadanWari of Nara Desert. *Pak J.Bot*, 33:801-812

Blum A, Johnson JW (1992) Transfer of water from roots into dry soil and the effect on wheat water relations and growth. *Plant Soil* 145:141–149

Borsani, O, Díaz, P. Agius, F. Valpuesta, V. and Monza, J. (2001) Water stress generates an oxidative streess through the induction of a specific Cu/Zn superoxide dismutase in *Lotus corniculatus* leaves. *Plant Sci.*161: 757-763.

Bubb, W. A, Urashima, T, Fujiwara, R, Sinnai, T, &Ariga, H. (1997) Structural characterisation of the exocellular polysaccharide produced by Streptococcus thermophilus OR 901.*Carbohydrate Research*, 301: 41–50.

Burke, J. J. and Mahan, J. R. (1991) Environmental regulation of cellular protection systems. InGausman H. W. (ed.) *Plant Biochemical Regulators,* 47-58.

Campbell SA, Close TL (1997) Dehydrin under water stress and its association with phenotypic traits. *Plant Physiology,* 47:61–74.

Cerning J (1990) Exocellular polysaccharides produced by lactic acid bacteria.FEMS *Microbiol.Rev,* 87: 113-130.

Chan, R., Lam, J. S. &Costerton, J. W. (1984) Influenceof culture conditions on expression of the mucoidmode of growth of *Pseudomonas aeruginosa. J. Clin.Microbiol.19*: 8-1.

Chandlee and Scandalios JG (1984) Analysis of variants affecting the catalase development program in maize scutellum. *Theoretical and Applied Genetics,* 69: 71–77.

Chang WS, van de Mortel M, Nielsen L, de Guzman GN, Li X, Halverson LJ (2007) Alginate production by *Pseudomonas putida* creates a hydrated microenvironment and contributes tobiofilm architecture and stress tolerance under water-limiting conditions. *J Bacteriol,* 189:8290–8299.

Chaves M.M., J.S. Pereira, J. Maroco, M.L. Rodriques, C.P.P. Ricardo, M.L. Osorio, I. Carvatho, T. Faria and C. Pinheiro (2002) How plantscope with water stress in the field photosynthesis and growth? *Ann. Bot*, 89: 907–916

Chen Y P, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities. *Applied Soil Ecology,* 3: 33-41.

Chen, J. and Dai, J. *Y. (*1994) Correlation among photosynthesis, lipid peroxidation and ultrastructural changes of mesophyll cells in corn leaves under water stress. *Maize Science (in Chinese),* 2:36-40.

Chenu C (1993) Clay or sand polysaccharide associations as models for the interface between microorganisms and soil: water-related properties and microstructure. *Geoderma,* 56:143–156.

Chenu C, Roberson EB (1996) Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. *Soil Biol Biochem*, 28:877–884.

Cheshire MV (1979) Nature and origin of carbohydrates in soils. Academic Press, London.

Close TJ (1996) Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiol Planta*, 97: 795–803.

COMTE S, GUIBAUD G and BAUDU M (2006) Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties Part I. Comparison of the efficiency of eight EPS extraction methods. *Enzyme Microb. Technol.* **38** (1-2) 237-245.

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR & Lappin-Scott HM (1995) Microbial biofilms. *Ann Rev Microbiol*,49: 711–745.

Critchley, W. and S. Klaus. 1991. A manual for the design and construction of water harvestingschemes for plant production. Rome: Food and Agriculture Organization of the United Nations.

D. Braun B. bohringer, and N. Eidam (1989) ATR-FTIR spectroscopy as a tool for studies of polymer-polymer miscibility.*Polymer Bulletin,* 21: 63-68.

De Vuyst L, Vanderveken F, Van De Ven S, Degeest B (1998). Production by and isolation of exopolysaccharides from *Streptococcus thermophilus*grown in a milk medium and evidence for their growth-associated biosynthesis.*J.of Appl. Micobiol*, 84: 1059- 1068.

Deka´nkova´ K, Luxova´ M, Gas˘para´kova´ O, Kolarovic˘ L (2004) Response of maize plants to water stress.*Biologia,* 13:151–155

Del Longo, 0. T, Gonzilez, C. A, Pastori, G. M. and Trippi, V. S. (1993) Antioxidant defences under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. *Plant and Cell Physiology,*34: 1023-1 028.

Del-Gallo, M. and A. Haegi (1990) Characterization and quantification of exocellular polysaccharide in *Azospirillum brasilense* and *Azospirillum lipoferum*. *Symbiosis*, 9: 155 161.

Dhillon, R.S., H.S. Thind, U.K. Saseena, R.K. Sharma and N.S. Malhi (1995) Tolerance to excess water stress and its association with other traits in maize. *Crop Improv*, 22(1): 22-28.

Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Science*, 135: 1–9.

Djibril, S, O.K. Mohamed, D. Diaga, D. Diégane, B.F. Abaye, S. Mauriceand B. Alain (2005) Growth and development of date palm (*Phoenixdactylifera*L.) seedlings under drought and salinity stresses. *African J. Biotechnol*, 4: 968–972.

Doco, T, Wieruszeski, J.-M, Fournet, B, Carcano, D, Ramos, P. & Loones, A (1990) Structure of an exocellular polysaccharide produced by Streptococcus thermophilus. *Carbohydrate Research,* 198: 313–321.

Dubois, M, Gilles, K.A, Hamilton, J.K, Rebers, P.A, and Smith, F (1956) Colorimetric method for determination of sugars and related substances. *Anal.Chem,* 28: 350.

Due nas-Chasco, M. T, Rodriguez-Carvajal, M. A, Tejero-Mateo, P, Espartero, J. L, Irastorza-Iberas, A, & Gil-Serano, A. M. (1998) Structural analysis of the exoploysaccharides produced by *Lactobacillus* spp G-77. *Carbohydrate Research,* 307: 125–133.

Duebel A, Gransee A, Merbach W. (2000) Transformation of organic rhizodeposits by rhizoplane bacteria and its influence on the availability of tertiary calcium phosphate. *J Plant Nutr Soil Sci,* 163: 387-392.

Dulbecco, R. et al. (1954) Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med,* 99 (2): 167-182

Edi-Premono, Moawad MA, Vleck PLG (1996) Effect of phosphate solubalizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Sciences,* 11:13-23.

Edwards AP, Bremmer JM (1967) Dispersion of soil particles by sonic vibrations. *J Soil Sci* 18:47–63

El-Komy, H.M., M.A. Hamdia and G.K.A. El-Baki (2003) Nitrate reductase in wheat plants grown under water stress and inoculated with *Azospirillum* spp. *Biol. Plant*, 46: 281–287.

F. Ganji Arjenaki, A. Morshedi, R. Jabbari (2012) Evaluation of Drought Stress on Relative Water Content, Chlorophyll Content and Mineral Elements of Wheat *(Triticum aestivum L.)* Varieties. *International Journal of Agriculture and Crop Sciences*, 4-11: 726-729.

Faber, E. J, van den Haak, M. J, Kamerling, J. P, & Vliegenthart, J. F. G. (2001b) Structure of the exopolysaccharide produced by *Streptococcus thermophilus* S3. *Carbohydrate Research,* 331: 173–182.

Faber, E. J, Zoon, P, Kamerling, J. P, & Vliegenthart, J. F. G. (1998) The exopolysaccharides produced by *Streptococcus thermophilus* Rs and Sts have the same repeating unit but differ in the viscosity of their milk cultures. *Carbohydrate Research,* 310: 269–276.

Faber, E. J. Kamerling, J. P, & Vliegenthart, J. F. G. (2001a) Structure of the exopolysaccharide produced by *Lactobacillu sdelbrueckii* subsp. *bulgaricus* 291. *Carbohydrate Research,* 331: 183–194.

Farooq, M, A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra (2009) Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev*, 29: 185–212.

Lei, Y., Yin, C. & Li, C. (2006) Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of Populus przewalskii. *PhysiologiaPlantarum*, 127: 182–191.

Lemoine, J, Chirat, F, Wieruszeski, J.-M., Strecker, G, Favre, N. & Neeser, J.-R. (1997) Structural characterisation of the exocellular polysaccharides produced by *Streptococcus thermophilus* Sfi39 and Sfi12. *Applied and Environmental Microbiology*, 63: 3512–3518.

Li, C.Y. and M.A. Castellano (1987) *Azospirillum* isolated from within sporocarps of the mycorrhizal fungi *Hebelomacrus tuliniforme*, *Laccarialaccata* and *Rhizopogonvinicolor*. *Trans Br Mycol Soc*, 88: 563-566.

Li, L. and Van Staden, J. (1998b) Effects of plant growth regulators on drought resistance of two maize cultivars. *South African Journal of Botany*, 64:116-120.

Liu Y, Fiskum G, Schubert D. (2002) Generation of reactive oxygen species by mitochondrial electron transport chain. *J. Neurochem*, 80: 780-787.

Liu, H, and Fang, H.H.P. (2002) Extraction of extracellular polymeric substances (EPS) of sludges. *J. Biotechnol*, 95: 249.

Liu, L. and He, R. T. (1995) Effects of water stress on membrane damage and changes of some related enzyme activities in leaf of maize seedling. *Journal of Guangzi Agricultural University (in Chinese)*.14:195-200.

Low, D, Ahlgren, J. A., Horne, D, McMahon, D. J, Oberg, C. J, & Broadbent, J. R. (1998) Role of *Streptococcus thermophilus* MR- 1C capsular exopolysaccharide in cheese moisture retention. *Applied and Environmental Microbiology*, 64: 2147–2151.

Lowry OH, Poesenbrough NJ, Fal AL, Randall RJ (1951) Protein measurement with folin phenol reagent. *The Journal of Biological Chemistry*,193 : 265-275.

M. L. H. Kaul, A. K. Bhan (1974) Studies on some genetic parameters of rice (*Oryza sativa L.*) *Theoretical and Applied Genetics* , pp 178-183

Manca de Nadra, M.C., A.M. Strasser de Saad, A.A. Pesce de Ruiz Holgado, and G. Oliver (1985) Extracellular polysaccharide production by *Lactobacillus bulgaricus* CRL 420. *Milchwissenschaft*, 40: 409-411.

Mantsch, H. H, Casal, H. L, and Jones, R. N. (1986) Spectroscopy of biological systems. Ed: Clark, R. and Hester, R. E, Willey & Sons, New York.

Marshall, V. M, Dunn, H, Elvin, M, Mc Lay, N, Gu, Y, & Laws, A. P. (2001a) Structural characterisation of the exopolysaccharides produced by *Streptococcus thermophilus* EU20. *Carbohydrate Research*, 331: 413–422.

Marshall, V. M, Laws, A. P, Gu, Y, Levander, F, Radstrom, P, DeVuyst, L, Degeest, B, Vaningelgem, F, Dunn, H, & Elvin, M. (2001b) Exopolysaccharide-producing strains or thermophilic lactic acid bacteria cluster into groups according to their EPS structure. *Letters in Applied Microbiology*, 32, 433–437.

Marulanda A, Porcel R, Barea JM, Azco´n R (2007) Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive Glomus species. *Microb Ecol* 54:543–552.

Matthysse AG & Kijne JW (1998) Attachment of Rhizobiaceae to plant cells. *The Rhizobiaceae, Molecular Biology of Model Plant- Associated Bacteria*, 235–249.

Mc Fadden (1980) Biochemical tests for identification of Medical bacteria. *Williams and Wilkins, Baltimore*, USA: 51-54.

McKeague JA (1978) Manual on soil sampling and methods of analysis. *Canadian Society of Soil Science*, 66-68.

McKee, G.W. (1964) A coefficient for computing leaf area in hybrid corn. *Agron. J*, 56:240-241.

McLean EO (1982) Soil pH and lime requirement Methods of soil analysis, Part 2: chemical and microbiological properties. *American Society of Agronomy*, 199-224.

Mendelson, R., and Mantsch, H. H. (1986) Fourier Transform Infrared studies of lipid-lipid interactions (Progress in protein-lipid interactions). Ed: A. W. And J. T. H. H., Elsevier, New York.

Miller KJ, Wood JM (1996) Osmoadoptation by rhizosphere bacteria. *Annu Rev Microbiol*, 50:101–136.

Mittleman, M.W. and Geesey, G.G. (1985) Copper-binding characteristics of exopolymers from a fresh water-sediment bacterium. *Appl Environ Microbiol* 49: 846–851.

Mohammad Hossein Ansary, HadiAsadi Rahmani, Mohammad Reza Ardakani, FarzadPaknejad, Davood Habibi and Saeed Mafakheri (2012) Effect of *Pseudomonas fluorescent* on Proline and Phytohormonal Status of Maize (*Zea mays* L.) under Water Deficit Stress. *Annals of Biological Research*, 3 (2):1054-1062

Mohammad HosseinAnsary, Hadi Asadi Rahmani, Mohammad Reza Ardakani, Farzad Paknejad, Davood Habibi and Saeed Mafakheri (2012) Effect of *Pseudomonas fluorescent* on Proline and Phytohormonal Status of Maize (*Zea mays* L.) under Water Deficit Stress. *Annals of Biological Research*, 3 (2):1054-1062

Morgan, J.W, Forster, C.F, and Evison, L. (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Res*, 24: 743.

Morizet, T., M. Pollucsek and D. Togola. (1983) Drought tolerance in four maize varieties (*Field Crops Abst*. 39: 306,1986),

Mundree S.G, Baker B, Mowla S, Peters S, Marais S, Wilingen C.V, Govender K, Maredza A, Muyanga S. Farrant J.M, Thomson J.A. (2002) Physiological and molecular insights into drought tolerance. *Afr.J. Biotechnol, 1*: 28–38.

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250.

Murphy J, Riley JR (1962) A modified solution method for determination of phosphate in natural water. *Analytica Chimica Acta*, 27:31-36.

Nagaoka M, Hashimoto S, Watanabe T, Yokokura T, Mori Y (1994) Anti-ulcer effects of lactic acid bacteria and their cell wall polysaccharides. *Biol Pharm Bull*. 17:1012–1017.

Nagy, Z, Tuba, Z, Zsoldos, F. and Erdei, L. (1995) COz-exchange and water relation responses of sorghum and maize during water and salt stress. *Journal of Plant Physiology*, 145: 539-544.

Nahas E. (1996) Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World J Microb Biotechnol*, 12: 18-23.

Nair, S, Chandramohan, D. & Bharathi, B. (1992) Differential sensitivity of pigmented and non-pigmented marine bacteria to metals and atibiotics. *Water Res*, *26*:431-434

Nakajima H, Toba T, Toyoda S (1995) Enhancement of antigen specific antibody production by extracellular slime products from slime-forming *Lactococcus lactis* subspecies *cremoris* SBT 0495 in mice. *Int J Food Microbiol*, 25:153–158.

Nakajima, H, Hirota, T. Toba, T, Itoh, T, & Adachi, S. (1992) Structure of the extracellular polysaccharide from slime-forming *Lactococcus lactis* subspecies *cremoris* SBT 0495.*Carbohydrate Research*, 224, 245–253.

Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R, Verma. D (2000) Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiol Lett*, 182:291- 296.

Navarini, L, Abatangelo, A, Bertocchi, C, Conti, E. Bosco, M, &Picotti, F. (2001) Isolation and characterisation of the exopolysaccharides produced by *Streptococcus thermophilus* SFi20. *Biological Macromolecules*, 28: 219–226.

Nemat M. Awad, lAzza Sh. Turky, Magdi T. Abdelhamid, Magdy Attia (2012) Ameliorate of Environmental Salt Stress on the Growth of *Zea mays* L. Plants By Exopolysaccharides Producing Bacteria. *Journal of Applied Sciences Research*, 8(4): 2033-2044.

Neyra, C.A, A. Atkinson and O. Olubayi (1995) Coaggregationof *Azospirillum* with other bacteria: basis for functional diversity. In: *Azospirillum* VI and related microorganisms, genetics– physiology–ecology. (Eds.): I. Fendrik, M. DelGallo, J. Vanderleyden, M. de Zamaroczy. NATO ASIseries, series G. *Springer, Berlin,* pp. 429-439.

Nielsen, P.H., and Jahn, A. (1999) Extraction of EPS. In J. Wingender,H.-C.Flemming, and T.R. Neu, Eds., *Microbial Extracellular Polymeric Substances: Characterization, Structure, and Function.* Berlin: Springer, p. 49.

Oades JM (1993) The role of biology in the formation, stabilization and degradation of soil structure. *Geoderma* 56:182–186

Oda M, Nakamura S, Komatsu S, Kambe M, Tsuchiya F, Komiyama K, Umezawa I (1982) Physiological activities of polysaccharide produced by *Lactobacillus* sp. *Jpn J Antibiot,* 35:2748–2754.

Okon, Y. and R. Itzigsohn (1992) Poly-β-hydroxybutirate metabolism in *Azospirillum* brasilense and the ecological role of PHB in the rhizosphere. FEMS. *Microbiol. Lett,* 103: 131-139

Osang Alfiana, L.O. and M. Alexander (1982) Differences among cowpea *Rhizobium* in tolerance to high temperature and desiccation in soil. *Appl Environ Microbiol,* 43: 435–439.

P.V. Bramhachari and S.K. Dubey (2006) Isolation and characterization of exopolysaccharides produced by Vibrio harveyi strain VB23 Laboratory of Bacterial Genetics & Environmental Biotechnology, Department of Microbiology,

Pal A, Paul AK (2008) Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. *Indian J Microbiol,* 48:49–64.

Pereyra, M.A, C.A. Zalaza and C.A. Barassi (2006) Root phospholipids in *Azospirillum* inoculated wheat seedlings exposed to water stress. *Plant Physiol. Biochem,* 44:873–87.

Peter Jeffries · Silvio Gianinazzi · Silvia Perotto ·KatarzynaTurnau · Jos_-Miguel Barea (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol Fertil Soils* 37:1–16

Postma J, Walter S, Van Veen JA (1989) Influence of different initial soil moisture contents on the distribution and population dynamics of introduced Rhizobium leguminosarum biovartrifolii. *Soil Biol Biochem* 21:437–442

Prado FE, Boero C, Gallardo M, Gonzalez JA (2000) Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* Willd seeds. *Bot Bull Acad Sinica*, 41: 27-34.

Qu, D, Wang, B. L, Shan, L, Wang, P. H. and Su, P. (1996) Effects of phosphorous on SOD and POD activities of maize under water stress and highly varied water conditions. *Acta University Agriculturae Boreali-Occidentalis (in Chinese)*,24:48-52.

Qureshi R and G.R. Bhatti (2005) Nara Desert, Pakistan: I: Soils, Climate and Vegetation. *Rangelands*, 27(5): 27-31.

Qureshi, R. (2004) Floristic and Ethnobotanical Study of Desert Nara region, Sindh.(Ph.D. Thesis),Department of Botany, Shah Abdul Latif University, Khairpur, Sindh, Pakistan. Vol. I-II:1-454 pp.

Reddy KJ, Theriappan P and Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curl. Sci.* 88: 3.

Reddy, A.R., K.V. Chaitanya and M. Vivekanandan (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol*, 161: 1189–1202.

Ren, Dai, W, Xuan, Z, Yao, Y. Korpelainen, H. & Li, C. (2007) The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species. *Forest Ecology Management*, 239: 112–119.

Ricciardi A, Clementi F (2000) Exopolysaccharides from lactic acid bacteria: Structure, production and technological applications. *Italian J. food Sci.* 1: 23-45.

Richads, R.A. (1978) Variation between and within species of rape seed (B. Campestris and B. napus) in response to drought stress. III. Physiological and physiochemical characters. *Aust. J. Agric. Res,* 29:491-501.

Ridge RW, Kim R & Yoshida F (1998) The diversity of lectin detectable sugar residues on root hair tips of selected legume scorrelates with the diversity of their host ranges for rhizobia. *Protoplasma* 2002: 84–90.

Rivas R, Peix A, Mateos PF, Trujillo ME, Martinez-Molina E, Velazqueze E (2006) Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. *Plant and soil,* 287: 23-33.

Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in soil *Pseudomonas* sp. *Appl Environ Microbiol,* 58:1284-1291.

ROBERTS, I. S. (1995) Bacterial polysaccharides in sickness and in health. *Journal of Microbiology,* vol. 141, 1995, p. 2023- 2031

Robertson, E. B. & Firestone, M. K. (1992) Relationshipbetween desiccation and exopolysaccharide productionin a soil *Pseudomonas* sp. *Appl. Environ.Microbiol, 58:* 1284-1291.

Robijn, G. W, Gutiérrez-Gallego, R, van den Berg, D. J. C, Haas, H, Kamerling, J. P, & Vliegenthart, J. F. G. (1996a) Structural characterization of the exopolysaccharide produced by *Lactobacillus acidophilus* LMG9433.*Carbohydrate Research,* 288: 203-218.

Robijn, G. W, van den Berg, D. J. C, Haas, H, Kamerling, J. P, & Vliegenthart, J. F. G. (1995a) Determination of the structure of the exopolysaccharide produced by *Lactobacillus sake* 0-1. *Carbohydrate Research,* 276: 117–136.

Robijn, G. W, Wienk, H. L. J, van den Berg, D. J. C, Haas, H, Kamerling, J. P, & Vliegenthart, J. F. G. (1996b) Structural studies of the exopolysaccharide produced by *Lactobacillus paracasei*34-1.*Carbohydrate Research,* 285: 129–139.

Robijn, G. W., Thomas, J. R., Haas, H., van den Berg, D. J. C., Kamerling, J. P., & Vliegenthart, J. F. G. (1995b). The structure of the exopolysaccharide produced *by Lactobacillus helveticus* 766.*Carbohydrate Research*, 276: 137–154.

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth promoting bacteria. *Plant and soil*, 287: 15 21.

Rosegrant, M.W, N. Leach and R.V. Gerpacio (1999) Alternative future for world cereal and meat consumption. Summer meeting of the Nutrition Society. *Proc. Nutr. Soc.* 58:1-16.

Rosenberg, E., Zuckerberg, A., Rubinovitz, C. and Gutnick, D.L. (1979) Emulsifier of Arthrobacter RAG-1: isolationand emulsifying properties. *Appl Environ Microbiol*, 37:402–408.

Rudiger H & Gabius HJ (2001) Plant lectins: occurrence, biochemistry, functions and applications. *Glycoconj J*, 18:589–613.

Sadasivan, L. and C. A. Neyra (1987) Cyst production and brown pigment formation aging cultures of *Azospirillum brasilense* ATCC 29145. *J. Bacteriol*, 169:16701677.

Sandhya V, Ali Sk Z, Grover M, Reddy G, Venkateswarlu B (2009b) Alleviation of drought stress effects in sunflower seedlings by exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biol Fert Soil*, 46:17–26.

Sandhya V, Ali SZ, Grover M, Kishore N, Venkateswarlu B (2009a) *Pseudomonas* sp. strain P45 protects sunflowers seedlings from drought stress through improved soil structure. *J Oilseed Res*, 26:600–601.

Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswaralu B (2010) Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul.doi:*10.1007/s 10725-010-9479-4

Schmitt, J, Flemming, H.C. (1998) FTIR-spectroscopy in microbial and materialanalysis. *Int Biodeteri or Biodegrad,* 41: 1–11

Schonfeld MA, Johnson RC, Carwer BF, Mornhinweg DW. (1988) Water relations in winter wheat as drought resistance indicators. *Crop.Sci.* 28:526-531.

Schwab K.B., Gaff D.F. (1990) Influence of compatible solutes on soluble enzymes from desiccation-*stapfianus* and desiccation-sensitive *Sporobolus pyramidalis*. *J. Plant Physiol.* 137:208–215.

Sheng, G.-P, Yu, H.-Q, Wang, C.-M. (2006) FTIR-spectral analysis of two photosynthetic H2-producing strains and their extracellular polymeric substances. *Appl Microbiol Biotechnol.*73: 204–210.

Shiralipour, A. and S.H. West, (1984) Inhibition of specific protein synthesis in maize seedlings during water stress. *Proc. Soil and Crop Sci. Soc. Florida.*43: 102–6

Shoushtari, N.H. and I.L. Pepper (1985) Mesquite rhizobia isolated from the Sonor and desert: competitiveness and survival in soil. *Soil Biol. Biochem.* 17: 803-806.

Sikkema J, Oba T (1998) Extracellular polysaccharides of lactic acid bacteria. *Snow Brand R&D Reports* 107: 1-31.

Simova-Stoilova L, Demirevska K, Petrova T, Tsenov N, Feller U (2008) Antioxidative protection in wheat varieties under severe recoverable drought at seedling stage. *Plant Soil Environ,*54:529–536.

Song, K. S, Li, S, Okamoto, T, Quilliam, L. A, Sargiacomo, M. & Lisanti, M. P. (1996) Co-purification and direct interaction of ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae membranes. *Journal of Biological Chemistry,*271: 9690-9697.

Staaf, M, Widmalm, G.Yang, Z, & Huttunen, E. (1996) Structural elucidation of an extracellular polysaccharide produced by Lactobacillus helveticus. *Carbohydrate Research,* 291:155–164.

Staaf. M, Yang, Z., Huttunen, E, &Widmalm, G. (2000) Structural elucidation of the viscous exopolysaccharide produced by *Lactobacillus helveticus* Lb161. *Carbohydrate Research,* 326: 113–119.

Steel KJ (1961) The oxidase reaction as a toxic tool. *Journal of General Microbiology,* 25:297.

Steel, R.G.D. and J.H. Torrie, (1980) *Principles and Procedures of Statistics: A Biometrical Approach. McGraw Hill Book Co.*, New York, USA

Stingele, F, Lemoine, J, & Neeser, J.-R. (1997) *Lactobacillus helveticus* Lh59 secretes an exopolysaccharide that is identical tothe one produced by *Lactobacillus helveticus* TY1-2. *Carbohydrate Research,* 302: 197–202.

Stingele, F, Neeser, J.-R., & Mollet, B. (1996) Identification and characterization of the eps (exopolysaccharide) gene cluster from *Streptococcus thermophilus* Sfi6. *Journal of Bacteriology,* 178:1680–1690.

Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiol,* 147: 3-9.

Sutherland, I.W. (1972) Bacterial Exopolysaccharides. *Advances in Microbial Physiology.*143-214.

Sutherland, I.W. (1977) Bacterial Exopolysaccharides – Their Nature and Production. *Surface Carbohydrates of the Prokaryotic Cell.*27-96.

Sutherland, I.W. (1996) Extracellular polysaccharides. *Biotechnology,* 615–657.

Sutherland, I.W. (1998) Novel and established applications of microbial polysaccharides. *Trends Biotechnol,* 16: 41–46.

Taylor, K.A, and Buchanan-Smith, J.G. (2001) A colorimetric method for the quantitation of uronic acids and a specific assay for galacturonic acid. *Anal. Biochem,* 2001, 190.

Tisdall JM, Oadea JM (1982) Organic matter and water stable aggregates in soils. *J Soil Sci*, 33:141–163.

V. Sandhya Sk. Z. Ali Minakshi Grover Gopal Reddy B. Venkateswarlu (2010) Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul*, 62:21–30

van Casteren, W. H. M., de Waard, P. Dijkema, C, Schols, H. A, &Voragen, A. G. J. (2000a) Structural characterisation andenzymic modification of the exopolysaccharide produced by *Lactococcus lactis* ssp. cremoris B891. *Carbohydrate Research*, 327:411–422.

van Casteren, W. H. M., Dijkema, C, Schols, H. A, Beldman, G, & Voragen, A. G. J. (1998) Characterisation and modification of the exopolysaccharides produced by *Lactococcus lactis* subsp. Cremoris B40. *Carbohydrate Polymers*, 37, 123–130.

Van Gestel M, Merckx R, Vlassak K (1993) Microbial biomass responses to soil drying and wetting: the fate of fast- and slow growing microorganisms in soils from different climates. *Soil Biol Biochem* 25:109–123

Van Hullebusch ED, Zandvoort MH, Lens PNL (2003) Metals immobilization in biofilms: Mechanisms and analytical tools. *Re/Views Environ. Sci. Bio/Technol*, 2, 9-33

vanCasteren, W. H. M, Dijkema, C, Schols, H. A, Beldman, G, & Voragen, A. G. J. (2000b). Structural characterisation andenzymic modification of the exopolysacchraide produced by *Lactococcus lactis* ssp. Cremoris B39. *Carbohydrate Research*, 324:170–181.

Vanhaverbeke, C, Bosso, C, Colin-Morel, P, Gey, C, Gamar-Nourani, L, Blondeau, K, Simonet, J-M, &Heyraud, A. (1998) Structure of an extracellular polysaccharide produced by *Lactobacillus rhamnosus* strain C83. *Carbohydrate Research*, 314: 211–220.

Vanhooren, P. and E.J. Vandamme (1998) Biosynthesis, physiological role, use and fermentation process characteristics of bacterial exopolysaccharides. *Recent Research Developments in Fermentation and Bioengineering*, 1: 253-300.

VanHullebusch ED, Zandvoort MH, Lens PNL (2003) Metal immobilisation by biofilms: mechanisms and analytical tools. *Rev Environ Sci Biotechnol*, 2:9–33.

Vetter JL, Steinberg MP, Nelson AI (1958) Quantitive Determination of peroxidase in sweet corn. *Journal of Agricultural and Food Chemistry*, 6: 39-41.

Vimala P, Lalithakumari D (2003) Characterization of exopolysaccharides (EPS) produced by *Leuconostoc*sp. V 41.*Asian J. Microbiol. Biotechnol. Environ. Sci*, 5(2): 161-165.

Vincent JM (1970) A Manual for the practical study of Root-Nodule Bacteria, Blackwell Scientific, Oxford. I.B.P Handbook, 15.

Vincent, S. J. F, Faber, E. J, Neeser, J. R, Stingele, F. & Kamerling, J. P. (2001) Structure and properties of the exopolysaccharides produced by *Streptococcus macedonicus* Sc136. *Glycobiology*, 11: 131–139.

Voetberg GS, Sharp RE 1991 Growth of the maize primary root tip at low water potentials. III. Role of increased proline deposition in osmotic adjustment. *Plant Physiol.* 96: 1125-1130.

Wahid A (2007) Heat tolerance in plants: An overview. *Environmental Experimental Botany*, 61(3): 199–223.

Wang Y, Nil N (2000) Changes in chlorophyll, ribulose biphosphate carboxylase–oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J. Hort. Sci. Biotechnol*, 75: 623-627.

Watt M, McCully ME, Jefferee CE (1993) Plant and bacterial mucilages of maize rhizosphere: comparison of their soil binding properties and histo chemistry in a model system. *Plant Soil* 151:151–165.

Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane (1991) 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.*173:697–703.

Welch, J. and M. McClelland (2000) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213-7218.

Williamson, D.H. and J.F. Wilkinson, (1958) The isolation and estimation of poly hydroxyl butyrate inclusions of *Bacillus* species. *J. Gen. Microbiol.* 19: 198-209.

Wingender J, Neu T.R, Flemming, H.C, 1999. Microbial Extracellular Polymeric Substances: Characterization, Structures and Function. Springer, Berlin Heidelberg, New York.

Wullschleger, S.D. T.M. Yin, S.P. DiFazio, T.J. Tschaplinski, L.E. Gunter,M.F. Davis and G.A. Tuskan (2005) Phenotypic variation in growthand biomass distribution for two advanced-generation pedigrees of hybrid poplar. *Canadian J. For. Res.* 35: 1779–1789.

Xiao, X, Xu, X. & Yang, F. (2008) Adaptive responses to progressive drought stress in two Populus cathayana populations. *Silva Fennica* 42: 705–719.

Xu, X, Yang, F, Xiao, X, Zhang, S, Korpelainen, H. & Li, C. (2008) Sex-specific responses of Populus cathayana to drought and elevated temperatures. *Plant, Cell and Environment* 31: 850–860.

Yamamoto, Y, Murosaki, S, Yamauchi, R., Kato, K, & Sone, Y. (1994) Structural study on an exocellular polysaccharide produced by *Lactobacillus helveticus* TY1-2. *Carbohydrate Research,* 261, 67–78.

Yamamoto, Y, Nunome, T, Yamauchi, R, Kato, K, & Sone, Y. (1995) Structure of an exocellular polysaccharide of *Lactobacillus helveticus* TN-4, a spontaneous mutant strain of *Lactobacillus helveticus* TY1-2.*Carbohydrate Research,* 275: 319–332.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte system. *Science* 217:122

Yang, Z, Huttunen, E, Staaf, M, Widmalm, G, & Tenhu, H. (1999) Separation, purification and characterisation of extracellular polysaccharides produced by slime-

forming *Lactococcus lactis*ssp. cremoris strains. *International Dairy Journal*. 9: 631 638.

Yang, Z, Staaf, M, Huttunen, E, & Widmalm, G. (2000) Structure of a viscous exopolysaccharide produced by *Lactobacillus helveticus* K16.*Carbohydrate Research*, 329, 465 469.

Yazdani M, Bahmanyar MA, Pirdashti H, Esmaili MA (2009) Effect of Phosphate Solubilization Microorganisms (PSM) and Plant Growth Promoting Rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays L). World Academy of Science, Engineering and Technology*, 49: 90-92.

Yoshiba Y, Kiyosue T, Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K. (1997) Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol*,38: 1095–1102.

Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (Pisum sativum) under drought conditions. *J Microbiol Biotechnol* 18(5): 958 963.

Zaidi, Khan MS, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiologicaet Immunologica Hungarica*, 56: 263-284.

Zhang, L.X. and S.X. Li. (2004) Research progress on relationship betaine and drought/salt resistance of plants. *Acta Bot Boreal-Occident Sin*, 24: 765-1771.

Zhao, C.X, L.Y. Guo, C.A. Jaleel, H.B. Shao and H.B. Yang (2008) Prospects for dissecting plant-adaptive molecular mechanisms to improve wheat cultivars in drought environments. *Comp. Rend. Biol*, 331: 579–586.

Appendix-1: Analysis of variance table for soil moisture content

Source	DF	SS	MS	F	P
replicate	2	2.02	1.010		
Treatment	15	4295.36	286.357	2166.25	0.0000
Error	30	3.97	0.132		
Total	47	4301.34			

Coefficient of Variation = 1.90

Appendix-2: Analysis of Variance Table for Relative Water Content

Source	DF	SS	MS	F	P
replicate	2	7.02	3.512		
Treatment	15	5685.02	379.001	127.77	0.0000
Error	30	88.99	2.966		
Total	47	5781.03			

Coefficient of Variation = 2.54

Appendix-3: Analysis of Variance Table for Leaf Area

Source	DF	SS	MS	F	P
replicate	2	9.95	4.9730		
Treatment	15	1148.08	76.5385	133.18	0.0000
Error	30	17.24	0.5747		
Total	47	1175.26			

Coefficient of Variation = 4.09

Appendix-4: Analysis of Variance Table for Shoot length

Source	DF	SS	MS	F	P
replicate	2	10.05	5.026		
Treatment	15	2859.46	190.631	133.67	0.0000
Error	30	42.78	1.426		
Total	47	2912.30			

Coefficient of Variation = 2.63

Appendix-5: Analysis of Variance Table for Shoot Fresh Weight

Source	DF	SS	MS	F	P
replicate	2	0.00937	0.00468		
Treatment	15	4.71673	0.31445	41.68	0.0000
Error	30	0.22632	0.00754		
Total	47	4.95242			

Coefficient of Variation = 8.14

Appendix-6: Analysis of Variance Table for Shoot Dry Weight

Source	DF	SS	MS	F	P
replicate	2	0.00005	0.00003		
Treatment	15	0.01528	0.00102	55.57	0.0000
Error	30	0.00055	0.00002		
Total	47	0.01588			

Coefficient of Variation = 6.17

Appendix-7: Analysis of Variance Table for Root length

Source	DF	SS	MS	F	P
replicate	2	4.34	2.172		
Treatment	15	1845.78	123.052	64.07	0.0000
Error	30	57.62	1.921		
Total	47	1907.74			

Coefficient of Variation = 5.49

Appendix-8: Analysis of Variance Table for Root Fresh Weight

Source	DF	SS	MS	F	P
replicate	2	0.00705	0.00353		
Treatment	15	1.82543	0.12170	181.82	0.0000
Error	30	0.02008	0.00067		
Total	47	1.85257			

Coefficient of Variation = 3.39

Appendix-9: Analysis of Variance Table for Root Dry Weight

Source	DF	SS	MS	F	P
replicate	2	0.00039	0.00019		
Treatment	15	0.05090	0.00339	32.55	0.0000
Error	30	0.00313	0.00010		
Total	47	0.05441			

Coefficient of Variation = 10.94

Appendix-10: Analysis of Variance Table for Protein Content of Leaves

Source	DF	SS	MS	F	P
replicate	2	16788.4	8394		
Treatment	15	1.062E+07	708604	18.78	0.0000
Error	30	1132077	37736		
Total	47	1.177E+07			

Coefficient of Variation = 7.14

Appendix-11: Analysis of Variance Table for Sugar Content of Leaves

Source	DF	SS	MS	F	P
replicate	2	273466	136733		
Treatment	15	2.648E+07	1765411	33.84	0.0000
Error	30	1565055	52168		
Total	47	2.832E+07			

Coefficient of Variation = 3.09

Appendix-12: Analysis of Variance Table for Proline Content of Leaves

Source	DF	SS	MS	F	P
replicate	2	169	84.3		
Treatment	15	155660	10377.3	78.82	0.0000
Error	30	3950	131.7		
Total	47	159778			

Coefficient of Variation = 2.88

Appendix-13: Analysis of Variance Table for Superoxide Dismutase

Source	DF	SS	MS	F	P
replicate	2	0.370	0.18492		
Treatment	15	113.617	7.57444	31.96	0.0000
Error	30	7.110	0.23701		
Total	47	121.097			

Coefficient of Variation = 15.72

Appendix-14: Analysis of Variance Table for Peroxidase

Source	DF	SS	MS	F	P
replicate	2	0.00010	5.063E-05		
Treatment	15	0.00630	4.198E-04	8.88	0.0000
Error	30	0.00142	4.726E-05		
Total	47	0.00782			

Coefficient of Variation = 28.51

Appendix-15: Analysis of Variance Table for Catalase

Source	DF	SS	MS	F	P
replicate	2	418	209		
Treatment	15	1751063	116738	409.91	0.0000
Error	30	8544	285		
Total	47	1760025			

Coefficient of Variation = 6.16

Turnitin Originality Report
Thesis by Hafsa Naseem
From Quick Submit (Quick Submit)

- Processed on 19-Jan-2013 14:55 PI
- ID: 298243502
- Word Count: 13838

Similarity Index
14%
Similarity by Source
Internet Sources:
3%
Publications:
12%
Student Papers:
0%

sources:

2% match (publications)
V. Sandhya. "Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress", Plant Growth Regulation, 05/07/2010

2

1% match (publications)
P.V. Bramhachari. "Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23", Letters in Applied Microbiology, 11/2006

3

1% match (publications)
Noshin Ilyas. "Azospirillum strains isolated from roots and rhizosphere soil of wheat (Triticum aestivum L.) grown under different soil moisture conditions", Biology and Fertility of Soils, 02/23/2010

4

1% match (publications)
V. Sandhya. "Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing Pseudomonas putida strain GAP-P45", Biology and Fertility of Soils, 09/04/2009

5

1% match (publications)
[illegible] "Extraction and Analysis of Extracellular Polymeric Substances: Comparison of Methods and Extracellular Polymeric Substance Levels in [illegible]", [illegible] Engineering Science, 10/2009

6

1% match (Internet from 4/6/12)
http://www.fspublishers.org/ijab/past-issues/IJABVOL_11_NO_1/22.pdf

7

1% match (Internet from 1/26/12)
[illegible]/CEREFT_AGROMULD/[illegible]

8

< 1% match (publications)

9

< 1% match (publications)
Singh, R.P., "Isolation and characterization of exopolysaccharides from seaweed associated bacteria Bacillus licheniformis", Carbohydrate Polymers, 20110317

10

< 1% match (publications)

11

< 1% match (publications)
Ahmed, , "Purification of Glycoproteins and Analyses of their Oligosaccharides", Principles and Reactions of Protein Extraction Purification and Characterization, 2004

12

< 1% match (Internet from 9/14/08)
http://las.perkinelmer.com/content/TechnicalInfo/TCH_FTIRATR.pdf

13

< 1% match (Internet from 9/22/09)
http://www.academicjournals.org/AJB/PDF/pdf2008/19Nov/Kamal%20and%20Sano.pdf

14

< 1% match (Internet from 11/15/10)
http://ejournal.sinica.edu.tw/bbas/content/2001/4/bot424-04.pdf

15

< 1% match (publications)
Anthony Adesemoye, "Evaluating Pseudomonas aeruginosa as plant growth-promoting rhizobacteria in West Africa", Archives of Phytopathology and Plant Protection, 12/21/2007

16

< 1% match (publications)

17

< 1% match (Internet from 10/15/10)
http://www.funpecrp.com.br/gmr/year2009/vol8-4/pdf/gmr606.pdf

18

< 1% match (publications)
Mirakshi Grover, "Role of microorganisms in adaptation of agriculture crops to abiotic stresses", World Journal of Microbiology and Biotechnology, 10/05/2010

19

< 1% match (publications)
Yassine Mabrouk, "The Potential Use of Rhizobium–Legume Symbiosis for Enhancing Plant Growth and Management of Plant Diseases", Microbes for Legume Improvement, 2010

20

< 1% match (publications)

21

< 1% match (publications)
Tripathi, P.. "Purification and biochemical characterisation of a novel protease streblin", Food Chemistry, 20110401

22

< 1% match (publications)
Ansande, N.M.. "Con-A conjugated mucoadhesive microspheres for the colonic delivery of diloxamide furoate", International Journal of Pharmaceutics, 20080709

23

< 1% match (publications)
Menalled, Liliana B., Andrea E. Kudwa, Sam Miller, Jon Fitzpatrick, Judy Watson-Johnson, Nicole Keating, Melinda Ruiz, Richard Mushlin, William Alosio, Kristi McConnell, David Connor, Carol Murphy, Steve Oakeshott, Mei Kwan, Jose Beltran, Afshin Ghavami, Dani Brunner, Larry C. Park, Sylvie Ramboz, and David Howland. "Comprehensive Behavioral and Molecular Characterization of a New Knock-In Mouse Model of Huntington's Disease: zQ175", PLoS ONE, 2012.

24

< 1% match (publications)
Dhirendra Katti. "Anionic polymerization of alkyl cyanoacrylates:In vitro model studies forin vivo applications", Journal of Applied Polymer Science, 10/10/1999

25

< 1% match (publications)
Kakumanu, A., M. M. R. Ambavaram, C. M. Klumas, A. Krishnan, U. Batlang, E. Myers, R. Grene, and A. Pereira. "Effects of Drought on Gene Expression in Maize Reproductive and Leaf Meristem Tissue Revealed by RNA-Seq", PLANT PHYSIOLOGY, 2012.

26

< 1% match (publications)
Shu-Mei Zhai. "Overexpression of the phosphatidylinositol synthase gene from Zea mays in tobacco plants alters the membrane lipids composition and improves drought stress tolerance", Planta, 08/10/2011

27

< 1% match (publications)
Andrew, D. R., R. R. Fitak, A. Munguia-Vega, A. Racolta, V. G. Martinson, and K. Dontsova. "Abiotic factors shape microbial diversity in Sonoran Desert soils", Applied and Environmental Microbiology, 2012.

28

< 1% match (publications)
Nookaew, Ni-on, Akkasaeng, Chutipong, Zhu, Yun J., Tantisuwichwong, Nathupapat, Roytrakul, Sittiruk and Sansenya, Wichai, Taksina. "Differentially expressed proteins in sugarcane leaves in response to water deficit stress", Plant OMICS: Journal of Plant Molecular Biology & Omics, 2012

29

< 1% match (Internet from 3/7/11)

30

< 1% match (Internet from 12/27/12)
http://ijpbs.net/vol-3/issue-4/Bio/92.pdf

31

< 1% match (publications)

32

< 1% match (publications)
Belene, Yayis, Setegn Gelebychu, and Habtamu Zelleke. "Morpho-physiological Response to Post-flowering Drought Stress in Small Red Seeded Common Bean (Phaseolus vulgaris L.) Genotypes", Journal of Plant Studies, 2012

33

< 1% match (publications)
S. Royan. "Exopolysaccharides of Pseudomonas mendocina P2d", Letters in Applied Microbiology, 11/1999

34

< 1% match (publications)
Kumar, R.. "Combined effects of Psoralens and ultraviolet-B on growth, pigmentation and biochemical parameters of Abelmoschus esculentus L.", Ecotoxicology and Environmental Safety, 200906

35

< 1% match (publications)
Yang, S., B. Vanderbeld, J. Wan, and Y. Huang. "Narrowing Down the Targets: Towards Successful Genetic Engineering of Drought-Tolerant Crops", Molecular Plant, 2010

36

< 1% match (publications)
Olubukola Oluranti Babalola. "Beneficial bacteria of agricultural importance", Biotechnology Letters, 07/16/2010

37

< 1% match (publications)

38

< 1% match (publications)
Esterhuizen-Londt, M.. "The effect of @b-N-methylamino-l-alanine (BMAA) on oxidative stress response enzymes of the macrophyte Ceratophyllum demersum", Toxicon, 201104

39

< 1% match (publications)
Miriam de Roman. "Elucidation of foliar resistance mechanisms transiently impairs root association with arbuscular mycorrhizal fungi: Impact of SAR on arbuscular mycorrhizas", Journal of Ecology, 01/2011

40

< 1% match (publications)
Ali, Qasim, Farooq Anwar, Muhammad Ashraf, Nazamid Saari, and Rashida Perveen. "Ameliorating Effects of Exogenously Applied Proline on Seed Composition, Seed Oil Quality and Oil Antioxidant Activity of Maize (Zea mays L.) under Drought Stress", International Journal of Molecular Sciences, 2013

41

< 1% match (publications)

42

< 1% match (publications)
Gilbert Lamothe, "Genetic and biochemical characterization of exopolysaccharide biosynthesis by Lactobacillus delbrueckii subsp. bulgaricus", Archives of Microbiology, 09/01/2002

43

< 1% match (publications)
Fatih Duman, "Biological responses of duckweed (Lemna minor L.) exposed to the inorganic arsenic species As(III) and As(V): effects of concentration and duration of exposure", Ecotoxicology, 03/11/2010

44

< 1% match (publications)
Amani, S., "Acetonitrile can promote formation of different structural intermediate states on aggregation pathway of immunoglobulin G from human and bovine", International Journal of Biological Macromolecules, 20110701

45

< 1% match (publications)
Thierry Fontaine, "Lipopolysaccharides from six strains of Acetobacter diazotrophicus", FEMS Microbiology Letters, 10/1995

46

< 1% match (publications)
Mamdouh M. Nemat Alla, "Physiological aspects of tolerance in Atriplex halimus L. to NaCl and drought", Acta Physiologiae Plantarum, 08/13/2010

47

< 1% match (publications)
Sandhya Vardhanabhuti, "Drought-tolerant plant growth promoting Bacillus spp.: effect on growth, osmotic, and antioxidant status of maize under drought stress", Journal of Plant Interactions, 2010

48

< 1% match (publications)
Su-Lian Lv, "Overexpression of Thellungiella halophila H+-PPase (TsVP) in cotton enhances drought stress resistance of plants", Planta, 03/2009

49

< 1% match (publications)
Saudinejad, Amir Hossein; Pourjamir, Farzin and Naghizadeh, Mahdi, "Improving Chilling Tolerance of Maize Seedlings under Cold Conditions by Spermine Application", Notulae Scientia Biologicae, 2012,

50

< 1% match (publications)

51

< 1% match (publications)
Michael Baum, "Molecular Approaches and Breeding Strategies for Drought Tolerance in Barley", Genomics-Assisted Crop Improvement, 2007

52

< 1% match (publications)

[illegible]

53

< 1% match (Internet from 11/13/11)
http://www.sipav.org/main/jpp/volumes/0110/011020.pdf

54

< 1% match (publications)
Yousef Sohrabi. "Changes of antioxidative enzymes, lipid peroxidation and chlorophyll content in chickpea types colonized by different Glomus species under drought stress", Symbiosis, 01/28/2012

55

< 1% match (publications)
Karatas, I., L. Ozturk, Y. Demir, A. Unlukara, A. Kurunc, and O. Duzdemir. "Alterations of antioxidant enzyme activities and proline content in pea leaves under long-term drought stress", Toxicology and Industrial Health, 2012.

56

< 1% match (publications)
Fogelman, E.. "Antioxidative activity associated with chilling injury tolerance of muskmelon (Cucumis melo L.) rind", Scientia Horticulturae, 20110411

57

< 1% match (publications)
Meng Guozhong. "Comprehensive Evaluation on Drought Resistance of Some Ground Covers", 2009 Second International Conference on Information and Computing Science, 05/2009

58

< 1% match (publications)
[illegible] "Isolation and identification of the antagonistic strain DM-54 of Bacillus [illegible] against Verticillium dahliae, and optimization of antifungal [illegible] conditions", [illegible] in China, [illegible]

59

< 1% match (publications)
Barzana, G., R. Aroca, J. A. Paz, F. Chaumont, M. C. Martinez-Ballesta, M. Carvajal, and J. M. Ruiz-Lozano. "Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions", Annals of Botany, 2012.

60

< 1% match (publications)
Eitel, J.U.H.. "Suitability of existing and novel spectral indices to remotely detect water stress in Populus spp.", Forest Ecology and Management, 20060701

61

< 1% match (publications)
Chen, J., W. Xu, J. Velten, Z. Xin, and J. Stout. "Characterization of maize inbred lines for drought and heat tolerance", Journal of Soil and Water Conservation, 2012.

62

< 1% match (publications)
[illegible]

63

< 1% match (publications)
Ali, Qurban, Mehboob Elahi, Muhammad Ahsan, Muhammad Tahir, and Shahzad Basra, "Genetic evaluation of maize Zea mays L genotypes at seedling stage under moisture stress", International Journal for Agro Veterinary and Medical Sciences, 2011.

64

< 1% match (publications)
Xu, Zhen-Zhu, and Guang-Sheng Zhou, "Photosynthetic Recovery of a Perennial Grass Leymus chinensis after Different Periods of Soil Drought", Plant Production Science, 2007

65

< 1% match (publications)
Tsukaguchi, Tadashi, Hiroshi Fukamachi, Kiyoshi Ozawa, Hiroyuki Takeda, Katsumi Suzuki, and Yoshinobu Egawa, "Diurnal Change in Water Balance of Heat-Tolerant Snap Bean (Phaseolus vulgaris) Cultivar and Its Association with Growth under High Temperature", Plant Production Science, 2005.

66

< 1% match (publications)
Govindan Selvakumar, "Bacterial Mediated Alleviation of Abiotic Stress in Crops", Bacteria in Agrobiology Stress Management, 2012

67

< 1% match (publications)
Hassan, A N., "Factors affecting capsule size and production by lactic acid bacteria used as dairy starter cultures", International Journal of Food Microbiology, 20010228

68

< 1% match (publications)
Sutton LA, "Increase of glycinebetaine synthesis improves drought tolerance in cotton", Molecular Breeding, 08/21/2002