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Faculty of Graduate Studies

The Effect of *Bacillus megaterium* on Barley Tolerance to Salinity

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Dedication

After thanking Allah for completed this thesis. My Humble effort I dedicate to my sweet and loving Father and Mother who spends their lives for me.

To My Brother and Sisters

To My Beloved Mentor

Thanks all for everything. Thanks for Supporting and Encouraging me. Thanks for patience and suffering for me. Without them none of my success would be possible.

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V
الإقرار

إننا الموقعة أدناه مقدمة الرسالة التي تحمل عنوان

The Effect of *Bacillus megaterium* on Barley Tolerance to Salinity

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص ، باستثناء ما تمت الإشارة إليه
حيثما ورد، وأن هذه الرسالة ككل أو من جزء منها لم يقدم من قبل لنيل أية درجة أو بحث علمي أو
بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the
researcher's own work, and has not been submitted elsewhere for any other
degree or qualification.

Student's name:

اسم الطالبة :

Signature:

التوقيع:

Date:

التاريخ:

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List of Abbreviations

Sympol	Abbreviation
$^{\circ}\text{C}$	Celsius
ABA	Absciscic acid
ACC	1-Aminocyclopropane-1-Carboxylic acid (ACC)
Al	Aluminum
Bac	Bacteria
B.megaterium	<i>Bacillus megaterium</i>
Ca^{+2}	Calcium
Cd	Cyanide
CFU	Colony forming units
CFU.ml^{-1}	Colony forming units per milliliter
Cl^{-}	Chloride
Cm	Centimeter
CRD	Complete Randomized Design
CV	Coefficient of Variation
d.f	degree of freedom
E.S.E	Estimated Standard Error
Ec	Electrical conduction
ePGPR	Extracellular Plant Growth Promoting Rhizobacteria
EPS	Exo-Polysaccharides
ESP	Equilibrium Exchangeable Sodium Percentage
FAO	Food and Agriculture Organization
Fe	Iron
Fpr	False Positive Ratio
IAA	Indole Acetic Acid
ICARDA	International Center for Agricultural Research in the Dry Areas
iPGPR	Intracellular Pant Growth Promoting Rhizobacteria
IST	Induced Systemic Tolerance
K^{+}	Potassium
l.s.d	Least significant differences
m.s	Mean Sequare
ml	Milli
mM	Millimolar
μm	Micrometer
Mmhos	Millimhos
N	Nitrogen
Na^{+}	Sodium
NaCl	Sodium Chloride

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NARC	National Agriculture Research Center
OD	Optical Density
P	Phosphate
PEG	Polyethylene Glycol
PGPR	Plant Growth-Promoting Rhizobacteria
Ph	potential of hydrogen
POD	Peroxidase
PSB	Phosphate Solubilizing Bacteria
SAR	Sodium Adsorption Ratio
SOD	Superoxide Dismutase
TDS	Total dissolved solids
VOCs	Volatile organic compounds

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Abstract

Barley is the forth important cereal crop in the world, and salinity is one of the most limiting factor for crop productivity. This research aimed to study the impact of *Bacillus megaterium* inoculation to three varieties of barley under 5 different salinity level (0, 50, 100, 150 & 200 mM).

This study revealed that *B. megaterium* have positive impact on agronomic traits of barley such as on leaf length, width and number, root weight, shoot weight and plant height and chlorophyll *B. megaterium* inoculation show increasing in the level of awn roughness slightly.

At moderate salinity level the response of plants to bacterial inoculation was positive on K⁺, Ca⁺, N⁺ and P accumulation this indicate that *B. megaterium* increase uptake of nutrient under saline condition to certain degree.

The study indicates that *B. megaterium* improve the growth of Nabawi and Icarda5 barley's varieties under saline condition and reduce the accumulation of Sodium and Chlorides compared to non-inoculated plants.

Chapter One

Introduction

Several stresses caused by complex environmental conditions, e.g. high and low temperatures, freezing, drought, bright light, UV, heavy metals and hypoxia, salinity, lead to substantial crop losses worldwide (Boyer, 1982; Mahajan & Tuteja, 2005; Mittler, 2006). Among the abiotic factors Soil salinity affects extensive areas of land in both developed and developing countries. Recent changes in global climate are likely to further exacerbate the problem of soil salinity. Variation in important climate variables including temperature and precipitation are expected to decrease water for irrigation and impose high evapotranspiration losses (Yeo, 1999). The resulting drier conditions will further raise irrigation demands which are often met with poor quality of water containing dissolved salts. These conditions will be more critical for arid and semi arid regions which are already at limit with respect to water availability (Chartzoulakis & Psarras, 2005; Sivakumar et al., 2005). The decrease in good water quality in these areas will accelerate the use of saline water for irrigation which will raise salt accumulation in soils, thus increase the extent of secondary salinization (Yeo, 1999).The agricultural intensification, together with unfavorable natural

conditions, has accelerated soil salinity in many part of the world. According to the FAO Land and Plant Nutrition Management Service, over 6% of the world's

land is salt-affected. (Table 1)

Table 1. Regional distribution of salt-affected soils, in million hectares the term salt-affected refers to soil that are saline or sodic (FAO, 2008).

Regions	Total area (Mha)	Saline soils		Sodic soil	
		Mha	%	Mha	%
Africa	1,899	39	2.0	34	1.8
Asia, the Pacific and Australia	3,107	195	6.3	249	8.0
Europe	2,011	7	0.3	73	3.6
Latin America	2,039	61	3.0	51	2.5
Near East	1,802	92	5.1	14	0.8
North America	1,924	5	0.2	15	0.8
Total	12,781	397	3.1	434	3.4

Source: FAO Land and Plant Nutrition Management Service.

Plants have different mechanisms to handle salinity tolerance that are grouped in three different categories. As a primary mechanisms in order to reduce osmotic stress plants decrease leaf area and stomatal conductance that benefits the plants only if there is sufficient soil water available. The second mechanism consists of Na^+ exclusion by roots in order to avoid its accumulation to toxic concentration in leaves. The third mechanism is the tissue tolerance that consists in accumulation of Na^+ , or in some species such as barley also Cl^- , by compartmentalization of these ions at cellular and intracellular level in order to avoid toxic concentration at cytoplasmic level. This process occurs especially in leaves mesophytic cells and leads to toxic levels of Na^+ with time (Munns & Tester, 2008). Barley (*Hordeum vulgare* L.) is rated as salt tolerant among the crop plants; however, a great genetic variation exists for salt tolerance in its cultivars (Niazi et al., 1987, 1992). Salt tolerance in Triticeae is generally considered to be associated with Na^+ ion exclusion and plant's ability to sustain acquisition and maintain adequate levels of K^+ during growth under saline conditions

(Kader & Lindberg, 2005; Colmer et al., 2005). Tavakoli et al., (2010) reported that salt tolerant barley genotype 'Afzal' produced higher dry mass compared to salt sensitive genotype under salt stress conditions (200 mM NaCl) and higher tolerance in genotype Afzal was associated with a higher K^+ / Na^+ ratio of the shoots. NaCl toxicity is largely attributed to the effects of Na^+ and only rarely to those of Cl^- (Tester & Davenport, 2003). Under saline field conditions, the plants may be subjected to different salt levels and ionic stresses.

Barley (*Hordeum vulgare L.*) is a highly adaptable cereal grain and ranks 5th among all crops for dry matter production in the world. In addition, it is an important food source in many parts of the world (Gupta et al., 2010). Although barley is regarded as salt tolerant among crop plants, its growth and development are severely affected by ionic and osmotic stresses in salt-affected soils (Mahmood, 2011).

Soil salinity induces water stress, nutritional imbalance, specific ion toxicity, hormonal imbalance and generation of reactive oxygen species which may cause membrane destabilization (Omar et al., 2009). Moreover, it decreases the yield of many crops as salt inhibits plant photosynthesis, protein synthesis and lipid metabolism (Paul and Lade, 2014). Plant growth under stress conditions may be enhanced by the application of microbial inoculation including Plant Growth Promoting Rhizobacteria (PGPR). These microbes can promote plant growth by regulating nutritional and hormonal balance, producing plant growth regulators, solubilizing nutrients and inducing resistance against plant pathogens (Boostani et al., 2014).

Certain strains of PGPR belong to *Bacillus*, *Enterobacter*, *Burkholderia*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* are now being used worldwide with the aim to enhance crop productivity (Bharti et al., 2013). There has been a great interest in eco-friendly and sustainable agriculture with emphasis on the use of beneficial microorganisms. The benefits of PGPR for plants growing in saline soils were reported as an enhancer of root and shoot growth, nutrient uptake, hydration, chlorophyll content, and resistance to diseases (Qurashi and Sabri, 2012). These PGPRs stimulate plant growth and enhance plant biomass and their beneficial impact have been demonstrated in many agricultural crop species such as wheat, tobacco, Brassica juncea, tomatoes, bell peppers, cucumbers and barley as reviewed by Kang et al. (2014). PGPRs are effective in colonizing the plant root and further multiply into microcolonies and/or produce biofilm as a result of a successful plant-microbe interaction. The plant associated biofilms are highly capable of providing protection from external stress, decreasing microbial competition, and giving protecting effects to the host plant supporting growth, yield and crop quality (Asari, 2015). *Bacillus* species have been reported previously in the rhizosphere of maize (Gao et al, 2004) and have been shown to act as bioprotectants and plant growth-promoting bacteria (Hayat et al ,2010). Isolate EBS8 was effective at enhancing Nitrogen content in maize seedlings, as reported previously for *B. megaterium* on wheat (Komy HMA, 2005). *Bacillus megaterium* the “big beast” described more than one century ago by De

Bary is a Gram-positive spore forming bacteria which is available in soil, (De Bary, 1884). Some strains of *B. megaterium* can improve plant growth and control pathogen invasion (Chakraborty 2006).

B. megaterium M3, *Bacillus* OSU-142, *Azospirillum brasilense* sp. 245, *Paenibacillus polymyxa* RC05, *B. megaterium* RC07, *Bacillus licheniformis* RC08, *Raoutella terrigena*, *Burkholderia cepacia* FS Tur showed increase plant root and shoot weight under greenhouse conditions. Single and combinations of PGPR increased yield up to 40.4% for wheat and 33.7% for barley under field conditions and in combination with N fertilizer (Çakmakçi et al., 2014). *Bacillus mucilaginosus* in coinoculation with the Phosphate Solubilizing Bacteria (PSB) *B. megaterium* promoted the growth of eggplant, pepper and cucumber (Han et al., 2005; Crowley et al., 2006). *B. megaterium* strains were found to produce cytokinins and promote cucumber growth (Sokolova et al., 2011). *B. megaterium* has ability to produce Indole Acetic Acid (IAA), siderophores, and antifungal metabolites and reduces the disease intensity (Chakraborty et al., 2006). The use of salt-tolerant PSB, *B. megaterium* increases the growth of rice and yield components. (Sapsirisopa et al., 2009)

There is no information about the impact of *B. megaterium* on barley under salinity stress has been reported (Cheng Zhou., 2016).

Objectives

General Objectives

Reduce the effect of salinity on barley through the inculcation with *B. megaterium* bacteria

Specific objectives

1. To reduce the effect of saline soil on crop productivity.
2. To determine the effect of *B. megaterium* on barley under saline water irrigation.
3. To determine best salinity tolerant variety on morphological parameter.

Research question and identified problems

Could *B. megaterium* reduce the deleterious effect of salinity on barley?

Problem:

The Jordan Valley is a fertile productive region, which constitutes 52% of the total irrigated land in the West Bank. It is described as the food basket of Palestine where citrus, bananas, date palms, vegetables and field crops are grow variety all over the year. Groundwater originating from the Quaternary Aquifer System forms the main water resource in the Jordan Valley. However, the quality of this groundwater is threatened by the high chloride concentration. Salinity leads to reduction in crop productivity and deterioration in fruit quality.

Chapter Two

Literature Review

2.1. Barley importance:

Barley (*Hordeum vulgare*) is one of the five major crop species of the world, which found at various sites in the Fertile Crescent (Palestine, Jordan, south Turkey, Iraqi Kurdistan, and southwestern Iran). Barley (*Hordeum vulgare* L.) is one of the world's most extensively cultivated crops, according to FAO, the European Union its highest producer. It is adapted to a wide range of conditions in the cool temperate zone. Its domesticated about 8000 B.C from its wild relative *Hordeum spontaneum*, *H. Spontaneum* and *H. Vulgare* have the same morphological such as cultivated form having broader leaves, short awns, short stem, tough ear rachis, larger grains, a shorter and thicker spike. (Roham Eshghi, et al., 2012).

Barley (*Hordeum Vulgare* L.) herbaceous monocotyledonous grass (Von Bothmer and Komatsuda 2011) and one of the first domesticated cereals, belong to the *Poaceae* (*Gramineae*) family and to the genus *Hordeum* which comprises more than 32 species, including diploid and polyploid, perennial and annual types, which are spread throughout the world (Von Bothmer et al. 1995)

The genus *H0ordeum* grows in different areas in central and southwestern Asia, southern South America, western North America, and in the Mediterranean. (Von Bothmer et al. 1992).

Barley is closely related to two other small-grain cereal species, wheat and rye (von Bothmer and Komatsuda 2011). Barley has nutritional and medicinal importance. The nutritional important due to the presence of beta-glucan (an anticholesterol substance), acetylcholine (a substance which nourishes our nervous system and recovers memory loss), easy digestibility (due to low gluten contents) and high lysine, thiamin and riboflavin . Barley food product provides cooling and soothing effect in body sustained for a longer time. Its alternate uses in malt and beer industry and health tonics (Nanak Chand et al., 2008)

In Palestine during the dry season, 84% of the farmers planted barley to use it as a feed for their livestock. Barley linked to the dominant crop-livestock farming systems. The old farming system is shifting towards intensive production methods due to the increased the mechanization, mainly for land preparation, sowing, harvesting and fertilizer. So barley production becomes more intensive and a larger proportion of the farm products, produced it for the market (Ihsan Abu-Alrub et al., 2004)

Although Palestine is a small geographic area, it has a different soil properties due to the variation in climate, the origin (parent material) and topographic features, In the Jordan Valley, the main soil type according to Reifenberg, is Lisan marls. Clay content approximately 10 to 20% and 25 to 50% of lime content (Dudeen B. 2001).

The main soil type In the Eastern Slopes region are the semi-desert soils, gravel is characteristic of desert weathering. As a result of the lack of rain, agriculture is only and terra rossa in the Central Highlands region in the West Bank and Gaza Strip (Dudeen, 2001). Its soil is generally neutral to moderately alkaline; and it has a high content of soluble salts, high iron content and the low organic matter are responsible for the red color. They are mainly of loamy texture (Dudeen B. 2001)

Barley grows well on a wide range soils, but the best soils that have neutral to slightly basic pH (7-8), and which have a high moisture holding capacity, well drained, (Abdel Gadir.O., 2003). Palestine show high salinity and a high susceptibility to contamination. Salinity poses a major problem for soils of the Jericho. Soil salinity is caused by two main factors. Salt can be added to the soil through the poor quality irrigation water. Salinity can be caused by ground water that is too close to the surface. When water is added to the soil it causes the ground water to rise by capillary action. When it evaporates it leaves salts behind, which can be form a crust on soil. Wheat and barley are the main cultivated field crops covering an area of 106.5 hectares in the Jericho district. (ARIJ, 1996)

2.2 Soil and water salinity

All natural waters contain soluble salts, Irrigation water varies in salt concentration and the availability of irrigation water depends on the characteristics of both water and soil. (Ragab et al.,2008), such as

Increasing the salinity of water in sandy soil up to 4.85 dS / m reduces the grain yield by 23 %, (Ragab et al., 2008)

Salinity is abiotic stresses and one of the most important environmental parameters that affect on Barley growth and more harmful to crop plants. Although barley is most drought and salinity tolerant among cereals (Ceccarelli et al., 1987; Belaid & Morris, 1991). Gorham, 1992 concluded that salinity is a complex phenomenon and to understand the salinity cooperation between plant physiologists, agronomists, soil scientists, molecular biologists are needed.

Salinity occurs due to soluble salts accumulation in the soil solution for a long time by molder the rocks and release soluble salts as Magnesium, Sodium Chloride which is the most soluble salt, Calcium, Sulphates and Carbonates or by salty water irrigation, insufficient drainage and transpiration (Bianco Carmen and Defez Roberto, 2011).

Loss of arable land via salinization is a major factor undermining the productivity of modern agricultural systems (Galvani, 2007). Salinization of agricultural soils occurs primarily due to agricultural practices, including poor water management, high evaporation, heavy irrigation and previous exposure to sea water (Pitman & Lauchli, 2002). Currently around 800 million hectares, is affected either by salinity or sodicity (FAO, 2008). In addition, salinity affects 20% of the world's irrigated land, which accounts for one-third of the world food production (Chinnusamy et al., 2005; FAO, 2008). It has been estimated that salinity is affecting 3 hectares of additional arable land each minute worldwide (FAO, 2008). This constant

salinization of arable land is expected to have overwhelming global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang et al., 2003). This progressive loss of arable land has potentially serious consequences for the expanding global population, which is steadily increasing towards seven billion, and set to increase by a further 50% by 2050 (FAO, 2009). Salinity can be abbreviated as E_{Ce} (Electrical Conductivity, TDS of the extract) with units of electrical conductance (e.g. deciSiemens per meter, dS m⁻¹ which is approximately equal to 10 mM NaCl), or in the old units of electrical resistance (e.g. millimhos per centimeter, mmhos/cm), which is expressed in numerically equivalent units, it is often expressed as concentrations (e.g. mM) (Veselov, 2009).

In salt affected soils the sodium absorption ratio (SAR) of soil solution extracts, irrigation waters and subsurface drainage waters has been an important tool for predicting the equilibrium exchangeable sodium percentage (ESP)(Robbins.,1983), SAR is usually defined as:

$$SAR = \frac{Na}{\sqrt{1/2(Ca+Mg)}}$$

When soil moisture is decreased and salinity of irrigation water are increased the EC values of the soil increased (El-Boraie.,1997)

The concentration of the salts determines if the water high quality (drinkable or usable for irrigation without need for special precautions) or low quality (brackish or saline) (Silva and Uchida, 2000). Water in the soil also contains soluble salts (sometimes called free or nonattached salts).

The Salt concentration in the root zone determines if the soil is “normal” or “salt-affected” (saline, sodic, or salinesodic). (Silva and Uchida ., 2000)

The most common salts in water and soil solutions are composed of the cations Sodium (Na^+), Potassium (K^+), Magnesium (Mg^{+2}), and Calcium (Ca^{+2}) and the anions Chloride (Cl^-) Sulfate (SO_4^{-2}), and Carbonate in the form of Bicarbonate (HCO_3^-). Boron, as non-dissociated boric acid. (Silva and Uchida, 2000; Veselov, 2009).

Ca^{+2} , Na^+ and Mg^{+2} which are soluble increased with increasing salinity level of irrigation water, while soluble K^+ decreased with increasing salinity levels and when decreasing irrigation frequency (El-Boraie. 1997).

Data on the total concentration of these constituents, their relative abundance (particularly that of Na^+), and their effect on soil pH are used to categorize the quality of water for irrigation, determine the suitability of soil for cultivation, select crops that are more adaptable for use, and assess the need for soil reclamation. (Silva and Uchida, 2000).

High concentrations of soluble salts in the root zone make a physiologic stresses on growing plants. These stresses may be caused by a salt present in soluble (or free) form (osmotic stress) (Fipps, 2003). They may also be due to toxic or specific-ion effects, or to nutritional imbalances. Most such stresses can be specified quantitatively. Quantifying a crop's sensitivity to salt requires that we define (Fipps, 2003). The first one the threshold salinity level below which the crop's performance is unaffected by salinity, the second one the incremental decline in yield per unit increase in salinity

above the threshold level finally the salinity level at which the crop ceases to grow (Fipps.,2003).

There are two types of salinity problem, high salinity water is toxic to plants and poses a salinity hazard and high level of salinity in the soil can result in a “physiological” drought condition due to the field have plenty of moisture, but the plants wilt because the roots are unable to absorb the water (Fipps. 2003).

The yield reduction increases by increasing salinity of irrigation water and reaches its maximum at 8.86 dSm⁻¹ salinity level. (Ragab et al., 2008). Develop salt tolerant plant is a way to protect plants from abiotic stresses such as salt stress it is a very important step to improve plant growth and crop production need.

2.2.1. Salinity in Palestine

In Palestine the Jordan Valley is a fertile productive region, described as the food basket of Palestine. Groundwater originating from the Quaternary Aquifer System forms the main water resource in the Jordan Valley. However, the quality of this groundwater is threatened mainly by the high chloride concentration (Da’as and Walraevens 2010).

EC is directly related to the concentration of total dissolved salts and ions and a good indicator for salinity mainly occurring in Jericho spring water. Salinity makes spring water unsuitable for irrigation as it affects crop productivity (Khayat, et al., 2006). Causes of salinity are derived from the discharge source of these springs resulting from: upwelling from deep

underlying brine aquifers as a result of over extraction or fresh water aquifers containing salt-bearing rocks, in-situ dissolution of salts from Lisan and Samara layers and anthropogenic sources such as agriculture return-flow and domestic sewage (Khayat, et al., 2006; Marie, and Vengosh, 2001). Wastewater facilities in the Jordan Valley are unlined or in need of repair, whereas, many communities lack connection to the sewerage network and families are forced to use septic tanks and holes (Maan Development Center, 2010). There is relatively little information concerning the impacts of the Dead Sea on the salinity of the groundwater in the Jordan Valley (Anayah, 2006). However, a study showed that salinity of groundwater was due to Dead Sea brine (Anayah, 2006; Marie, and Vengosh, 2001). Salinity considered one of the most limiting factors for plant growth and production.

2.2.2. Salinity effect on plant growth and development

Salinity limits the water availability to plants due to reducing the total water potential in the soil. Salinity has an effect on plant physiology and on the yield, when salt concentration increases the yield gradually decreases until reach to zero. (Haman Dorota Z., 2008). Under stress most of the cultivated plants declined yields even at values that are lower than the defined value for salinity ($EC = 4 \text{ dS m}^{-1}$) (Maas, 1990). Salt-sensitive plants when exposure for few days to salt will reduce the plant growth rate with no many visible changes. Extended exposure effects of few weeks will become evident by the yellowing or death of older leaves and a more

evident reduction of growth. On the other hand under moderate salinity salt-tolerant plants are able to grow for several months, although flowering or decreased production of florets may result (Munns, 2002).

According to the tolerance of salinity plants can be classified into two groups: Halophytes which are salt tolerant plants, plant belongs to this group can grow and reproduce under high salinity ($> 400\text{mM NaCl}$), and the salt sensitive plants, termed as ‘Glycophytes most of the major crops of the world are glycophytes; that cannot grow in saline habitats where salt concentrations are above $\sim 100\text{ mM NaCl}$. (Greenway and Munns, 1980). These glycophytes have evolved in habitats with very low soil Na^+ content, and may never have possessed the mechanisms or features to enable them to cope with the water deficits and ion levels prevailing in saline habitats (Greenway and Munns, 1980)

Barley, cotton, and sugar beet are considered tolerant because they can grow in the salinity range of 6.9 to 8.0 dS m^{-1} ($77\text{-}88\text{ mM NaCl}$) (Maas and Hoffman, 1977). Sugar beets and barley are highly sensitive to salinity during germination but are highly tolerant during the later phases of crop development (Maas, E.V. 1990). Other researchers (Table 2) concluded that barley, cotton, olive, rye and wheat grass can tolerate salinity range of $8\text{-}12\text{ dS m}^{-1}$ (Brady & Weil, 2008).

Table.2 Tolerance threshold values of some crops to saline soils. Salinity expressed as electrical conductivity of the saturation extract (Brady & Weil, 2008)

Sensitive (0-4 ds m⁻¹)	Moderately tolerance (4-6 ds m⁻¹)	Tolerance (6-8 ds m⁻¹)	Highly tolerance (8-12 ds m⁻¹)
Almond	Corn	Fig	Barley
Bean	Grain Sorghum	Oats	Cotton
Clover	Lettuce	Pomegranate	Olive
Onion	Soybean	Sunflower	Rye
Potato	Tomato	Wheat	Wheat grass

Salt sensitive varieties have lower antioxidant enzyme activities than the salt tolerant varieties such as Barley (Xiaoli et al., 2009). Antioxidant enzymes such as Superoxide Dismutase (SOD) and Peroxidase (POD) activities, and Lipid Peroxidation MDA content in barley plants are increased Under Salinity stress condition (Turkyilmaz unal B. et al., 2014). Antioxidant defense system induced by salinity plays prominent role particularly in early growth periods and its efficiency decrease with age of the plants (Turkyilmaz unal B. et al., 2014). It is important to study the effect of salinity on barley and salt tolerance during germination and growth stages of plant for determining saline limits at each developmental phase (Zapata et al., 2004) the response of barley to the salinity varies according to the stages of growth. Seed germination is the most important phases in the life cycle of barley and is highly responsive to the existing environment (Saritha et al., 2007).

Inhibitory effects of salinity on plant growth are due to decreased of water availability that imposed by an osmotic stress or to toxic effect of excessive Na^+ or Cl ions (Veselov, 2009).

Water deficiency and salinity are decreased and delayed germination and it is not significantly affected up to 16.3 dS m^{-1} , but was inhibited when salinity increased to 22 dS m^{-1} (Heenan et al., 1988). Seedlings are sensitive to salinity, germination decrease at high salt levels might be mainly due to decrease the osmotic stress that induces the inhibition uptake of certain nutrients such as K^+ , Ca^{+2} and NO_3^- and accumulation of Na^+ and Cl to toxic levels within cells and around the roots. Salinity have negative effect on plant growth due to low osmotic potential of soil solution, changes in nutrient uptake and pecific Sodium and Chloride ion effects, and the effect depends on the salt concentration in addition to the growth conditions (Kalaji and Pietkiewicz, 1993)

Salinity decrease photosynthesis assimilation, the transpiration rate, and the stomatal conductance, the K^+ / Na^+ and didn't induce significant variability on the intracellular CO_2 concentration. (Abdennaceur et al., 2014) increase the level of the Sodium ions in the plant leaves makes ion toxicity decreases in Photosynthetic activity is leading to reduced growth and productivity of plants through reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent through a decrease in photosystem II efficiency (Netondo, 2004).

Salinity reduce the growth and biomass accumulation of plants, the epidermis thickness, diameter of the vascular bundles of leaves and the

central cylinder of roots and increased the ratio of Exodermis/ Endodermis roots in some varieties (Atabayeva et al., 2013), also it is reduce evaporation, photosynthesis, depression in carbon uptake, and inhibition of photochemical capacity due to stomatal closure (Kaouther et al., 2012; Horie et al., 2012).

Under saline conditions 31 to 53 % the area of midrib along the leaf axis are reduced due to salt stress and reduction in the number of small veins may be resulted to lower growth in the growth zone (25-50 mm above the leaf base) (Bijanzadeh .E .,2014)

Salinity causes nutritional imbalance in plant growth, development and yield mainly because salt affects nutrient availability, competitive uptake and mineral transport inducing nutritional disorders (Grattan & Grieve, 1999). Salinity reduces N uptake/accumulation (Feigin, 1985), reduction of phosphate uptake/accumulation by reducing phosphate availability (Sharpley et al., 1992), reducing K^+ net uptake and its translocation by lowering K^+ content in shoot and increasing K^+ in root (Botella et al., 1997).

Limitation of plant growth by salinity is primarily due to reduction of water uptake from soil by osmotic effects. Damage is mainly caused by excess of Na^+ and Cl^- ions and nutrient deficiencies caused by Na^+ competition with other ions (K^+ , NO_3^- and $H_2PO_4^-$), needed for plant nutrition (Tester & Davenport, 2003). Toxicity by Na^+ affects plants more than toxicity caused by Cl^- because Na^+ causes cell swelling and several disorders at enzyme activation and protein synthesis processes resulting in reduced energy

production and other physiological changes (Tester and Davenport, 2003; Larcher, 1980). Increasing of Sodium Chloride concentration resulted in the reduction of number of tillers, spike length, number of spikelets per spike, biomass per plant and grain yield per plant and made greater damage in barley (Ahmad et al., 2003). Increasing in salinity level showed decreased in leaf area, dry weight of shoot, dry weight of root, length of shoot, fresh weight of stem and fresh weight of root (Taghipour and Salehi, 2008). The contents of Na^+ in shoots of barley increased significantly under NaCl treatment and Cl^- contents in shoots increased under saline treatments (Mahmood K., 2011).

Excess of Cl^- in plants accumulates in shoots inhibiting photosynthesis mainly by inhibition of nitrate reductase activity (Xu et al., 2000; Flowers, 1988). Effects of salinity on plants lead to anatomical and morphological changes, leaf discoloration, inhibition of seed germination, seedling growth, flowering and fruit set (Tester and Davenport, 2003; Sairam and Tyagi, 2004). In order to maintain water homeostasis and normal physiological functions produced by salinity plants overproduce compatible organic solutes such as proline and glycine betaine (Serraj & Sinclair, 2005). Proline maintains higher leaf water potential and protects plants against oxidative stress by adjusting osmotic pressure and stabilizing membranes, constitutive proteins and enzymes, scavenging free radicals, and buffering cellular redox potential during salt stress (Ashraf and Foolad, 2007; Peng et al., 2008; Kohler et al., 2009). The time frame of salt effects on plants have been described by Munns and Sharp (1993) and it is

proposed as a two-phase growth response concept. First phase or osmotic phase is of short duration and reduce growth by the water stress due to the root surrounding salt. The second or ion-specific phase takes time to develop and it is caused by the excessive levels of salt accumulation in cell vacuoles of transpiring leaves leading to the reduction of growth of younger leaves by the lack of carbohydrates supply to growing cells (Munns, 2002). Plants have different mechanisms to handle salinity tolerance that are grouped in three different categories. As a primary mechanisms in order to reduce osmotic stress plants decrease leaf area and stomatal conductance that benefits the plants only if there is sufficient soil water available. The second mechanism consists of Na^+ exclusion by roots in order to avoid its accumulation to toxic concentration in leaves. The third mechanism is the tissue tolerance that consists in accumulation of Na^+ , or in some species such as barley also Cl^- , by compartmentalization of these ions at cellular and intracellular level in order to avoid toxic concentration at cytoplasmatic level. This process occurs especially in leaves mesophytic cells and leads to toxic levels of Na^+ with time (Munns & Tester, 2008).

Barley is the most salt tolerant cereal, reported to die only after extended periods at salt concentrations higher than 250 mM NaCl (equivalent to 50 % seawater) (Munns et al., 2006). Due to its salt tolerance barley crops may be suitable to be used in salt remediation of salt impacted soils (Chang et al., 2014).

2.2.3. Raising crop productivity under salinity

Numerous physical and chemical approaches exist for improving agricultural productivity in saline environments (Rains & Goyal, 2003). These include drainage and leaching of excess salt from the root zone, chemical amelioration of soils, and crop-based management practices (Goyal et al., 1999). However, apart from being extremely costly and time-consuming, these techniques are non-applicable at many instances due to the unavailability of improved irrigation and drainage systems (Sharma & Manchanda, 1996). Alternatively, researchers have been working towards developing salt-tolerant crop varieties using selective breeding techniques over the past century; however, none of those efforts has proven successful (Ashraf, 2010; Yamaguchi & Blumwald, 2005). During the last decade, scientists are also using transgenic approaches to obtain genetically modified plants (Ashraf & Akram, 2009; Mittler & Blumwald, 2010; Valliyodan & Nguyen, 2006; Vinocur & Altman, 2005; Zhang et al., 2000). These approaches are time consuming and costly due to the impressive charges required to validate the consumption or cultivation of genetically modified plants.

Plants in their natural environment are colonized both by endocellular and intracellular microorganisms (Gray & Smith, 2005). Classification based on their degree of association with plant root cells divides PGPR in extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR). ePGPR are found as part of the rhizosphere, rhizoplane or endophytic bacteria located

at the spaces between root cortex cells. iPGPR are found as intra cellular endophytic bacteria that are located inside specialized nodular structure of plant root cells (Gray & Smith, 2005). Rhizosphere microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments and, consequently, enhance yield both directly and indirectly (Dimkpa et al., 2009). Some Plant Growth-Promoting Rhizobacteria (PGPR) may exert a direct stimulation on plant growth and development by providing plants with fixed Nitrogen, Phytohormones, Iron that has been sequestered by Bacterial Siderophores, and Soluble Phosphate (Hayat et al., 2010; Rodriguez & Fraga, 1999). Inoculation of various plant species with such bacteria lead to increased root growth and/or enhanced formation of lateral roots and root hairs that can result in enhanced tolerance to abiotic stress. A fruitful strategy to alleviate negative effects of salt stress in plants might be the coinoculation of seeds with different PGPR species was shown to increase the total nodule number of several legumes, acetylene reduction activities, and the total N content of mineral macro- and micronutrients (Burdman, 1996; Molla et al., 2001; Remans et al., 2008).

2.3. Plant growth promoting rhizobacteria (PGPR)

The term Plant Growth-Promoting Rhizobacteria (PGPR) is used to define bacteria that colonize the rhizosphere and stimulate plant growth (Kloepper & Schroth, 1981). The rhizosphere soil surrounding plant roots contains many times more microbes than the bulk soil (Lugtenberg & Kamilova,

2009). Among the rhizosphere bacteria there is a category named PGPR characterized by their ability to promote plant growth and health. Figure 1 and 2 demonstrate the interaction between PGPR and plant, which endophytic and ectophytic respectively and the following paragraphs will describe them in more detail. Inoculation with non-pathogenic root zone bacteria can have various consequences within the plant as well as in the rhizosphere Figure 1. (a) Upon bacterial inoculation, the selectivity for Na^+ , K^+ and Ca^{+2} is altered, resulting in higher K^+/Na^+ ratios. (b) Inoculation with Rhizobacteria can lead to changes in membrane phospholipid content and alterations in the saturation pattern of the lipids. Membrane potential is, thus, reduced. (c) Nitric oxide and indole acetic acid (IAA) produced by bacteria promote lateral root development in the host plant, resulting in increased root surface area. (d) Bacteria-produced osmolytes, such as glycine betaine, can act synergistically with plant osmolytes, accelerating osmotic adjustment. (e) Inoculation with non-pathogenic rhizobacteria can induce signalling cascades that put the host plant in a ‘primed’ physiological state as part of a phenomenon of induced systemic resistance (ISR). (f) Bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity reduces ‘stress ethylene’ levels within the plant (DIMKPA et al., 2009). In figure 2 the rhizosphere-dependent mechanisms underlying enhanced abiotic stress tolerance by plants shows (a) Host plant Nitrogen uptake can be positively influenced by bacterial Nitrogen fixation. (b) The mobility of heavy metals in contaminated soils can be significantly reduced through root zone bacteria. (c) Migration of bacteria from the

rhizoplane to the rhizosphere plays a role in reducing plant uptake of Cd. (d) Iron–siderophores complexes can be taken up by the host plant, resulting in a higher fitness. (e) Bacterial Exo-Polysaccharides (EPS) lead to the development of soil sheaths around the plant root, which reduces the flow of Sodium into the stele. (f) Root zone bacteria can influence pH and redox potential in the rhizosphere, for instance, through the release of organic acids. This can have positive effects on the availability of nutrients for the plant (DIMKPA et al., 2009).

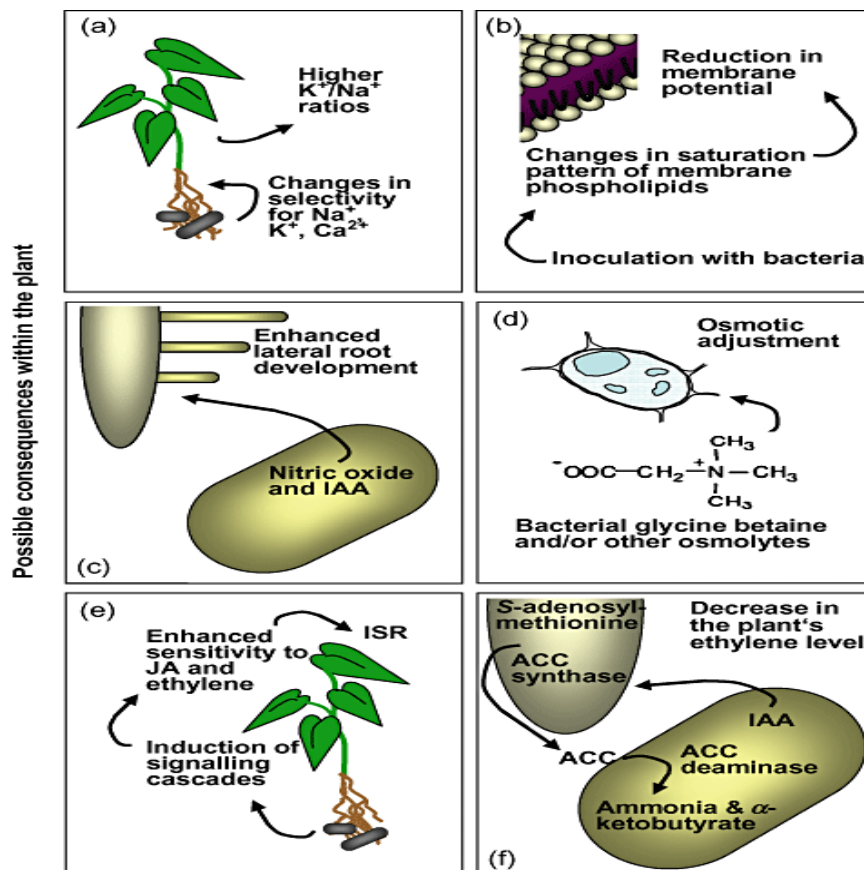


Figure 1. Proposed mechanisms underlying enhanced abiotic stress tolerance within the plant.

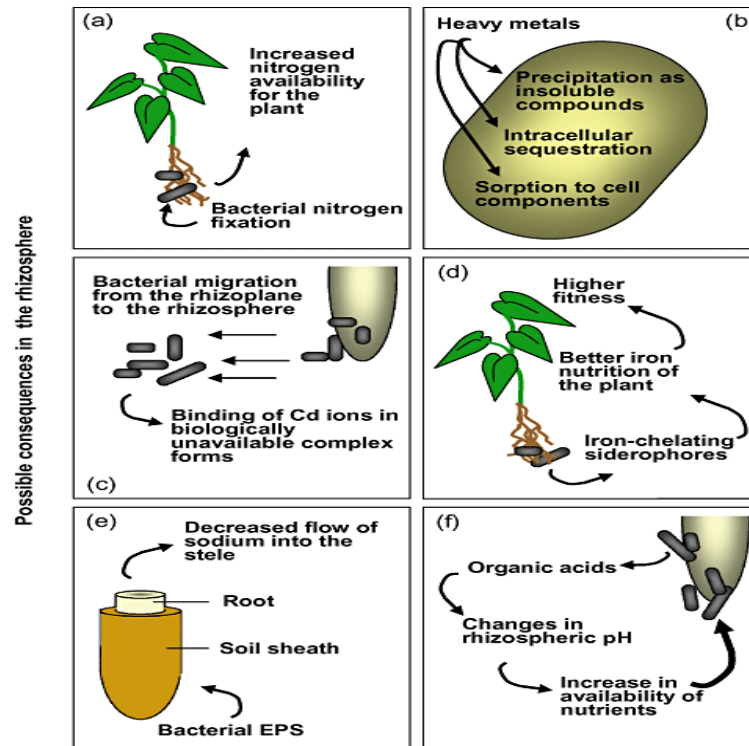


Figure 2. Proposed rhizosphere-dependent mechanisms underlying enhanced abiotic stress tolerance by plants.

These PGPR can be classified into rhizospheric and endophytic bacteria based on the colonization behavior. The former ones only colonize the root surface (rhizoplane), such as some *Azospirilli* (Bloemberg and Lugtenberg 2001) while the latter ones can additionally penetrate into roots and grow inside of plants such as *Gluconacetobacter diazotrophicus* (Alqueres et al. 2013). Growth of various plants was shown to be affected by their root associated PGPR, for instance maize, rice, sugarcane, sorghum, wheat, lettuce, radish, pine, and rape. PGPR species are widely distributed across the phylogentic tree; however, many of the isolates can be classified as *Pseudomonas* or *Bacillus* (Vessey 2003). *Azospirillum brasilense* improve epidermal cell differentiation and enhance root development, increased

root branching and increase root hair density, root dry matter, providing increased surface for nutrient absorption and rhizobacterial colonization (Okon 1997., Molla et al., 2000) in soybean crops (Molla et al., 2000).

PGPR activity is influenced by or even dependent on plant root exudates. The roots provide soluble nutrients for their growth which includes mostly organic acids, making up 83% of the total amount of exudates, as well as photosynthates, sugars and the polyamine putrescine. Also a vast range of insoluble chemical compounds are released from roots (e.g. cellulose, lignin, proteins) (Liu et al. 2012). Due to this high abundance of nutrients, PGPR can multiply in the rhizosphere and colonize the root surface. For example, *Pseudomonas putida* PCL1444 can reach a tenfold increase in cell numbers in the presence of grass seedling in soil (Liu et al. 2012a). On the other hand, the exudates can also include some toxic secondary metabolites that inhibit some microbes, providing a selection advantage to the resistant ones.

2.3.1. Bacterial osmotic stress tolerance

Microorganisms have developed different adaptations to counteract the outflow of water which enables them to also grow in high osmolarity environments that cause a rapid loss of cell water along the osmotic gradient causing reduction in turgor and dehydration of the cytoplasm. When bacterial cells are exposed to high osmolarity the cytoplasm is exposed to high ionic strength, in order to maintain osmotic equilibrium accumulation of K^+ could serve as a second messenger activating additional

osmotic responses. As response, cells upregulate genes involved in adaptive, protective, metabolic, and amino acids transport processes and production of organic compatible solutes in order to equilibrate the intracellular Potassium concentration. (Miller and Wood, 1996; Shabala, 2009). Osmolytes produced by bacteria as organic compatible solutes can be sugars and derivatives, polyols, α - and β -amino acids and their derivatives, betaines and/or ectoines (Paul and Nair, 2008; Lamosa et al., 1998; Roesser and Müller, 2001). Compatible solutes function as osmoprotectants and also supporting protein stability, folding and function in vitro and in vivo (Street et al., 2006). Other mechanisms to survive under salt stress is the production of exopolysaccharides to enhance water retention to protect cells from osmotic stress and fluctuations in water potential (Sandhya et al., 2010) changes in the fatty acid composition of the bacterial membrane (Klein et al., 1999) and/or shortening peptidoglycan interpeptide bridges (Piuri et al., 2005).

2.3.2. Plant interaction with PGPR

The use of PGPR is a promising strategy to alleviate salt stress in horticultural crops and to maintain an acceptable level of productivity under higher salt concentrations (Nadeem et al., 2012; Singh et al., 2011). PGPR have been related to influence plant health under salt stress on several parameters such as increasing biomass, root system surface, improving germination rate, enhancement of chlorophyll content and resistance to diseases. Among PGPR mechanisms reported to influence

plant growth under salt stress are enhancement of plant nutrient uptake, production of phytohormones, increase K^+ ion concentration and ion homeostasis mediation (Ryu et al., 2005; Yang et al., 2009; Nadeem et al., 2012; Paul and Lade, 2014). Previous studies demonstrated that the bacteria isolated from the rhizosphere of wild plants grown on saline was used to reduce salt stress in Tomato. (Pengfei Fan et al., 2016)

2.3.2.1. Enhancement of plant nutrient uptake

PGPR improve Nitrogen and Phosphorus uptake, Solubilizing Inorganic Phosphate and mineralizing organic Phosphate (Diby et al., 2005; Ogut et al., 2010, Upadhyay et al., 2011). PGPR inoculation influencing positively plant biomass, increase of N, P, K^+ , and Ca^{+2} absorption and decrease of Na^+ absorption have been reported in cotton by *Klebsiella oxytoca* Rs-5 and *Pseudomonas putida* Rs-198 under salt stress (Yue et al., 2007; Yao et al., 2010). For example strain GR12-2, *P. putida*, isolated from the rhizosphere of plants growing in the Canadian High Arctic, was found to promote growth of canola cv. Tobin by fixing nitrogen and enhancing the uptake of phosphate under gnotobiotic conditions (Lifshitz et al. 1986; Lifshitz et al., 1987), by synthesizing siderophores that can solubilize and sequester iron from the soil and supply it to the plants Glick (1995). *Pseudomonas putida* GR12-2 and *Azospirillum* enhance the plant root system and water uptake and improve mineral by the roots (Patten and Glick, 2002)

B. megaterium increase the availability of solubilize Phosphorus (Sandeep et al., 2011; Xiang et al., 2011) and mineralizes the organic Nitrogen (Sakurai et al., 2007) thereby make it available to plants (Armada et al., 2014; Hu et al., 2013; Kieselburg et al., 1984). Plant Growth-Promoting Rhizobacteria that fix Nitrogen in non-leguminous plants are diazotrophs that form a non-obligate interaction with the host (Glick et al. 1999). The process of Nitrogen fixation is carried out by the Nitrogenase enzyme coded by *nif* genes (Masepohl and Klipp, 1996; Kim and Rees, 1994). *Bacillus Mucilaginosus* produce Exo-polysaccharides. It was utilized in agriculture as a Multifunctional microbial fertilizer, which can make K, P, silicate and other beneficial elements available by dissolving insoluble minerals in soil (Lian et al., 2000)

Combined inoculation of *A. brasilense* with *Pseudomonas striata* significantly increased grain yield, Nitrogen and Phosphorus uptake of sorghum (Alagawadi and Gaur, 1992). All the strains belonged to the genus *Pseudomonas* and also demonstrated the ability to colonize roots of canola cv. Tobin under field conditions. However, the amount of Nitrogen fixed by these bacteria was minimal and the positive plant growth response observed may be due to other factors such as phytohormone production and enhanced mineral uptake (James and Olivares, 1997).

2.3.2.2. Plant growth regulators

PGPR promote plant growth through improving the synthesis of vitamins, phytohormones (Gibberellic Acid, Indole Acetic Acid (IAA),

Cytokinins)(Russo et al., 2008) and inhibition ethylene synthesis by production 1-aminocyclopropane-1-carboxylate (ACC) deaminase this enzyme present on the surface of plant roots (rhizospheric) the activities of this enzyme is protect plants from growth inhibition by flooding, drought, high salt level, presence of metals and organic contaminants, flower wilting ,making easy the nodulation of legumes (Gamalero et al., 2015). Various authors have identified the production of Indole-3-Acetic Acid by microorganisms in the presence of the precursor tryptophan or peptone. Eighty percent of microorganisms isolated from the rhizosphere of various crops have the ability to produce auxins as secondary metabolites (Kampert et al. 1975; Loper and Schroth, 1986). *Bacillus Thuringiensis* help in produce of IAA (Indole acetic acid) & 1-Aminocyclopropane- 1- Carboxylate (ACC) deaminase and in iron and phosphate Solubilization, (Raddadi, 2008)

Salamone (2000) reported the growth-promoting effect of *P. fluorescens* strain G20-18 on wheat and radish plants by production of cytokinin phytohormones. Beside the effect of PGPR lowering the ethylene concentration and thereby stress signal for the plant Glick (2014) suggested a cross-talk between IAA and ACC deaminase where by lowering plant ethylene levels, ACC deaminase facilitates the stimulation of plant growth by IAA (Fig. 2). There are several reports of ethylene emission reduction by inoculation ACC deaminase producing bacteria e.g. *Achromobacter piechaudii* on tomato plants (Mayak et al., 2004), *Achromobacter xylosoxidans* on *Madagascar periwinkle* (*Catharanthus roseus*)

(Karthikeyan et al., 2012) and *Bacillus licheniformis*, *Brevibacterium iodinum* and *Zhihengliuella Alba* on red pepper seedlings (Siddikee et al., 2011). Also a *Streptomyces* strain reported to promote growth in wheat under salt stress by production of indole acetic acid and auxin, phosphate solubilization and siderophore production even though no ACC deaminase is evaluated (Sadeghi et al., 2012). Bacterially-mediated plant tolerance to salt stress has been reviewed and includes diverse functional and taxonomical groups of bacteria (Dimkpa et al., 2009). Diversity of rhizobacteria mediated plant tolerance to salinity stress involving ACC deaminase activity in different plant species is reviewed in Table.3. ACC deaminase production has been reported in strains belonging to Proteobacteria, Actinobacteria, Firmicutes and 'Bacteroidetes' (Glick, 2014; Nadeem et al., 2010). *B. megaterium* has a beneficial effect as PGPR it provides plant with IAA and enzymes that promote growth (Armada et al., 2014; Sadiq and Ali 2013; Shaharoon 2006).

2.3.2.3. Induced systemic tolerance

Yang et al. (2009) proposed the term induced systemic tolerance (IST) to the effect of VOCs, produced by PGPR, that induce physical and chemical changes in plants enhancing tolerance to abiotic stresses, including salt stress (Farag et al., 2013). Zhang et al., (2008) reported that plant growth promotion triggered by VOCs from *Bacillus subtilis* GB03 confers salt tolerance in *Arabidopsis thaliana* reducing Na^+ levels and recirculation of Na^+ in the whole plant under salt condition by accumulation of tissue

specific high affinity Potassium transporter HKT1, that mediate Na^+ transport, expression down regulated in roots and upregulated in shoots. Furthermore, PGPR inoculation increased iron uptake, redistributed whole-plant auxin, increased leaf cell expansion, and influenced root branching (Zhang et al., 2007; Zhang et al., 2008a). Similar effects have been also studied in white clover and wheat (Han et al., 2014; Zhang et al., 2014).

2.3.2.4. Ion homeostasis mediation

As an effect of salinity the availability, transport and mobility of Ca^{+2} and K^+ are affected in growing parts of plants. Potassium can act as a cationic solute responsible for stomatal movements as a response to changes in water status on bulk leaf (Caravaca et al., 2004) and Ca^{+2} regulates early signaling processes at the onset of salt stress. PGPR can influence in host physiology and in the foliar reduction of Na^+ and Cl^- ions accumulation by increasing K^+ and Ca^{+2} . Wheat plants separately inoculated with *Pseudomonas putida*, *Enterobacter cloacae*, *Serratia ficaria* and *Pseudomonas fluorescens* have been reported to increase the K^+/Na^+ ratio by increasing K^+ effectively influencing salinity tolerance (Nadeem et al., 2013). Inoculation with *Pseudomonas* sp. on eggplant (*Solanum melongena* L.) significantly increased K^+ and Ca^{+2} , and decreased Na^+ shoot concentrations under saline conditions but not under non stress conditions (Fu et al., 2010). Similar results in cotton by inoculation of *Pseudomonas putida* Rs-198 increased K^+ and Ca^{+2} , and decreased Na^+ in leaves and roots (Yao et al., 2010).

2.3.2.5. Increase disease tolerance

Breakthrough research in the field of PGPR occurred in the mid 1970s with studies demonstrating the ability of *Pseudomonas* strains capable of controlling soil-borne pathogens to indirectly enhance plant growth and increase the yield of potato and radish plants (Burr et al. 1978; Kloepper et al. 1980; Kloepper and Schroth 1981; Howie and Echandi 1983).

When the plant under Saline condition or water stress the pathogenic fungi will be active and cause disease (Lugtenberg et al. 2001) and uses PGPR will improve the resistance of plant against disease (Germida JJ., 1998) by the production of antibiotics, lytic enzymes, hydrogen cyanide, siderophores that induced systemic resistance (Gupta et al. 2000 and Gamalero et al., 2015) and reduce the use of chemical fertilizers , improving the uptake of nutrient including N, P, K, and microelements by stimulating the ion uptake and increase the root surface area by increasing root hair size and number (Defreitas and Germida ., 1992 and Burdman et al., 2000)

2.4. Effect of *Bacillus megaterium* as PBPR on plant.

Gram's positive bacterium called phosphobacterium (Cooper., 1959) aerobic spore forming bacterium available in agricultural fields sediment , fish and dried food , seawater, rice paddies, honey (vary et al., 2007) *B. megaterium* have Large size with a volume approximately 100 times that of *Escherichia coli* (De Bary, 1884) with rod shape and deep-rooted in the phylogeny of *Bacillus* (Rossler., 1991) and it is non

pathogenic bacteria (Eppinger et al., 2011). First described over 100 years ago, *B. megaterium* has recently been gaining more and more importance in scientific as well as industrial applications. The source of the significant name "*megaterium*" was the large size of the vegetative cells (over 1 μm) and the spores. The capability of sporulation has made *B. megaterium* an important tool for examining spore-mediated disease and cell development. *B. megaterium* is able to grow on a wide variety of carbon sources and thus has been found in many ecological niches, such as waste from meat industry or petrochemical effluents. Also documented has been the degradation of persistent insecticides by *B. megaterium* (Sexana et al., 1987) offering potential applications as detoxifying agent.

B. megaterium use in biochemistry, Bacteriophages (Clarke and Cowles. 1952) industrial applications such as industrial protein production (Vary et al. 2007) for more than 50 years and it possesses some very useful and unusual enzymes, and a high capacity for the production of Exoenzymes. The importance of enzymes is used in the production of new synthetic antibiotics by penicillin Amidase and amylases which used in starch processing industries, for example baking industry (Nagao et al., 1992) It is the major aerobic producer of vitamin B₁₂ and anaerobically (Raux et al., 1998).

B. megaterium has many advantages such as no endotoxins found in the cell wall, uses inexpensive substrates and alkaline protease not present. (Vary et al. 2007)

Availability of this Bacteria in the field make Increase in crop yield is reported on various crops, including sugar beet (Cakmakci et al., 1999) , barley (Salantur et al., 2005) clover, wheatgrass (Holl F.B et al., 1988). Inoculation of *Arabidopsis* plants with *B. megaterium* BOFC15 on increased plant biomass, improved root system architecture, and augmented photosynthetic capacity. Inoculated plants also displayed stronger ability to tolerate drought stress than non-inoculated (control) plants. Absciscic acid (ABA) content was notably higher in the inoculated plants than in the control plants under drought stress and Polyethylene Glycol (PEG)-induced stress conditions (Zhou et al., 2016).

Chapter Three

Materials and methods

3.1 Experimental Set Up

3.1.1. Location of the experiment

This study was carried out during 2015/2016 At National Agriculture Research Center NARC in Jenin area- West Bank in Palestine under greenhouse condition.(Fig.3)

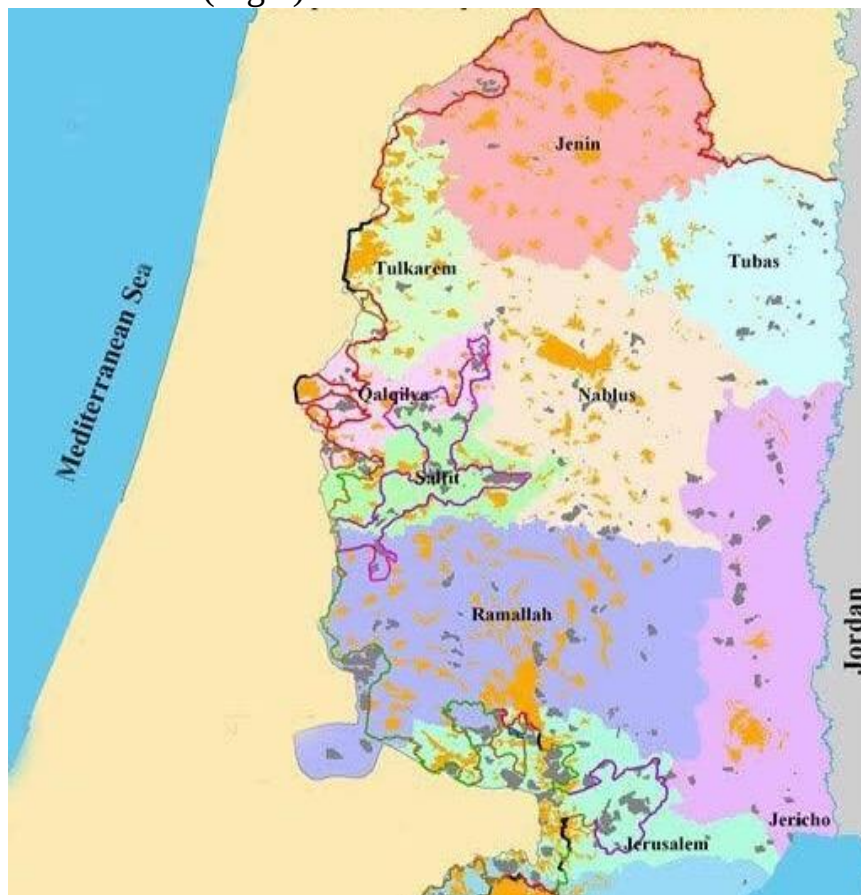


Fig.3: Map of Jenin, West Bank, Palestine

3.1.2. Plant Materials

Three barley cultivars Reehan (R), ICARDA 5 (Ic5) and Nabawi (N) was used in this experiment kindly provided by ICARDA. The study was carried out in a pot experiment in greenhouse conditions, Five seeds per pot was planted and every cultivar has treated with five salinity levels 0, 50, 100, 150 and 200 mM of NaCl.

Salinity treatment was applied after three week. The irrigation with NaCl solution carried out twice a week. The plant was fertilized with N ,P ,K (13:13:13) and with multi micronutrient (S, B, Cu, Fe, Mn, Mo, Zn, Mg) 3cm /liter twice per entire period of cultivation.

3.2.3. *Bacillus megaterium* growth and preparation:

Bacillus megaterium (ATCC® 14581™) Freeze Dried bacteria purchased from American Type Culture Collection company (ATCC).

It was activated in 100 ml nutrient broth Nutrient Broth (BD 234000) in tap water and incubated for 24-48 h in a rotary shaker, 200 rpm at $30 \pm 2^\circ\text{C}$. The bacteria were subjected to several subcultures to increase the total colony forming units and the quantity of bacteria. Then, every culture was diluted to 10^8 colony forming units (cfu) /ml. the number of colony forming units was measured by obtaining the Optical Density (OD) (1 ml) using spectrophotometer (at 600 nm, Model V530, Jasco Corporation, Japan). The final OD unit (at 600 nm) of 1.0 is equivalent to approximately 7×10^8 CFU.ml⁻¹ used for plant inoculation. Bacterial inoculation was carried out by injecting 1.5 ml/plant of *B. megaterium*. Each pot was placed

on gravel which was spread on the soil surface to prevent airborne dispersal of bacteria within the controlled greenhouse. The experiment consists of two parts, one of them with bacteria and the other part without bacterial inoculation.

Summary of the Treatments

For each plant cultivar five salinity levels were subjected to the following treatments (Fig.4):

- 1) T1: Various levels of saline water with microorganism
- 2) T2: Various levels of saline water without microorganism
- 3) T3: Control (Fresh water) with microorganism
- 4) T4: Control (Fresh water) without microorganism

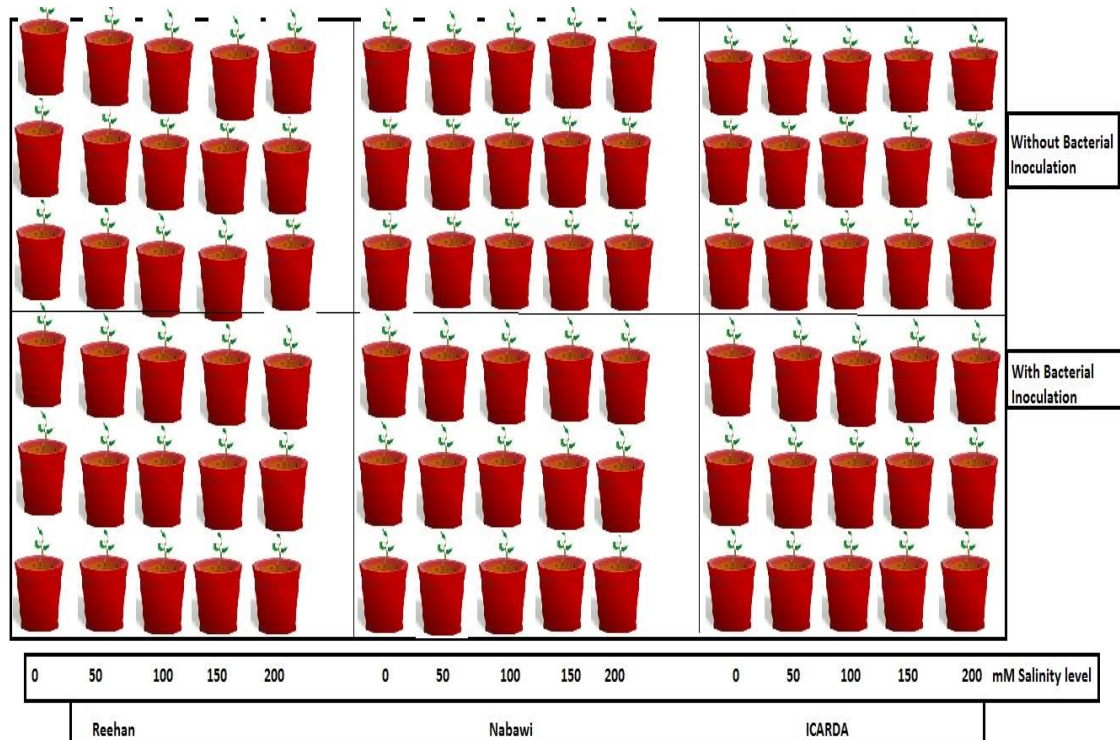


Figure.4a Summary of the treatments



Figure.4b Summary of the treatments

3.3. Measured growth and production parameters

3.3.1. Morphological and yield parameters

The response of plants to the treatments will be monitored at frequent periods and samples will be collected for the following parameters; vegetative growth at maturity, including plant height, number of tillers, leaf area, leaf number, peduncle length, awn softness, total dry weight per plant, total fresh weight per plant and chlorophyll contents.

First leaf area:

Leaf length (cm): should be measured from the top of the sheath to the tip of the blade

Leaf width (cm): should be measured at the widest point.

Tillering:

Number of fertile tillers/ plant

Plant Height at Maturity (cm): This character is measured from the surface of the ground to the tip of the spikelet, excluding awns.

Spike characteristics:

Awn softness: A tangible score at full maturity, using a scale from 1= soft to 5 = very rough.

Peduncle length (cm): Length of the top internodes of the culm, measured from the base of the spike to the ligules of the flag leaf

Leaf number: The average number of leaf per five plants

Chlorophyll content: This character is measured by Chlorophyll meter type SPAD 502 Plus.

Root weight (g): Weight of roots per five plants

3.4. Chemical analysis

The Chemical analysis of Calcium using AA500 Atomic Absorption Spectrophotometer, Sodium and Potassium using Jenway PFP7 Flame photometer, total Nitrogen using the Kjeldahl method. VAPODEST 20s, and chlorine for this study carried out according to International Center for Agricultural Research in the Dry Area (ICARDA) Third Edition, George Estefan, Rolf Sommer, and John Ryan. Analysis of total Phosphorus using Jenway 7305 Spectrophotometer done according to the Standard Analytical Procedures for Water Analysis.

3.4.1. Plant material digestion

3.4.1.1. Nitric acid digestion

Plant material collected from all field trial harvests was digested with concentrated Nitric Acid (BDH, Poole, and Dorset) on a Tecator digestion block.

3.4.1.2. Digestion procedure

Approximately 1 g (oven dried) plant material was weighed accurately in 4 digit balance and placed into digestion block tubes. 5 ml concentrated HNO_3 was added and the tubes left to pre-digest overnight. The samples were boiled for 3 hours at 120 °C with baffles on the system to promote refluxing conditions and ensure complete digestion. 10 ml distilled water was added after cooling and the sample was filtered through Whatman No.50 filter paper into 100 ml volumetric flasks and made up to volume with distilled water. The samples were stored in plastic bottles at 4 °C until analysis.

3.4.2 Determination of Total Nitrogen

Total Nitrogen was determined using Kjeldahl method by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hydroxides in the reagent system. Digestion of the samples was carried out by the standard Kjeldahl and the salicylic acid modification methods of Bremner and Mulvaney (1982).

Salicylic Acid Modification Method

Reagents

Analytical grade reagents and Nitrogen free deionized water were used throughout.

Salicylic acid-sulphuric acid mixture 25g salicylic acid was dissolved in 1 liter of concentrated sulphuric acid in a beaker. The mixture was poured carefully into a 1 liter glass bottle fitted with a 5 ml acid dispenser.

Sodium Thiosulphate Pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) 500 g of Sodium Thiosulphate was obtained from FSA Laboratory Supplies, Bishop Meadow Road, Loughborough, LE11 ORG, England.

Sodium Sulphate-Copper Sulphate combination of 100g Sodium Sulphate was finely ground with 10.0 g of Copper Sulphate in a mortar and pestle. This mixture was stored in a plastic bottle.

Kjeldahl

Procedure

Approximately 0.2 g of plant materials were weighed on a boat made from aluminium foil. The boat was placed on the end of a pipette, pushed down to the end of a Tecator Kjeldahl tube and emptied. This was done to ensure that the entire sample reached the base of the tube. The boat was reweighed to allow correction for the small amount of sample remaining in the weighing boat. A 16 ml of concentrated sulphuric acid (H_2SO_4) with 5 gm Salicylic Acid mixture was added to the sample and a tablet of catalyst $\text{Na}_2\text{SO}_4/\text{CuSO}_4$: 1 g of Sodium Sulphate and Copper Sulphate Pentahydrate

mixture (10:1 by weight) and 10 ml Distilled water was also added. The tubes were gently shaken to mix well. The 8 tube rack was placed in the Tecator block digester and heated at 375 °C for 2.5 hours. Following digestion the tubes were removed from the block digester and left to cool until they were able to be handled. The digest was first made up to approximately 30 ml with deionized water and shaken well to dissolve all digest. The extracts were diluted in a ratio of 1:50 prior to analysis. The procedure for filtration and storage was also the same for both methods. After mixing, approximately 40 ml of the digest was Stored in a plastic bottle for determination of the Nitrogen content by steam distillation. The collected sample was titrated with 0.1N HCl. The amount of HCl added until the color change used in the equation to calculate total Nitrogen.

Equation

$$\%Nitrogen = N Hcl * (VHCL - V Blank) * \frac{1.4}{weight\ of\ sample(gm)}$$

Where:

N HCl: Normality of titrate

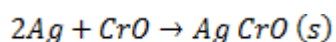
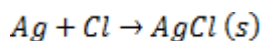
VHCL: volume of HCl

V Blank: volume of Blank

3.4.3 Chlorine determination in barley tissue

Argentometric methods (Titrimetric methods) based upon silver nitrate according to Mohr titration method

The reactions of this titration method for Cl determination was the silver nitrate react with Chloride ion, and silver Chloride precipitated quantitatively before formation the red silver chromate from excess silver nitrate



Reagents preparation

5% of Potassium Chromate (indicator) Preparation: 1.0 g of K_2CrO_4 was dissolved in 20 mL of distilled water.

Standard $AgNO_3$ solution preparation: weighed out 9 g of $AgNO_3$ and transferred to a 500 mL volumetric flask and filled distilled water up to 500 ml mark and the solution was approximately 0.1 M. and the solution stored in a brown bottle and should not expose to light the Silver Nitrate is the most important precipitating reagent .

Procedure

The amount of Chloride which present in plant determined by making an extraction from digested plant materials by added 50 ml from Hot distilled water to specimen container which contain 1 gm of weighted plant material and mixed them together through putting them on shaker for 30 minute for homogenization. After that the supernatant filtered through filtration apparatus, the sample would have pH near to 7.0 because of the ion of chromate is conjugate base of the weak Chromic Acid (Sheen R.T. and Kahler H. L.,1938) ,then pipette out 2ml of the sample and added 2ml of

distilled water and added (3-5)ml from Potassium Chromate indicator (K_2CrO_7) to get light yellow color then the burette filled with silver nitrate 0.01 N $AgNO_3$ and titrated the sample against silver nitrate solution until the color changed from yellow to permanent reddish-brown and noticed the volume of silver nitrate .

Blank Titration by the same way above but without using plant materials

Equation

$$\text{Chloride } \left(\frac{\text{mg}}{\text{l}} \right) = 35.45 * \text{Normality of } AgNO_3 * \text{Volume} * \frac{1000}{\text{Volume of extract used for titration (ml)}}$$

3.4.4 Calcium Ca , Sodium Na^+ and Potassium K^+ determination in barley tissue

3.4.4.1. Determination of Na^+ and K^+ in solution by flame photometry

Flame photometry was devised by Barnes et al in 1945 and it also called flame atomic emission spectrometry. A traditional, old and simple analytical method for determining Sodium and Potassium in biological fluids involves the technique of emission flame photometry using Jenway PFP7 Flame photometer device.

Procedure

Approximately 2 g of oven-dried plant materials were weighed on a porcelain crucibles and entered them into Muffen furnace at $450^\circ C$ over night for Burning the organic matter and obtained the ash and wait until cool the ash then dissolved it in 10 ml of hydrochloric acid and filled with distilled water up to 50 ml mark and allow it to digest for

approximately 30 minutes . Then extraction has done by a filtration apparatus .after that prepares flame photometer by Set up as detailed in the instruction manual, and then read off the sample Potassium concentration, and Sodium concentration.

3.4.4.2. Determination of Calcium in solution by atomic absorption

Spectroscopy

The same samples that were used to examine the Sodium and Potassium concentrations were used to check the Calcium concentration but by using Atomic Absorption Spectrophotometer 500.

3.4.5 Total Phosphorus determination in barley tissue using Ascorbic Acid/Molybdate Method

Phosphate was measured based on the formation of a Phospho-Molybdate complex which is reduced using ascorbic acid to give a blue colour which may be measured at 660 or 880 nm by using 7305 Spectrophotometer. In order to speed up the formation of the complex, a small amount of antimony is added. The intensity of the blue colour is proportional to the Phosphorus concentration in the original solution. The method is applicable to water samples and a wide range of soil extract solutions and acid digests of plant or soil material.

Reagents

Phenolphthalein indicator aqueous solution prepared by the addition of 300 mL concentrated H_2SO_4 (10 N) to approximately 600 mL distilled water

and dilute to 1 Liter. Preparation of diluted concentration of H_2SO_4 by the mixing 70 mL concentrated H_2SO_4 (5 N) with 500 mL with distilled water

Potassium Antimonyl Tartrate solution: prepared by dissolving 1.3715 g K (SbO) $\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$ in 400 mL Distilled water and diluted to 500 mL.

Ammonium Molybdate solution: 20g $(\text{NH}_4)_6 \text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ was dissolved in 500 mL distilled water.

Ascorbic acid 0.1 M: 1.76g Ascorbic Acid was dissolved in 100 mL distilled water

Combined reagents was prepared by a mixture of 50 mL 5N, H_2SO_4 , 5 mL Potassium Antimonyl Tartrate, 15 mL Ammonium Molybdate solution, and 30 mL ascorbic acid solution, in the order given and at room temperature stable for 4 hours.

Stock phosphate solution: 219.5mg Anhydrous KH_2PO_4 was dissolved in distilled water and dilute to 1 L P.

Standard Phosphate Solution: prepared by diluting 50 mL of stock solution to 1L with distilled water; 1 mL = 2.5 kg P.

Procedure

The filtrates were analysed for phosphate using the manifold shown in Figure 2.4 along with standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour. The colour was developed in the water bath at 37 °C. The intensity of the colour was measured at 880 nm. The Phosphate calibration graph is linear in the range of 0-5 mg $\text{PO}_4\text{-P}$ 1-1. Samples having phosphate concentrations higher than 5 mg 1-1 were diluted by an inbuilt dilution system.

Equation:

$$\text{Total P as mg P/L} = g \text{ P from the calibration} * \frac{1000}{\text{ml sample}}$$

3.5. Statistical analysis and experimental design

The experimental design was CRD factorial with 3 replication X 5 salinity level X 3 varieties X 2 bacterial treatments. The data was statistically analyzed using GenStat Software.

Chapter Four

Results

4.1.1 Effect of salinity and bacterial inoculation on Chlorophyll content in Reehan

According to the statistical analysis the bacteria showed a positive significant

($P \leq 0.05$) impact on plant chlorophyll content. Bacteria inoculation effect on chlorophyll content was highly associated with the level of salinity.

In general as the level of salinity increase the quantity of chlorophyll decrease. When Reehan inoculated with bacteria and without salinity the chlorophyll content was 13.03 while at higher salinity level 200 mM the amount was reduced to 4.47 in contrast plant without inoculation showed lower contents ranged from 6.83 at zero level salinity to 2.62 at 200 mM level salinity (Table3).

Table (3) Means, Standard Errors and Analysis of Variance for chlorophyll content in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	6.83	8.34	7.73	8.25	2.62
With Bacteria	13.03	12.18	9.98	16.45	4.47
Fpr					
Bacteria	0.003				
Salinity	0.006				
Bac. Salinity	0.52		LSD	6.1	

4.1.2 Effect of salinity and bacterial inoculation on awn softness in Reehan

Bacteria inoculation showed no significant ($P > 0.05$) impact on awn softness. However salinity showed significant effect on awn softness. Based on mean separation the data showed that as the level of salinity increase the awn softness decrease. (Table 4)

Table (4) Means, Standard Errors and Analysis of Variance for of awn softness in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	2.0	2.93	1.33	1.33	0.0
With Bacteria	1.8	3.0	0.93	0.80	0.27
Fpr					
Bacteria	0.693				
Salinity	<.001				
Bac. Salinity	0.502		LSD 0.9308		

4.1.3. Analysis of Variance for the effect of salinity and bacterial inoculation on leaf number in Reehan

Statistical analysis of leaf number with and without salinity showed no significant ($P > 0.05$) difference with or without bacterial inoculation. The means for the highest number of leaf was 5.93 at 100 mM in plant inoculated with bacteria and the lowest number was 3.67 at 200 mM salinity and without bacteria (Table 5)

Table (5) Means, Standard Errors and Analysis of Variance for leaf number in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	5.00	5.47	4.73	4.53	3.67
With Bacteria	5.73	5.67	5.93	5.33	4.14
Fpr					
Bacteria	0.138				
Salinity	0.165				
Bac. Salinity	0.964	LSD 2.058			

4.1.4. Analysis of Variance for the effect of salinity and bacterial inoculation on leaf area length in Reehan

Leaf length measurement and statistical analysis showed no significant difference ($P > 0.05$). The range of means for plant inoculated with bacteria 4.78cm at 100 mM to 3.30cm at 200 mM while plant without inoculation 4.43 cm at 50 mM to 3.01 cm at 200 mM (Table6).

Table (6) Means, Standard Errors and Analysis of Variance for leaf area length in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	3.03	4.43	4.18	3.39	3.01
With Bacteria	4.11	3.81	4.78	4.32	3.30
Fpr					
Bacteria	0.164				
Salinity	0.126				
Bac. Salinity	0.485	LSD 1.482			

4.1.5. Analysis of Variance for the effect of salinity and bacterial inoculation on leaf area width in Reehan

Table (7) show that Leaf width was highly affect by salinity level as the statistical analysis showed high significant difference ($p \leq 0.05$) for salinity level while the bacterial inoculation have no significant effect on leaf width. The leaf width range 0.25 cm at 200mM to 0.44 cm at 50 mM and 0.27 cm at 200 mM to 0.43cm at 50 mM without and with inoculation respectively.

Table (7) Means, Standard Errors and Analysis of Variance for leaf area width in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	0.29	0.44	0.41	0.33	0.25
With Bacteria	0.30	0.43	0.41	0.31	0.27
Fpr					
Bacteria	0.845				
Salinity	<.001				
Bac. Salinity	0.957		LSD	0.0943	

4.1.6. Analysis of Variance for the effect of salinity and bacterial inoculation on peduncle length in Reehan

Table (8) the effect of salinity levels and bacterial inoculation on peduncle length was highly significant ($p \leq 0.05$). The mean separation for Reehan without inoculation showed that as the level of salinity increase the peduncle length decrease 3.16 cm at zero salinity to 0cm at 200 mM in contrast the response for Reehan inoculated with bacteria was variable 3.59 cm at zero level to 0.69cm at 200mM with slight increase 3.35 cm at 100 mM.

Table (8) Means, Standard Errors and Analysis of Variance for peduncle length in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	3.16	2.35	1.27	0.17	0.00
With Bacteria	3.59	2.59	3.35	2.16	0.69
Fpr					
Bacteria	0.001				
Salinity	<.001				
Bac. Salinity	0.163	LSD		1.365	

4.1.7. Analysis of variance for the effect of salinity and bacterial inoculation on plant height in Reehan

Plant height showed a significant difference based on the statistical analysis for salinity and bacterial inoculation. However no interaction between salinity and bacterial inoculation was detected. Mean separation showed that height of plant inoculated with bacteria range from 24.63 cm at zero level to 15.8 cm at 200 mM while without inoculation 19.81 cm at zero level to 14.07 cm at 150 mM (Table 9).

Table (9) Means, Standard Errors and Analysis of Variance for plant height in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	19.81	18.17	17.53	14.07	17.37
With Bacteria	24.63	18.17	19.15	20.01	15.8
Fpr					
Bacteria	0.049				
Salinity	0.021				
Bac. Salinity	0.172	LSD		4.878	

4.1.8. Analysis of Variance for the effect of salinity and bacterial inoculation on root weight in Reehan

Analysis of variance for root weight indicated no significant difference ($P > 0.05$)

for salinity and bacterial inoculation. However the range for inoculated plant and non inoculated plant range was 9.5 g at 100 mM to 18.7g at 50 mM and 8.3 g at 100 mM to 23.5g at 150 mM respectively (Table 10).

Table (10) Means, Standard Errors and Analysis of Variance for root weight in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	10.9	10.7	8.3	23.5	23.2
With Bacteria	14.1	18.7	9.5	14.2	17.7
Fpr					
Bacteria	0.869				
Salinity	0.143				
Bac. Salinity	0.401		LSD	14.06	

4.1.9. Analysis of Variance for the effect of salinity and bacterial inoculation on spike length in Reehan

The analysis of variance showed high significant difference for spike length under different salinity level. In addition the response of plant to bacterial inoculation was also significant. According to mean separation the highest length was 1.85cm at zero level to 0.07 cm at 150 mM and 1.77 cm at 50 mM to 0.21 at 200 mM without and with bacterial inoculation respectively (Table 11).

Table (11) Means, Standard Errors and Analysis of Variance for of spike length in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	1.85	1.67	0.70	0.07	0.0
With Bacteria	0.90	1.77	1.18	0.57	0.21
Fpr					
Bacteria	0.693				
Salinity	<.001				
Bac. Salinity	0.015	LSD		0.6059	

4.1.10. Analysis of Variance for the effect of salinity and bacterial inoculation on tiller number in Reehan

Tiller number showed a highly significant ($P \leq 0.05$) difference under salinity level in addition to the interaction between bacteria and salinity. The number of tiller range between zero tiller at highest salinity level ≥ 100 mM to 0.33 at 50mM and zero tiller at salinity level equal or greater than 50 mM to 0.783 at zero level salinity without and with bacterial inoculation respectively (Table 12) .

Table (12) Means, Standard Errors and Analysis of Variance for of tiller in Reehan

Salinity Concentration	0	50	100	150	200
Without Bacteria	0.133	0.33	0.0	0.0	0.0
With Bacteria	0.783	0.0	0.0	0.0	0.0
Fpr					
Bacteria	0.334				
Salinity	<.001				
Bac. Salinity	0.002	LSD		0.2987	

4.1.11. Analysis of Variance for the effect of salinity and bacterial inoculation on Chlorophyll content for Nabawi

Nabawi landrace showed significant ($P \leq 0.05$) reduction in chlorophyll content in response to salinity while bacterial inoculation showed no significant difference. showed that when Nabawi inoculated with bacteria and without salinity the chlorophyll content was 16.25 and the amount was reduced to 2.78 when salinity level was 200 mM , in contrast plants without inoculation showed a ranged from 16.81 at zero level salinity to 2.53 at 200 mM level salinity. (Table 13).

Table (13) Means, Standard Errors and Analysis of Variance for chlorophyll content in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	16.81	16.13	6.70	5.08	2.53
With Bacteria	16.25	17.73	7.23	3.99	2.78
Fpr					
Bacteria	0.934				
Salinity	<.001				
Bac. Salinity	0.991	LSD		8.266	

4.1.12. Analysis of Variance for the effect of salinity and bacterial inoculation on awn softness in Nabawi

Bacteria inoculation showed significant effect ($P \leq 0.05$) on awn softness. In addition salinity showed highly significant effect on awn softness (Table 36). The analysis of variance indicated that the interaction between salinity and bacteria was not significant. Mean separation in showed that the awn softness decrease when the level of salinity increase (Table 14).

Table (14) Means, Standard Errors and Analysis of Variance for awn softness in Nabawi

Salinity concentration	0	50	100	150	200
Without Bacteria	0.53	1.60	0.20	0.0	0.0
With Bacteria	1.60	2.33	1.07	0.0	0.0
Fpr					
Bacteria	0.016				
Salinity	<.001				
Bac. Salinity	0.333	LSD		0.945	

4.1.13. Analysis of Variance for the effect of salinity and bacterial inoculation on leaf number in Nabawi

The differences was highly significant ($P \leq 0.05$) on leaf number with and without salinity or bacteria based on the statistical analysis of leaf number. The means for highest number of leaf was 6.01 at zero level salinity with bacteria inoculation. However leaf number was .073 at 200 mM salinity and without bacteria while at the same salinity level with bacteria the average number was 2.11 (Table 15).

Table (15) Means, Standard Errors and Analysis of Variance for leaf number in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	3.87	1.93	1.60	1.2	0.73
With Bacteria	6.07	5.00	5.53	1.20	2.11
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	<.001	LSD		1.102	

4.1.14. Analysis of Variance for the effect of salinity and bacterial inoculation on leaf area length in Nabawi

According to leaf length measurement and statistical analysis highly significant difference ($P \leq 0.05$) was observed. The means number for plant inoculated with bacteria was 3.86 cm at 50 mM and 1.41 cm at 150 mM in contrast the mean for plant without inoculation with bacteria was 1.75cm at 50 mM and 0.0 cm at 150 mM (Table 16).

Table (16) Means, Standard Errors and Analysis of Variance for leaf area length in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	2.43	1.75	0.21	0.0	0.0
With Bacteria	5.63	3.86	2.72	1.41	0.0
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.004		LSD	1.102	

4.1.15. Analysis of Variance for the effect of salinity and bacterial inoculation on leaf area width in Nabawi

Statistical analysis showed high significant difference ($P \leq 0.05$) for salinity level and inoculation with bacteria in respect to leaf area width. The leaf width range 0.3 cm at 50 mM to 0.087 cm at 150 mM and 0.14 cm at 50 mM to 0.00 cm at 150 mM with and without inoculation respectively (Table 17).

Table (17) Means, Standard Errors and Analysis of Variance for leaf area width in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	0.20	0.14	0.013	0.0	0.0
With Bacteria	0.42	0.30	0.20	0.087	0.0
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.005		LSD	0.08109	

4.1.16. Analysis of Variance for the effect of salinity and bacterial inoculation on peduncle length in Nabawi

The effect of salinity levels and bacterial inoculation on peduncle length was significant ($P \leq 0.05$)

The mean separation for Nabawi without inoculation showed that as the level of salinity increase the peduncle length decrease 1.28 cm at zero salinity to 0 cm at 200 mM slight increase 1.31 cm at 50 mM in contrast the response of Nabawi inoculated with bacteria was 3.47 cm at zero level to 0 cm at 200 mM (Table 18).

Table (18) Means, Standard Errors and Analysis of Variance for peduncle length in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	1.28	1.31	0.15	0.0	0.0
With Bacteria	3.47	3.38	0.93	0.0	0.0
Fpr					
Bacteria	.003				
Salinity	<.001				
Bac. Salinity	0.050		LSD	1.388	

4.1.17. Analysis of Variance for the effect of salinity and bacterial inoculation on plant height in Nabawi

Statistical analysis for salinity and bacterial inoculation showed highly significant differences ($P \leq 0.05$) in plant height. Mean separation showed that height of plant inoculated with bacteria range from 23.01 cm at zero level to 2.39 cm at 200 mM while without inoculation 15.31 cm at zero level to 2.87 cm at 200 mM as a result the means of plant height decreased when salinity level increased.(Table 19)

Table (19) Means, Standard Errors and Analysis of Variance for plant height in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	15.31	12.30	4.67	2.39	2.87
With Bacteria	23.01	22.49	17.81	7.89	2.39
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.002	LSD 4.335			

4.1.18. Analysis of Variance for the effect of salinity and bacterial inoculation on root weight in Nabawi

No significant difference for salinity and bacterial inoculation was observed by the analysis of variance for root weight. According to the mean separation the lowest root weight was 8.6g at 200 mM highest root weight was 26.0g at 50 mM for inoculated plant while in non-inoculated plants the lowest root weight was 15.5 g at 200 mM and highest root weight was 21.4 g at 0 mM. (Table 20)

Table (20) Means, Standard Errors and Analysis of Variance for root weight in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	21.4	19.3	18.8	17.2	15.5
With Bacteria	12.7	26.0	12.2	18.5	8.6
Fpr					
Bacteria	0.276				
Salinity	0.168				
Bac. Salinity	0.296	LSD		12.06	

4.1.19. Analysis of Variance for the effect of salinity and bacterial inoculation on spike length in Nabawi

The analysis of variance showed high significant difference for spike length under different salinity level. In addition the response of plant to bacterial inoculation was also highly significant. The highest length of spike was 1.12 cm at 50 salinity level to 0 cm at 200 mM and 2.19 cm at 50 mM to 0 at 200 mM without and with bacterial inoculation respectively according to mean separation (Table 21).

Table (21) Means, Standard Errors and Analysis of Variance for spike length in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	0.63	1.12	0.14	0.0	0.0
With Bacteria	1.77	2.19	0.99	0.0	0.0
Fpr					
Bacteria	0.002				
Salinity	<.001				
Bac. Salinity	0.095	LSD		0.7851	

4.1.20. Analysis of Variance for the effect of salinity and bacterial inoculation on tiller number in Nabawi

Tiller number showed a highly significant difference ($P \leq 0.05$) under saline condition, in addition to the interaction between bacteria and salinity (Table 60). The number of tiller was zero at highest salinity level ≥ 100 mM without bacterial inoculation and 0.333 tiller at 50 mM to 1.667 tiller at zero mM with bacterial inoculation. (Table 22)

Table (22) Means, Standard Errors and Analysis of Variance for tiller in Nabawi

Salinity Concentration	0	50	100	150	200
Without Bacteria	0.0	0.13	0.0	0.0	0.0
With Bacteria	1.67	0.33	0.0	0.0	0.0
Fpr					
Bacteria	0.004				
Salinity	<.001				
Bac. Salinity	<.001		LSD	0.5386	

4.1.21. Analysis of Variance for the effect of salinity and bacterial inoculation on chlorophyll content under saline condition in Icarda 5

Salinity significantly reduces the chlorophyll content in Icarda5. A significant interaction was observed between salinity and bacterial inoculation. Means separation provides evidence that as salinity increase chlorophyll content decrease significantly at zero level salinity chlorophyll was 34.75 mM without bacterial inoculation while with bacterial the range was from 35.47 at zero level to 3.49 at 200 mM. (Table 23)

Table (23) means of chlorophyll content in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	34.75	35.32	10.41	3.93	3.97
With Bacteria	35.47	15.17	24.18	3.79	3.49
Fpr					
Bacteria	0.461				
Salinity	<.001				
Bac. Salinity	0.051	LSD		7.505	

4.1.22. Analysis of variance for the effect of salinity and bacterial inoculation on awn softness in Icarda 5

Bacteria inoculation and salinity showed highly significant effect ($P \leq 0.05$) on awn softness (Table 66).

Table 67 based on mean separation the data showed that as the level of salinity increase the awn softness decrease with and without bacterial inoculation range from 2.6 at no salinity treatment to 1.2 at 100 mM and from 1.73 at no salinity to 0.2 at 100 mM with and without bacteria respectively. However bacterial inoculation significantly reduces the effect of salinity on awn softness.

Table (24) Means, Standard Errors and Analysis of Variance for awn softness in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	1.73	2.27	0.2	0.0	0.0
With Bacteria	2.6	2.13	1.2	0.0	0.0
Fpr					
Bacteria	0.008				
Salinity	<.001				
Bac. Salinity	0.008	LSD		0.5658	

4.1.23. Analysis of Variance for the effect of salinity and bacterial inoculation leaf number in Icarda 5

Highly significant difference at P level 0.001 based on the analysis of variance for leaf number under salinity condition and bacterial inoculation. The means for highest number of leaf was at zero mM level salinity and with bacterial inoculation 5.93 and the lowest number at 150 mM was 1.00 while without bacterial inoculation the range was 3.73 to 1.67 at the same level of salinity (Table 25).

Table (25) Means, Standard Errors and Analysis of Variance for leaf number in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	3.73	3.47	3.73	1.67	1.73
With Bacteria	5.93	5.87	5.13	1.00	1.53
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.003		LSD	1.337	

4.1.24. Analysis of Variance for the effect of salinity and bacterial inoculation for leaf area length in Icarda 5

The analysis of variance for leaf length indicated a highly significant difference for bacterial inoculation and the level of salinity. The range of means for plant inoculated with bacteria was 3.23 cm at 0 mM to 0.25 cm at 200 mM while plant without inoculation was 2.58cm at 100mM to 0 cm at 200 mM (Table 26).

Table (26) Means, Standard Errors and Analysis of Variance for leaf area length in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	2.28	1.86	2.58	0.48	0.0
With Bacteria	3.23	3.2	2.86	1.11	0.25
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.361	LSD		0.8742	

4.1.25. Analysis of Variance for the effect of salinity and bacterial inoculation for leaf area width in Icarda 5

Leaf width was highly affected by salinity level as the statistical analysis indicated high significant difference ($P \leq 0.05$) for salinity level while the interaction between bacterial inoculation and salinity have no significant difference on leaf width. The leaf width range 0.28 cm at 100 mM to 0 cm at 200 mM and 0.37cm at 0 mM to 0.05cm at 200 mM without and with inoculation respectively (Table 27).

Table (27) Means, Standard Errors and Analysis of Variance for leaf area width in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	0.27	0.18	0.28	0.07	0.0
With Bacteria	0.31	0.29	0.25	0.09	0.05
Fpr					
Bacteria	0.043				
Salinity	<.001				
Bac. Salinity	0.286	LSD		0.08378	

4.1.26 Analysis of Variance for the effect of salinity and bacterial inoculation for peduncle length in Icarda 5

No significant effect of salinity levels and bacterial inoculation was observed in relation to peduncle length. The mean separation for Icarda 5 without inoculation showed that as the level of salinity increase the peduncle length decrease 3.46 cm at zero salinity to 0 cm at 200 mM in contrast the response for Icarda 5 inoculated with bacteria was variable 4.73 cm at zero level to 0.53 cm at 200 mM (Table 28).

Table (28) Means, Standard Errors and Analysis of Variance for peduncle length in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	3.46	4.31	0.65	0.0	0.0
With Bacteria	4.73	4.10	3.20	0.21	0.53
Fpr					
Bacteria	0.011				
Salinity	<.001				
Bac. Salinity	0.088		LSD	1.494	

4.1.27. Analysis of variance for the effect of salinity and bacterial inoculation for plant height in Icarda 5

Statistical analysis for salinity and bacterial inoculation for plant height showed a highly significant difference. However no interaction between salinity and bacterial inoculation was detected. Mean separation showed that height of plant inoculated with bacteria range from 25.07 cm at zero level to 4.12 cm at 200 mM while without inoculation 18.19 cm at 50 mM level to 2.29 cm at 200 mM (Table 29).

Table (29) Means, Standard Errors and Analysis of Variance for plant height in Icarda

Salinity Concentration	0	50	100	150	200
Without Bacteria	17.64	18.19	12.79	3.29	2.29
With Bacteria	25.07	22.28	17.77	7.35	4.12
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.615	LSD		4.883	

4.1.28. Analysis of Variance for the effect of salinity and bacterial inoculation for root weight in Icarda 5

Root weight showed no significant difference ($P > 0.05$) based on the analysis of variance for salinity and bacterial inoculation. The range for root weight 21.8g at 100 mM to 17.3g at 0 mM and 31.3g at 50 mM to 18.6g at 0 mM for inoculated plant and non-inoculated plant respectively (Table 30).

Table (30) Means, Standard Errors and Analysis of Variance for root weight in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	18.6	31.3	16.5	25.7	24.2
With Bacteria	17.3	19.7	21.8	20.7	18.9
Fpr					
Bacteria	0.109				
Salinity	0.207				
Bac. Salinity	0.199	LSD		10.05	

4.1.29. Analysis of variance for the effect of salinity and bacterial inoculation for spike length in Icarda 5

The analysis of variance showed high significant difference for spike length under different salinity level. In addition the response of plant to bacterial inoculation was also highly significant. The mean separation showed that the highest length was 1.51 cm at 50 mM level to 0 cm at 200 mM and 2.35 cm at 0 mM to 0.17 at 200 mM without and with bacterial inoculation respectively (Table 31).

Table (31) Means, Standard Errors and Analysis of Variance for spike length in Icarda 5

Salinity Concentration	0	50	100	150	200
Without Bacteria	1.31	1.51	0.20	0.0	0.0
With Bacteria	2.35	1.75	1.29	0.17	0.17
Fpr					
Bacteria	<.001				
Salinity	<.001				
Bac. Salinity	0.024		LSD	0.5595	

4.1.30. The effect of bacteria inoculation and salinity level on tiller number for Icarda 5

During the measurement of tiller number it was observed that no tillering records for Icarda 5.

4.2. Chemical analysis for plant nutrients.

4.2.1. Total Nitrogen

4.2 .1.1 Total Nitrogen % in the Root and Shoot of Reehan

The chemical analysis for Nitrogen content showed reduction in the percentage of Nitrogen accumulation in plant inoculated with bacteria at highest salinity level 0.64 % at 100 mM while the level of Nitrogen was very high in plant root without inoculation 1.38 % at 100 mM (Fig.5). The trend of Nitrogen accumulation in the shoot was in general the opposite as the level of salinity increase the amount of Nitrogen in plant inoculated with bacteria was higher in root and opposite in shoot (Fig.6).

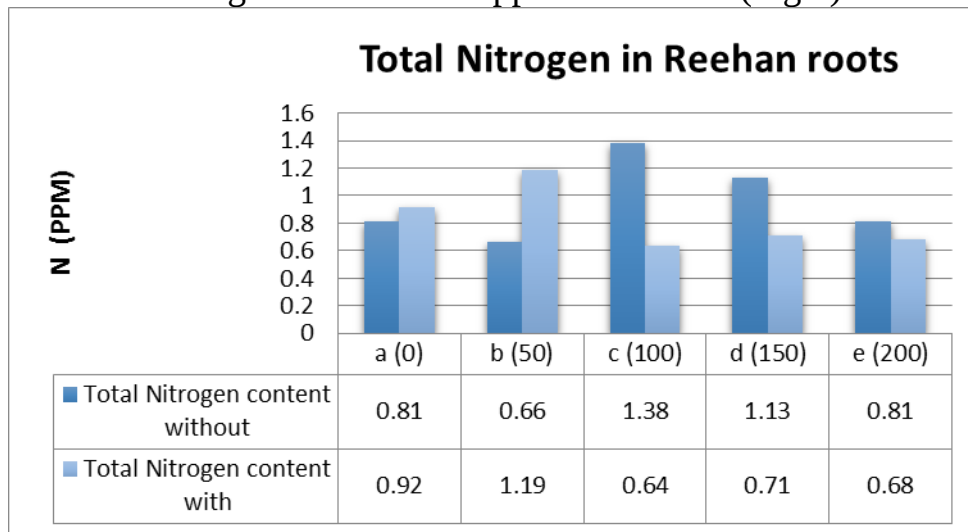


Fig .5: Total Nitrogen % in root of Reehan at different levels of salinity

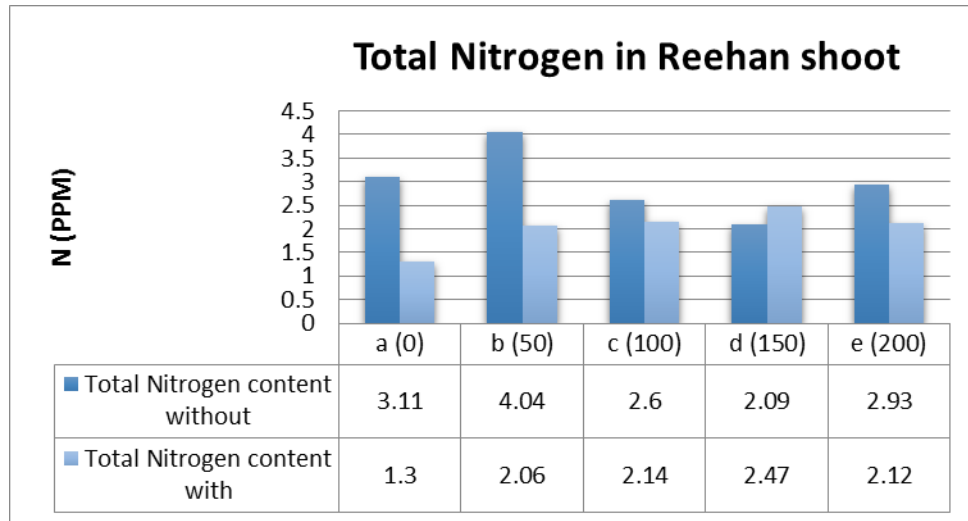


Fig.6 Total Nitrogen percentage in the shoot of Reehan at different levels of salinity .

4.2 .1.2 Total Nitrogen % in the Root and Shoot of Nabawi

The chemical analysis for Nitrogen content in the Root of Nabawi showed raising in the percentage of Nitrogen accumulation in plant inoculated with bacteria at zero and 50 mM salinity level (0.87 to 1.12%) while the level of Nitrogen was low in plant root with bacterial inoculation at high level of salinity (0.77 to 0.68%) (Fig .7). The trend of Nitrogen accumulation in the shoot was in general the opposite as the level of salinity increase the amount of Nitrogen in plant inoculated with bacteria was higher (Fig .8)

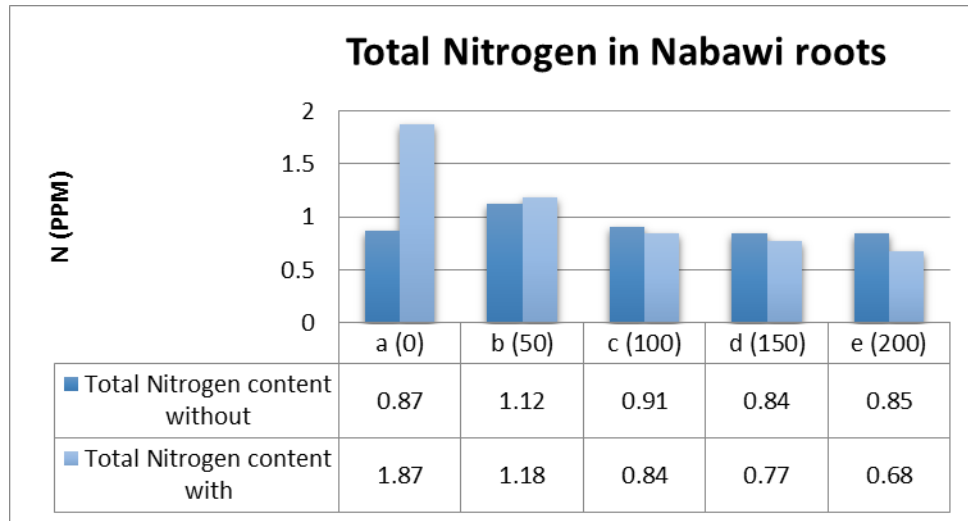


Fig .7 Total Nitrogen % in root of Nabawi at different levels of salinity

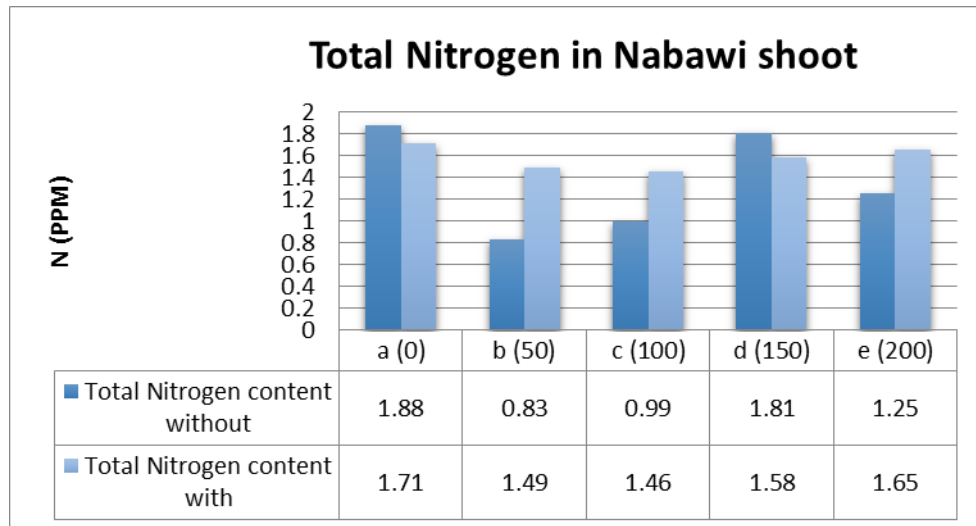


Fig .8 Total Nitrogen percentage in shoot of Nabawi at different levels of salinity

4.2 .1.3 Total Nitrogen % in the Root and Shoot of Icarda 5

At the highest salinity level the amount of Nitrogen was very high in plant root of Icarda 5(Fig .9), and low percentage at zero level of salinity (0.63% N) with bacterial inoculation, while in shoot N % was very high at zero level without bacterial inoculation (0.99 %) in contrast the amount of Nitrogen in plant inoculated with bacteria was higher at 200 mM level

salinity (1.93%) in shoot compared to 1.281 %at the same salinity level without bacteria according to the chemical analysis for Nitrogen content (Fig .10)

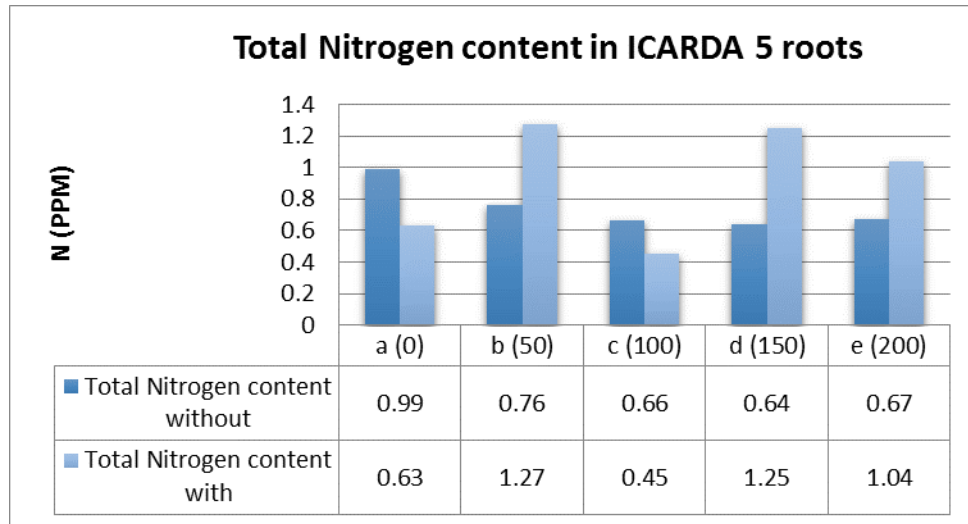


Fig .9 Total Nitrogen % in root of (Icarda 5) at different levels of salinity

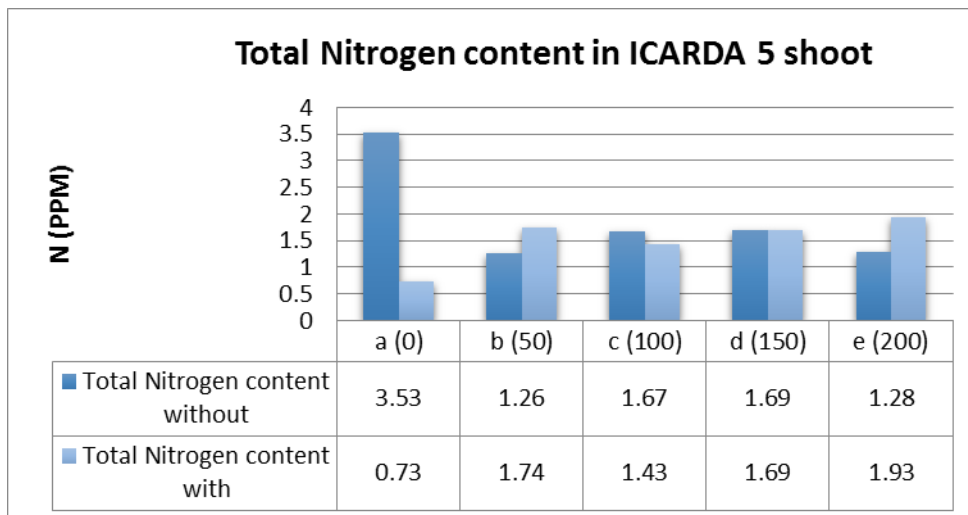


Fig .10 Total Nitrogen % in shoot of (Icarda 5) at different levels of salinity

4.2.2 Chloride

4.2 .2.1 Chloride concentration in Root and Shoot of Reehan

The chemical analysis for Chloride in root of Reehan was very high at 100 mM (4254 ppm) salinity level and low at 200mM (355ppm) with inoculation of bacteria (Fig .11). The Chloride accumulation in the shoot was higher at different level of salinity without bacterial inoculation the highest Cl content in shoot was 3367.75 ppm at 200mM to 620 ppm at zero salinity (Fig .12).

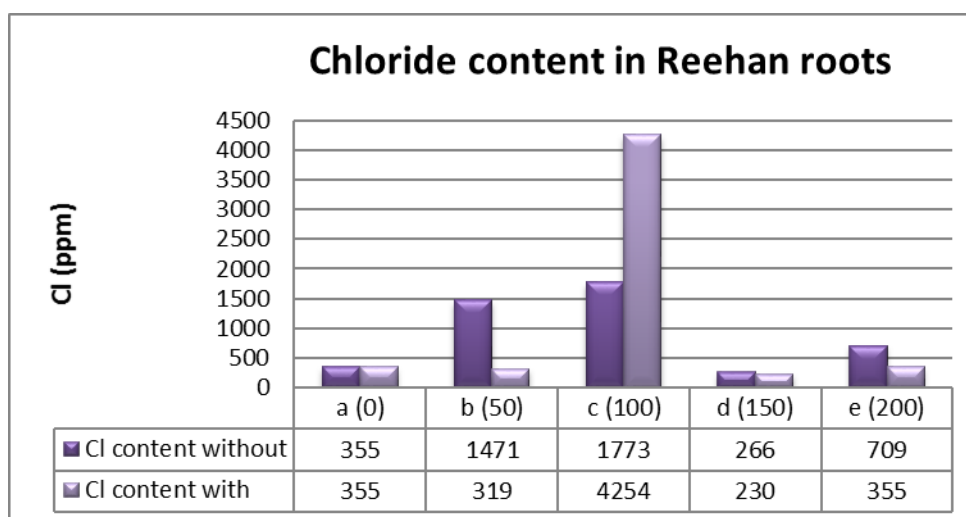


Fig .11 Chloride concentration in roots of Reehan at different levels of salinity

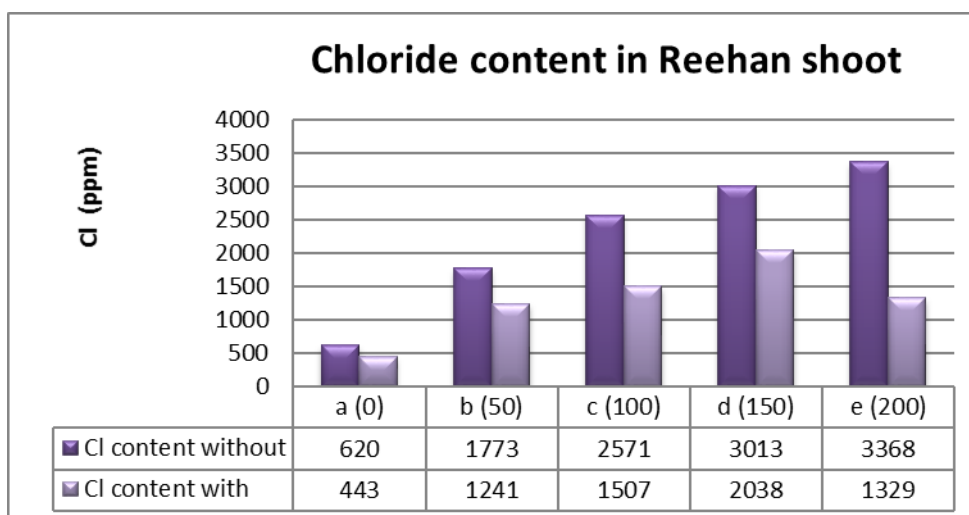


Fig .12 Chloride concentration in shoot of Reehan at different levels of salinity.

4.2 .2.2 Chloride concentration in Root and Shoot of Nabawi

The chemical analysis for Cl in the root of Nabawi show that the highest accumulation of Cl was at 50 mM (1595.25 ppm) with inoculation of bacteria then reduced when salinity level increased and therefore raised the Cl accumulation with plant not treated with Bacteria, in contrast non inoculated plant showed higher Cl content as salinity increase ranged (177to 5325 ppm) (Fig .13)

.In the shoot Nabawi have the heighest level of Cl at 100mM with bacteria (4431ppm) and 200 mM without Bacterial inoculation (5211ppm) (Fig .14)

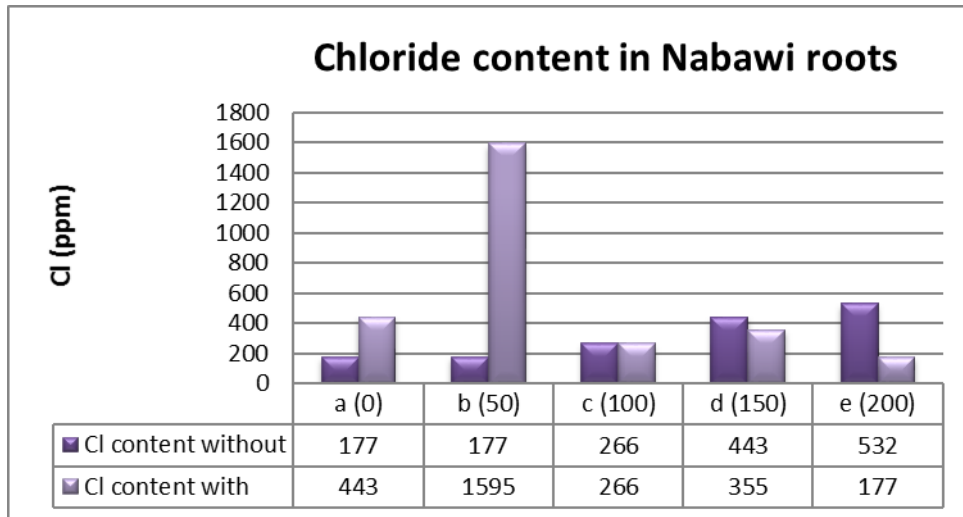


Fig. 13 Chloride concentration in roots of Nabawi at different levels of salinity

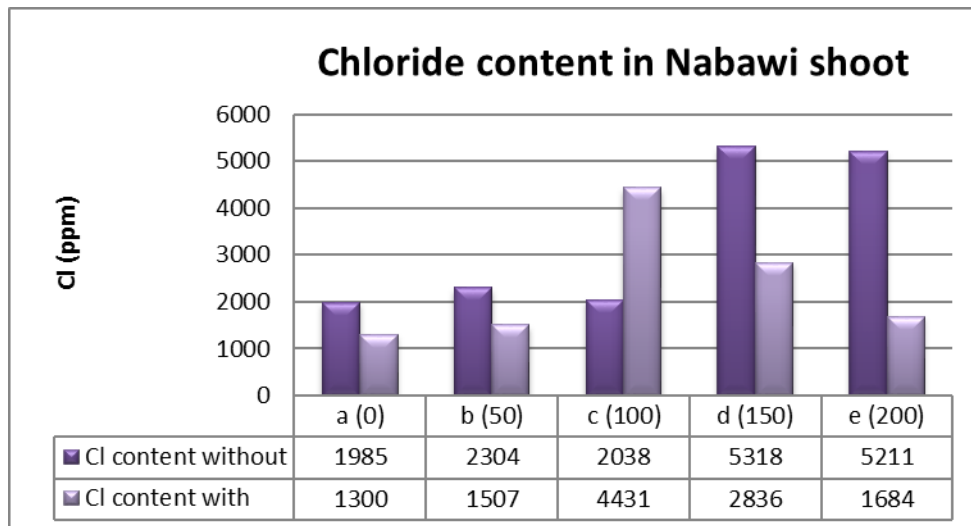


Fig. 14 Chloride concentration in shoot of Nabawi at different levels of salinity

4.2 .2.3 Chloride concentration in Root and Shoot of Icarda 5

At highest salinity level 200 mM with bacterial inoculation root analysis showed extremely high (655 ppm) Cl content the level decrease drastically in the root of Icarda 5 (Fig .15). Similar in the shoot the accumulation of Cl was high at 200 mM 6470 with bacterial inoculation compared to 1418 ppm Cl at same salinity level without bacterial inoculation (Fig .16).

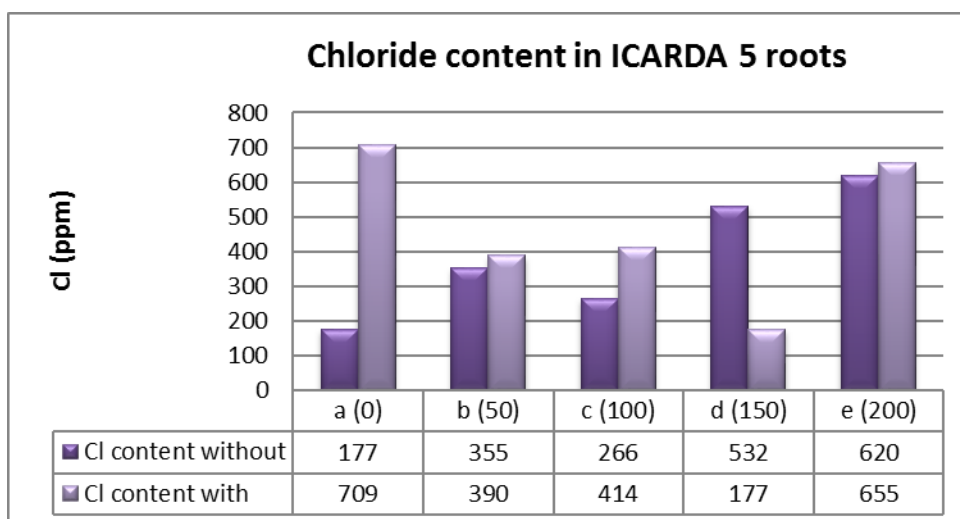


Fig 15 Chloride concentration in roots of (Icarda 5) at different levels of salinity

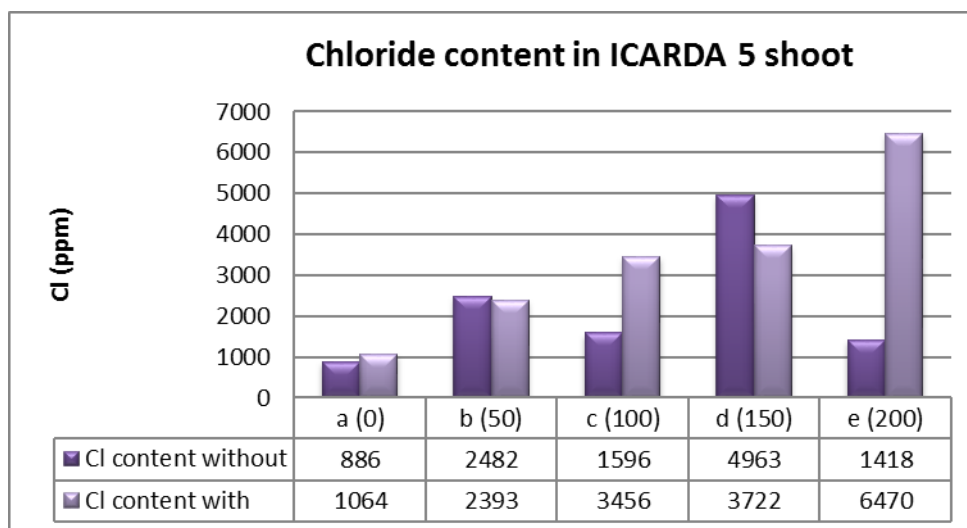


Fig 16 Chloride concentration in shoot of (Icarda 5) at different levels of salinity

4.2.3 Calcium “Ca”

4.2 .3.1 Calcium concentration in Root and Shoot of Reehan

The chemical analysis for root showed reduction in the percentage of Calcium accumulation in plant inoculated with bacteria at highest salinity

level 200mM (873 ppm) while the level of Calcium was very high in plant root without inoculation 2250 ppm at salinity level ≥ 100 mM (Fig .17).

The trend of Calcium accumulation in the shoot was in general the opposite as the level of salinity increase the amount of Calcium in plant inoculated with bacteria was higher except at 100 mM level was very low.

(Fig .18)

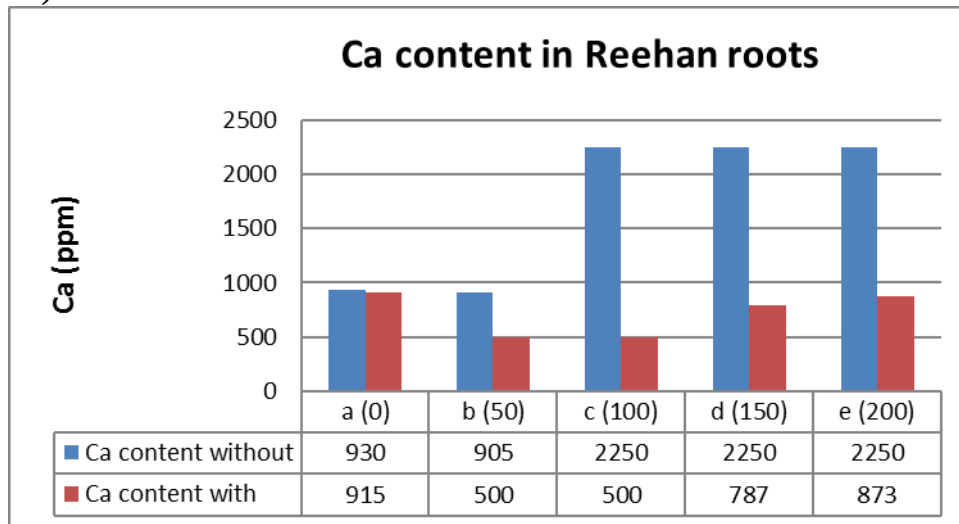


Fig.17 Calcium concentration in root of Reehan at different levels of salinity

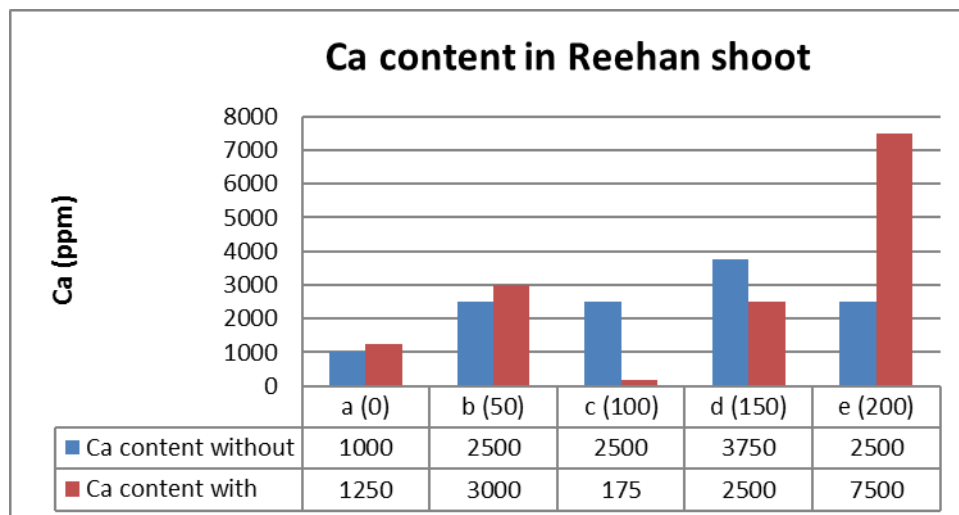


Fig. 18 Calcium concentration in shoot of Reehan at different levels of salinity

4.2 .3.2 Calcium concentration in Root and Shoot of Nabawi

The chemical analysis for root showed reduction in the percentage of Calcium accumulation in plant inoculated with bacteria at highest salinity level salinity ranged from 50 to 150mM (805.25 to 334 ppm) while the level of Calcium was very high in plant root without inoculation at salinity level ranged from 50 to 200mM (846 - 888ppm) (Fig .19). The trend of Calcium accumulation in the shoot was higher at high level of salinity with bacteria (Fig .20)

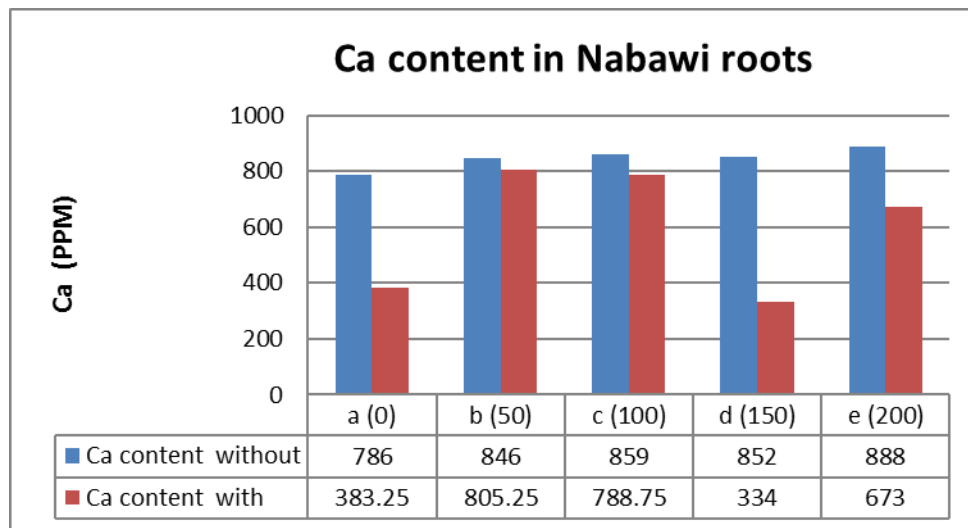


Fig.19 Calcium concentration in root of Nabawi at different levels of salinity.

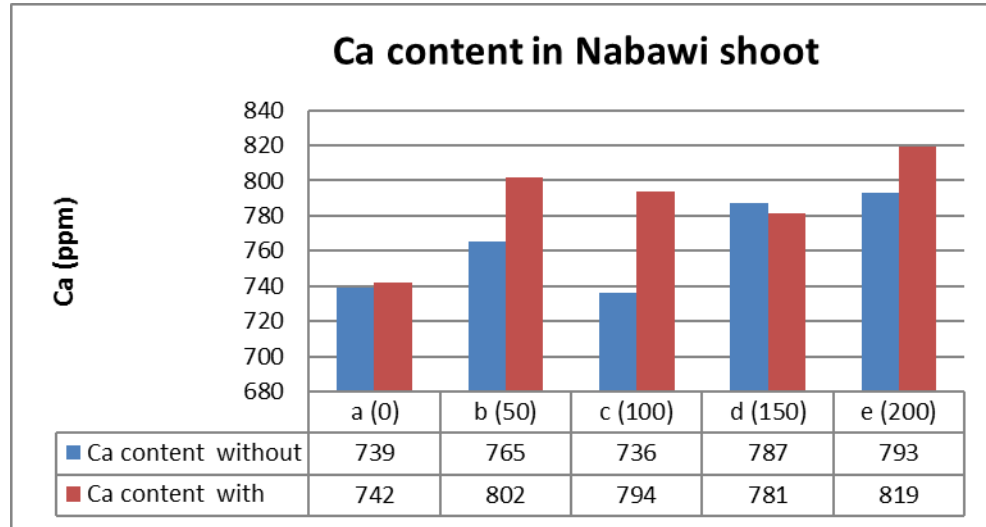


Fig .20 Calcium concentration in shoot of Nabawi at different levels of salinity

4.2 .3.3 Calcium concentration in Root and Shoot of Icarda 5

The percentage of Calcium accumulation in the root according to the chemical analysis was slightly lower in plant which inoculated with bacteria 923 at 200mM compared to non inoculated plant 930 ppm at the same level of salinity (Fig.21). The trend of Calcium accumulation in the shoot was in general the opposite as the level of salinity increase the amount of Calcium in plant inoculated with bacteria was higher the range was 862.25 at zero salinity to 879 ppm at 200mM (Fig.22).

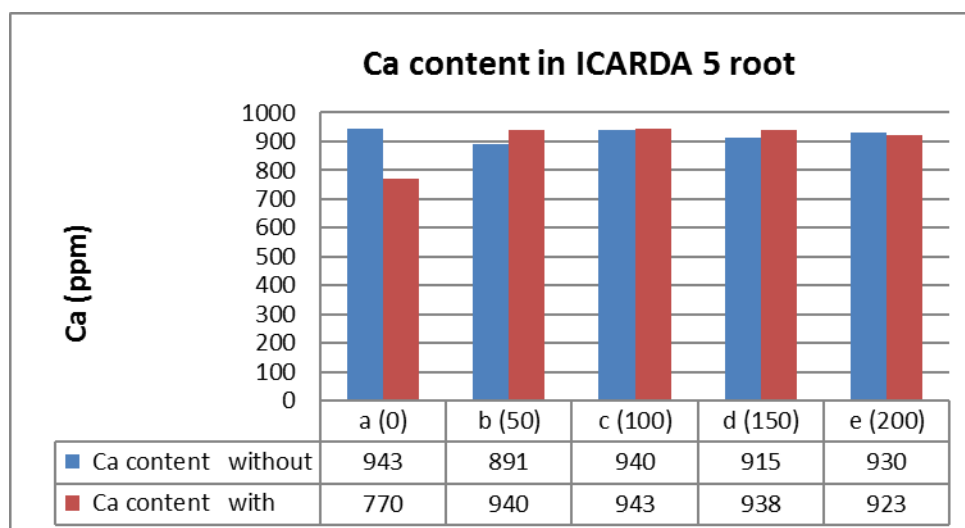


Fig.21 Calcium concentration in root of (Icarda 5) at different levels of salinity

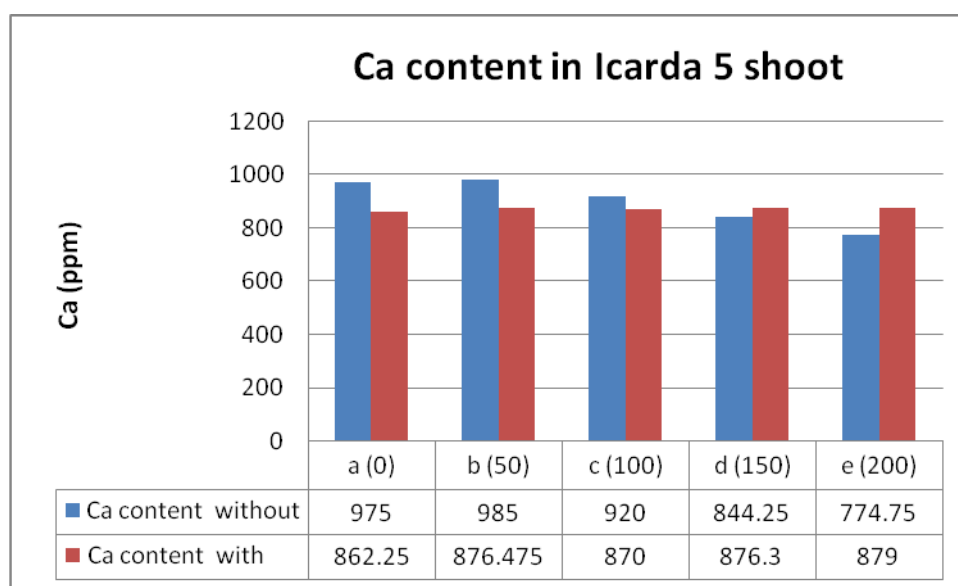


Fig.22 Calcium concentration in shoot of (Icarda 5) at different levels of salinity

4.2 .4.Sodium “Na⁺”

4.2 .4.1 Sodium concentration in Root and Shoot of Reehan

The chemical analysis for root showed raise in the percentage of Sodium accumulation in plant inoculated with bacteria at highest salinity level (250 ppm) while the level of Sodium was very high in plant root without inoculation 500 ppm at 100 mM (Fig. 23). Conversely Sodium accumulation in the shoot was higher at high level of salinity with bacteria (5000 ppm at 200 mM) (Fig .24).

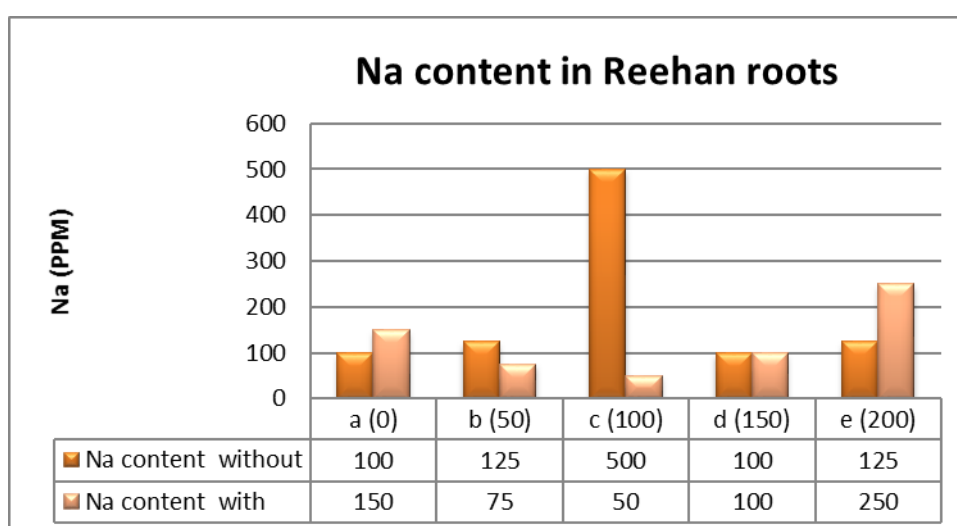


Fig .23 Sodium concentration in root of Reehan at different levels of salinity

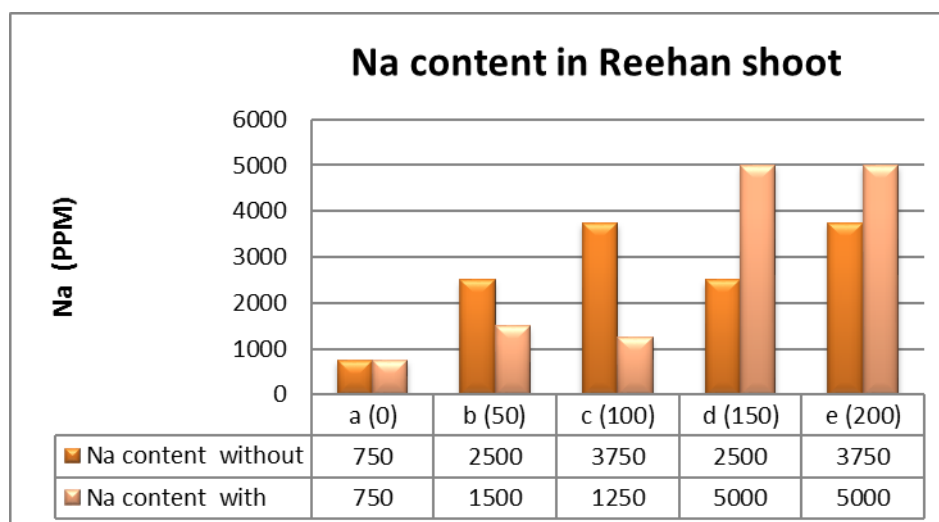


Fig .24 Sodium concentration in shoot of Reehan at different levels of salinity

4.2 .4.2 Sodium concentration in Root and Shoot of Nabawi

The chemical analysis for root showed high in the percentage of Sodium accumulation in plant inoculated with bacteria 750 ppm at 50mM while the level of Sodium was very low in plant root with bacterial inoculation 50 ppm at 200mM (Fig .25). Conversely Sodium accumulation in the shoot was higher 15000 ppm at 50mM of salinity without bacteria and 5000 ppm at 150 mM without bacterial inoculation (Fig .26).

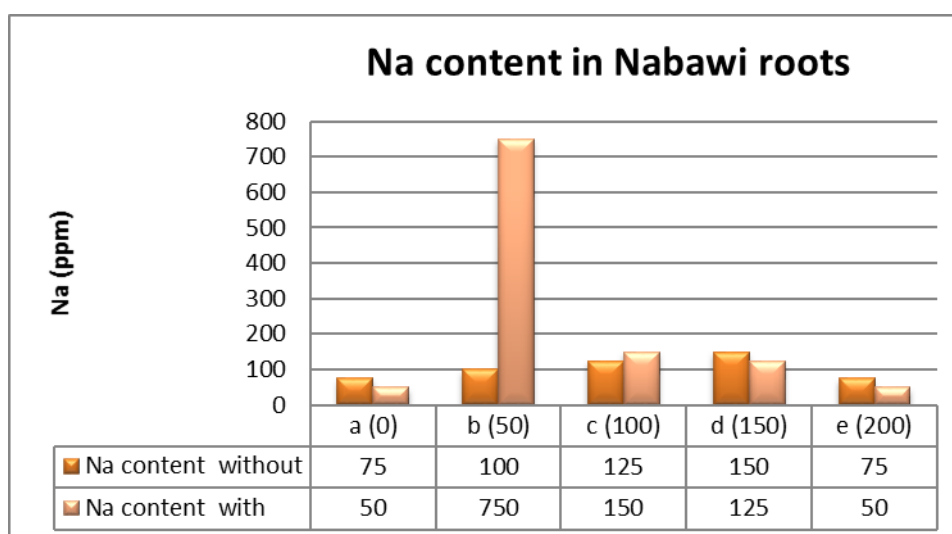


Fig.25 Sodium concentration in the root of Nabawi at different levels of salinity

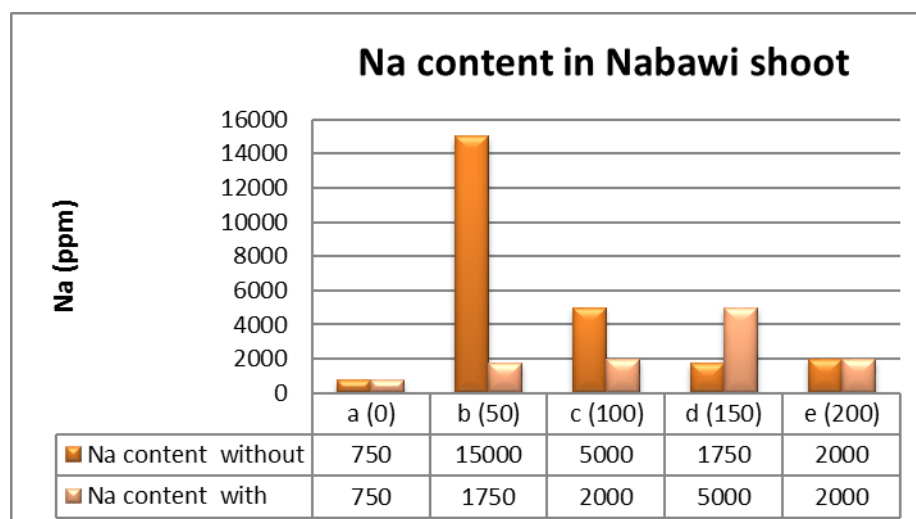


Fig.26 Sodium concentration in shoot of Nabawi at different levels of salinity

4.2 .4.3 Sodium concentration in the Root and Shoot of Icarda 5

The percentage of Sodium accumulation in the root of plant inoculated with bacteria at 150mM was high (Fig .27). The level of Sodium was very high in plant root without inoculation at 200mM. The highest Sodium content in shoot inoculated with bacteria was 1500 ppm at 100 mM. (Fig .28).

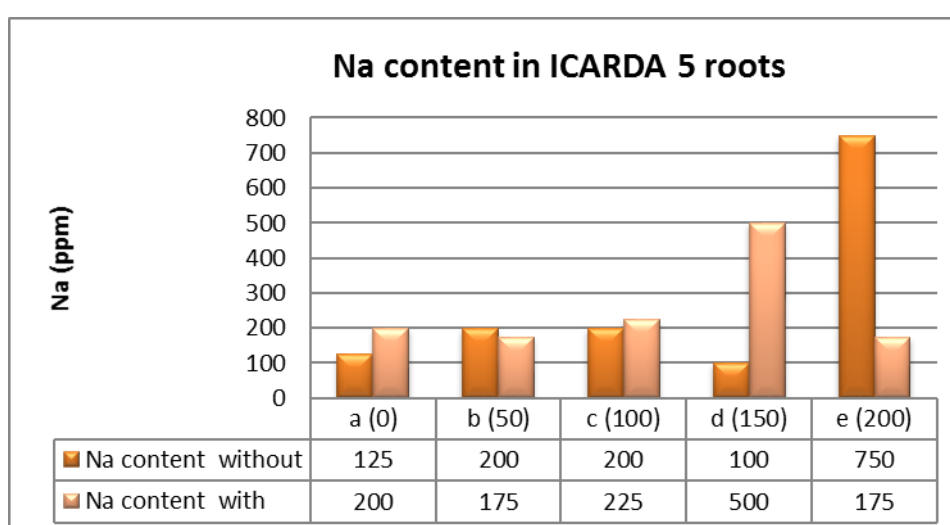


Fig. 27 Sodium concentration in root of (Icarda 5) at different levels of salinity

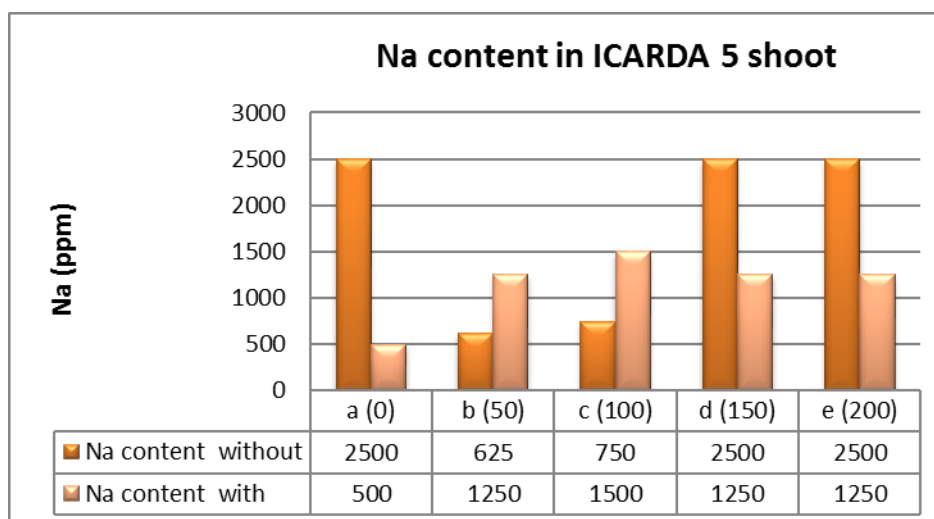


Fig .28 Sodium concentration in shoot of (Icarda 5) at different levels of salinity

4.2 .5 Potassium “K⁺”

4.2 .5.1 Potassium concentration in Root and Shoot of Reehan

The chemical analysis of root showed that the highest Potassium accumulation was at 100mM in Reehan inoculated with bacteria 11250 ppm in contrast it was very low 225 PPM at 200mM (Fig.29) however without bacterial inoculation the highest level of K⁺ 2500 ppm was at 200mM. In shoot the chemical analysis showed that the effect of bacterial inoculation was pronounced on Potassium content (5000ppm) at higher salinity level 150-200mM compared to non-inoculated plants (2500 ppm) (Fig .30).

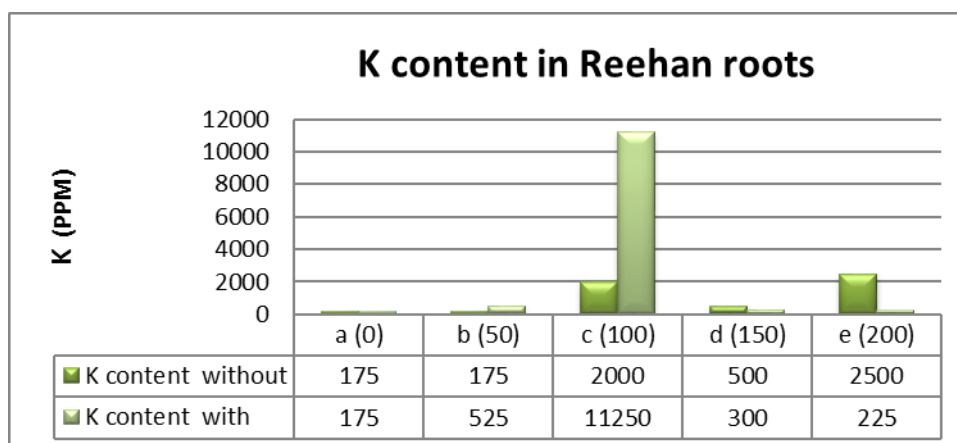


Fig .29 Potassium concentration in root of Reehan at different levels of salinity

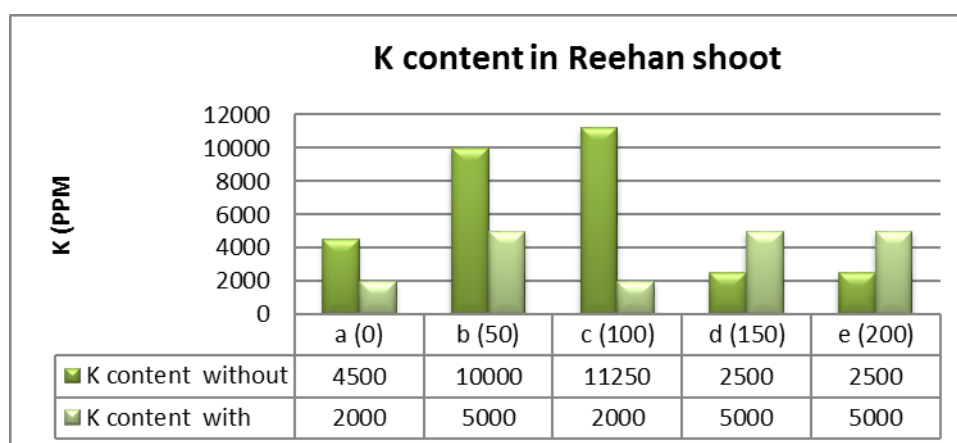


Fig .30 Potassium concentration in shoot of Reehan at different levels of salinity

4.2 .5.2 Potassium concentration in Root and Shoot of Nabawi

The chemical analysis of root showed higher K^+ content 750 ppm at 50 mM in plant inoculated with bacteria while the level of Potassium was high 275 ppm in plant root without bacterial inoculation at 150 mM salinity level (Fig .31).

On the other hand Potassium accumulation in shoot of plant with bacterial inoculation ranged from 2500 ppm at zero salinity to 500 ppm at 200mM

and ranged from 2250 ppm at zero salinity to 750 ppm at 200mM in plant shoot without bacterial inoculation (Fig .32)

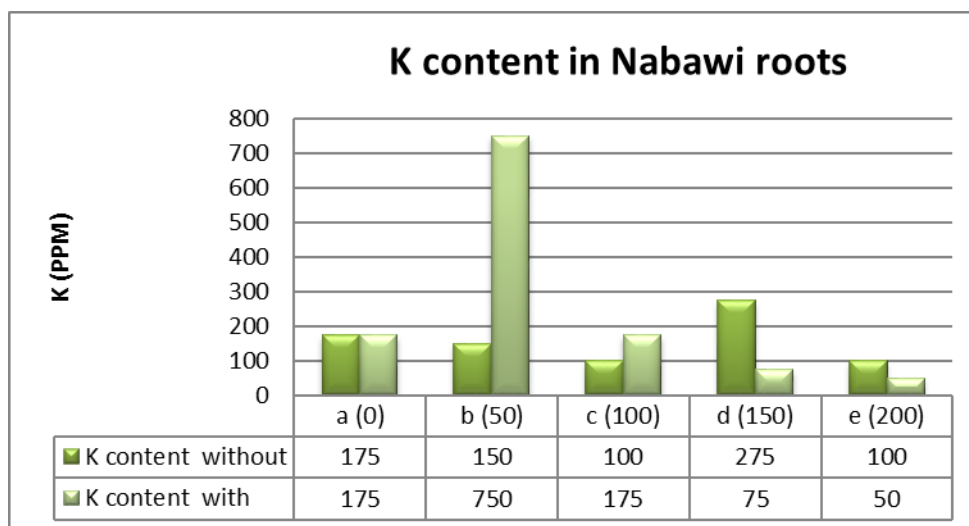


Fig .31 Potassium concentration in root of Nabawi at different levels of salinity.

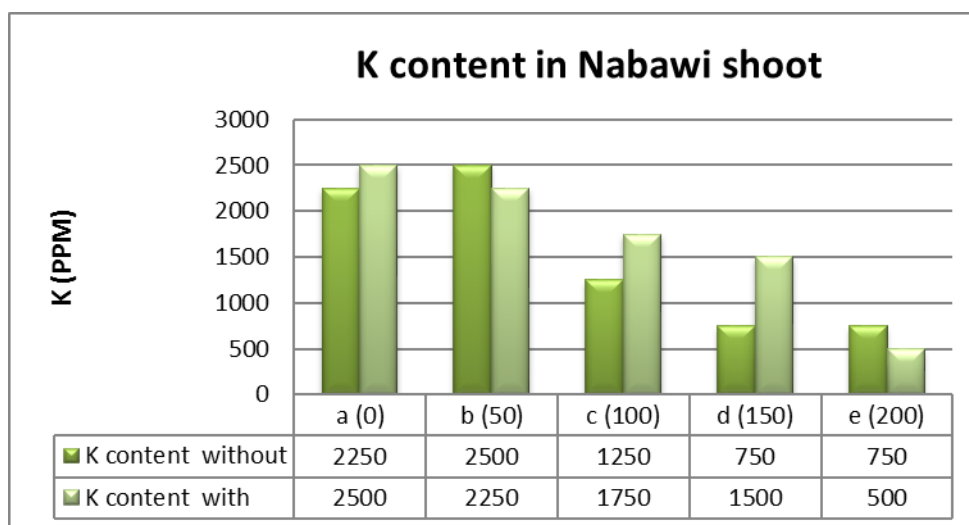


Fig .32 Potassium concentration in the shoot of Nabawi at different levels of salinity .

4.2 .5.3 Potassium concentration in Root and Shoot of Icarda 5

The chemical analysis for root showed that the percentage of Potassium accumulation in plant inoculated with bacteria increased at highest salinity

level 2500ppm at 200mM while the level of Potassium was 750ppm root without bacterial inoculation at same salinity level. (Fig .33)

The Potassium accumulation in shoot without bacterial inoculation was higher 11250 mM at zero salinity and 750ppm at 200mM salinity, and ranged from 2250ppm at zero salinity to 750ppm at 200mM in inoculated plant with bacteria (Fig .34)

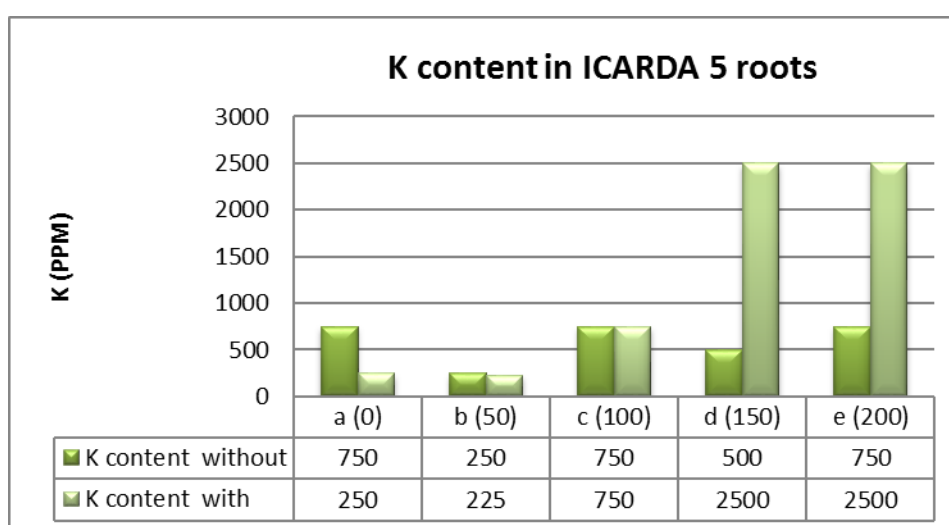


Fig.33 Potassium concentration in root of (Icarda 5) at different levels of salinity

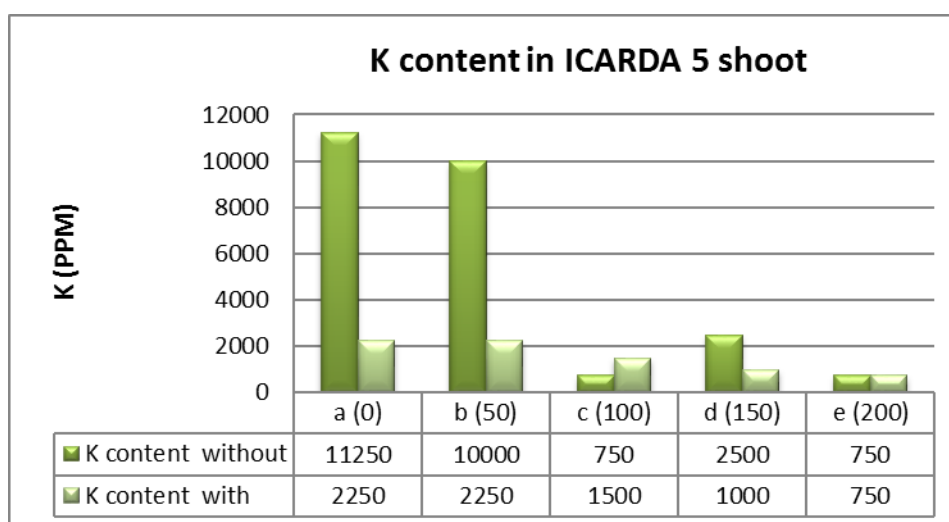


Fig.34 Potassium concentration in shoot of (Icarda 5) at different levels of salinity.

4.2 .6 Total Phosphorus

4.2 .6.1 Total Phosphorus concentration in Root and Shoot of Reehan

In the root of plant with bacterial inoculation total Phosphorus was very high 3.391 ppm at 100mM, and low .0543 ppm at 100mM and without bacterial inoculation the highest content of was 1.72 ppm at zero salinity and low(-0.502 at 200mM) (Fig .35).

In the shoot of plant inoculated with bacteria highest Phosphorus content was 4.63 ppm at 50 mM and the lowest was 1.83 ppm at 200mM in contrast plant without bacterial inoculation showed highest Phosphorus concentration 5.26 ppm at 100mM and reduced to 1.47 ppm at 150mM (Fig .36).

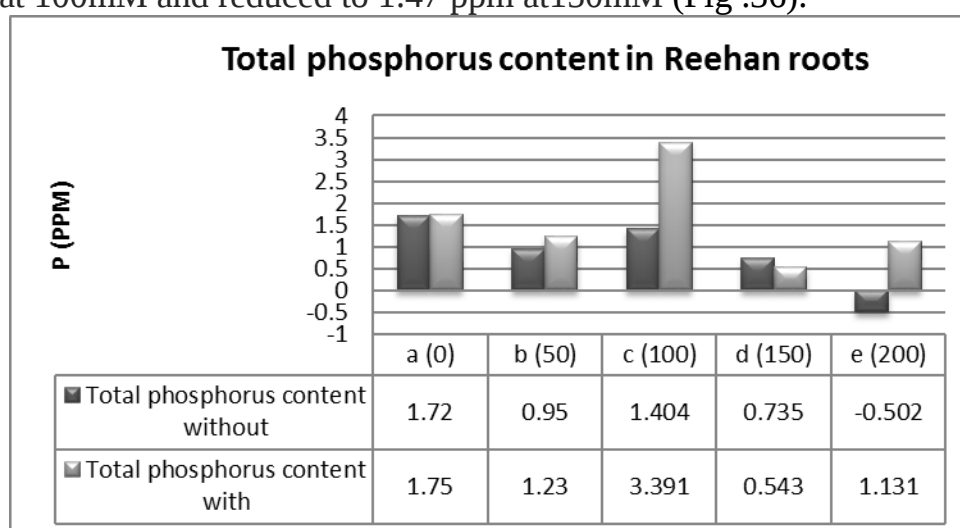


Fig.35 Total Phosphorus concentration in Root of (Reehan) at different levels of salinity

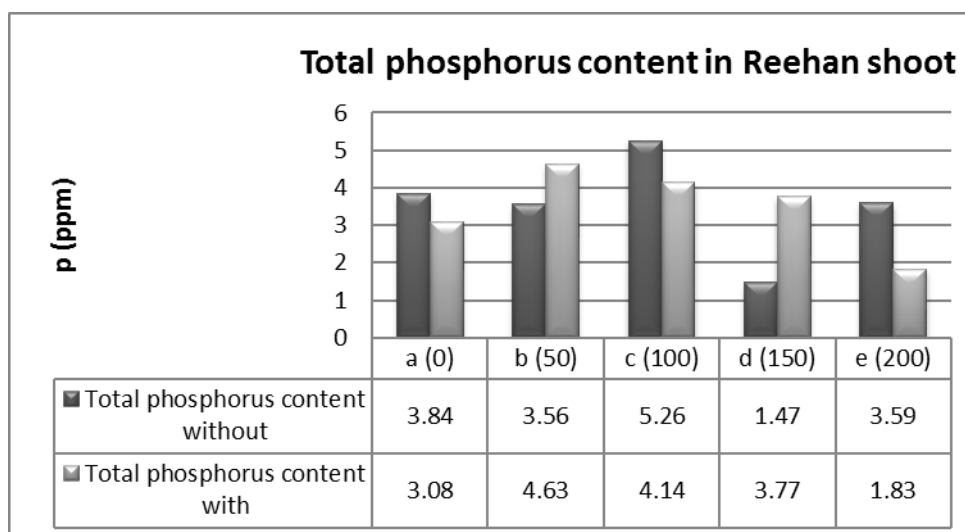


Fig.36 Total Phosphorus concentration in shoot of (Reehan) at different levels of salinity

4.2 .6.2 Total Phosphorus concentration in Root and Shoot of Nabawi

The chemical analysis for total phosphorus in the root of Nabawi was high at zero salinity level (2.52ppm), and low at 100mM (0.43ppm) with inoculation of bacteria (Fig .37).The highest total Phosphorus in the shoot with bacterial inoculation was 5.73 ppm at 100 mM ,and 6.67 ppm at 200 mM in shoot without bacterial inoculation (Fig .38).

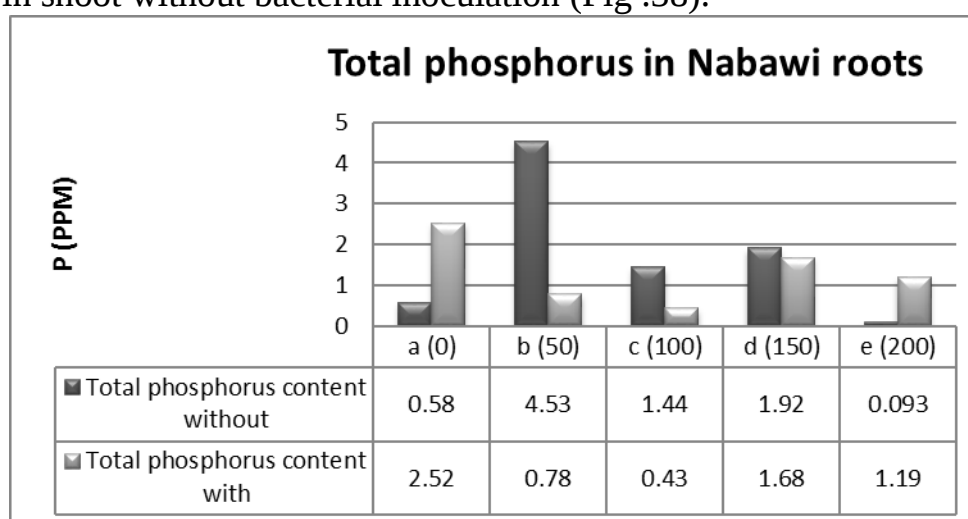


Fig.37 Total Phosphorus concentration in root of Nabawi at different levels of salinity

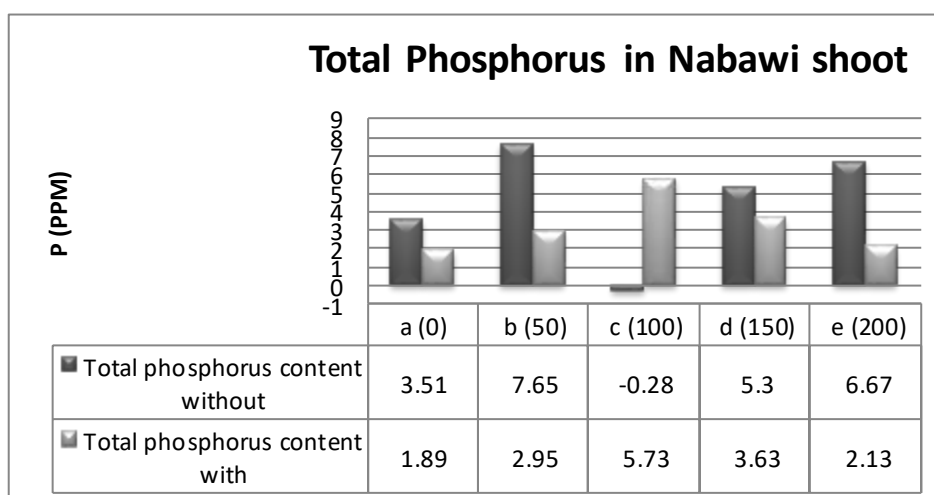


Fig.38 Total Phosphorus concentration in shoot of Nabawi at different levels of salinity.

4.2 .6.3 Total Phosphorus concentration in Root and Shoot of Icarda 5

Chemical Analysis of root inoculated with bacteria showed that Phosphorus accumulation increased at 150 mM (4.25ppm) and at zero salinity (11.02) in root without bacterial inoculation (Fig .39). In shoot without bacterial inoculation Phosphorus was very high 3.04ppm at 150mM salinity level and the highest Phosphorus content was 9.97 ppm in shoot of plant with bacterial inoculation (Fig .40).

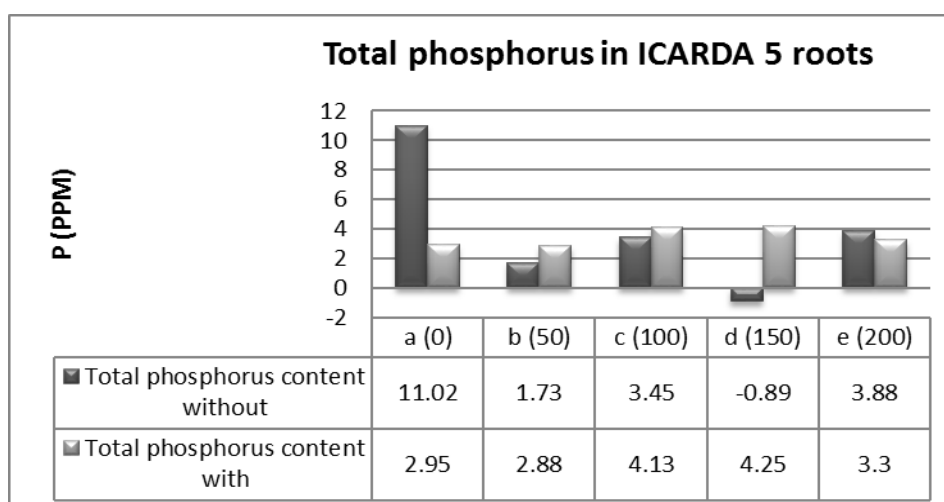


Fig.39 Total Phosphorus concentration in root of Icarda 5 at different levels of salinity

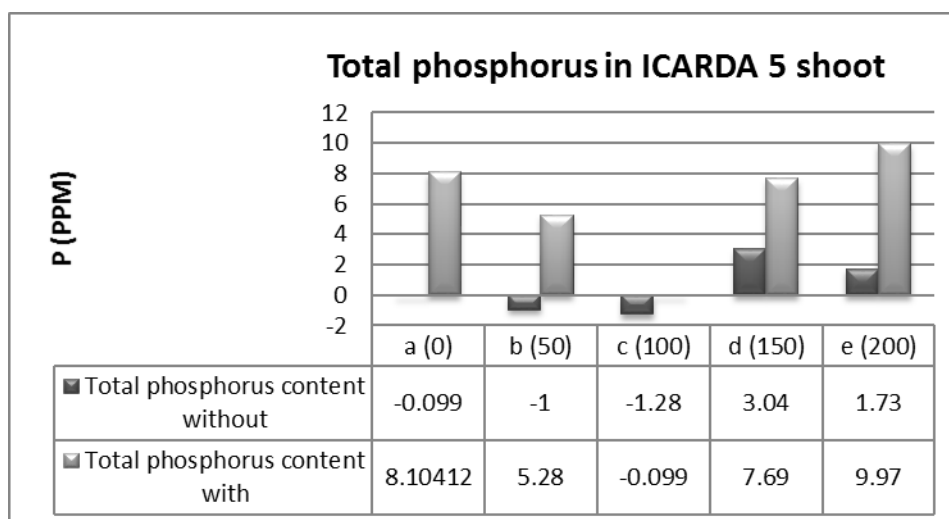


Fig.40 Total Phosphorus concentration in shoot of Icarda5 at different levels of salinity

Chapter Five

Discussion

5.1. Effect of salinity and *B. megaterium* on agronomic traits of barley:

Salinity affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent through a decrease in photosystem II efficiency (Netondo et al., 2004). Inhibited leaf growth is among the earliest visible effects of salinity stress in grasses, which was attributed to decreased cell division and expansion in the leaf's growth zone (Ortega et al., 2006; Taleisnik et al., 2009; Bernstein et al., 2010). In our study salinity significantly decrease the chlorophyll content in the three barley variety used in this study. Chlorophyll content in general increased with increasing salinity up to the 50mM without bacterial inoculation. The fluctuation in Reehan might be attributed to sensitivity to light intensity. Several researchers reported the effect of light intensity on photosynthetic pigments. In crop plants, pigment composition of barley did not change significantly in response to salt stress when grown under low light (Morales et al 1992). On the other hand, some studies have demonstrated that salinity in the presence of high light induced significantly changes in pigment composition in sorghum (Mosojidek et al 1991). Other researcher explained the decrease in chlorophyll content at higher salinity levels possibly due to changes in the lipid protein ratio of pigment-protein complexes or increased chlorophyllase activity (Iyengar

and Reddy 1996). Our results are in agreement with several reports of decrease content of chlorophyll by salinity as reported in a number of glycophytes (Agaistian et al., 2000) and in certain halophytes such as *Suaeda salsa* (Congming et al., 2002) and *Aegiceras corniculatum* (Parida et al 2004). In addition salinity induces partial stomatal closure, decreasing carboxylation efficiency and CO₂ saturated photosynthesis and inhibiting the light reaction mechanism (Mudrik et al., 2003).

The bacterial inoculation significantly reduces the effect of salinity on chlorophyll content in the three landrace. No significant interaction was observed at zero level with or without bacteria. However, the inoculated plants had higher chlorophyll content than the control plants at higher salinity levels. Our results were consistent with previous studies on high-level photosynthetic pigments that allowed the plants to withstand harsh conditions, including drought, salinity, oxidative stress, and heavy metal stress (Wimalasekera et al., 2011, Wen et al., 2008; Jang et al., 2012; Zhou et al., 2014; Gupta et al., 2012; Parvin et al., 2013; Liu et al., 2016). The data shown in this study imply that *B. megaterium* enhanced the ability of plants to tolerate salinity stress, which was likely attributed to chlorophyll contents. Percival et al., (2003) reported that leaf chlorophyll concentration is an indicator of salt tolerance and responds to increasing salinity. Sheikh et al., (2016) proposed that chlorophyll content was significantly higher in okra plants receiving bacterial suspension compared with control plant. Hassine et al., (2010) and Abbas et al., (2013) suggested that in control plants, chlorophyll is destroyed due to excessive amount of salts, ions (Na

and Cl), or reactive oxygen species (ROS) which disturb the cellular metabolism and result in the degeneration of cell organelles in the leaf tissue. On the other hand, the inoculated salt stressed okra plants exhibited higher chlorophyll content and dark green leaves owing to the presence of ACC deaminase-containing PGPR isolates that maintain the photosynthetic efficiency of plants by reducing ethylene biosynthesis. Ali and colleagues (2014) observed that inoculation of plants with wild-type *P. fluorescens* YsS6 and *P. migulae* 8R6 significantly increased the total chlorophyll content of tomato plants compared to control plants under salt stress.

Awn softness was showed significant difference in relation to salinity and bacteria. The awn type was smooth to moderate. Texture was influences with salinity level. As the level of salinity decrease the smoothness increase. Jana' et al., (1980) indicted that smooth awned were tolerant of salt stress as compared to rough-awned barleys. *B. megaterium* inoculation increases the level of awn roughness slightly. No previous study was investigating the effect of bacterial inoculation and salinity on awns softness.

Different growth parameters; leaf length, width and number, root weight, shoot weight and plant height was highly influenced by salinity levels. The general trends was reduction in the growth parameters as the level of salinity increase with or without *B. megaterium*, however bacterial inoculation reduce the effect of salinity a slight fluctuation at 100-150mM

was also observed. Taghipour and Salehi (2008), who found that leaf area, shoot dry weight, root dry weight, shoot length, stem and root fresh weight were decreased in 12 barley varieties with increasing in salinity level. Reehan landrace was not affected by bacteria inoculation for leaf length, leaf number and width while the other two varieties significantly influenced by bacteria inoculation with positive effect. The effect of bacteria on root weight was positive but not significant. **Under saline conditions, plant growth is usually reduced by reducing the rate of leaf elongation, enlargement and leave cells division. The reduction in fresh weight of root and shoot in barley varieties with increasing salt level was also reported by Naseer et al. (2001). Ravikumar et al. (2007) found that *Ceriops decandra* inoculated with *Azotobacter beijerinckii* enhanced root dry biomass at a maximum of 75.8% at 30 g/L NaCl.**

Metin et al., (2010) concluded that PGPR (*B. megaterium* M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245, and *Raoultella terrigena*) treatments positively affected dry weight and physiological parameters.

The plants were grown in soil with salinity levels of 1.3, 6.0, and 9.5 dS m⁻¹ in the saturated soil paste extract. While the high salinity decreased grain and stem & leaf weight. Cowpea subjected to 80 mM NaCl showed reduction in stem elongation, plant fresh and dry weight and leaf area were inhibited under salinity (Jaleel et al., 2007). Whereas, Mozafar and Oertli (1990) found that the percent dry weight of tops was increased by salinity. On the other hand, Allen et al. (1998) found that salts in the soil water

solution can reduce evapotranspiration by making soil water less available for plant root extraction.

5.2. Effect of salinity and *B. megaterium* on chemical composition of barley

Chemical composition of plants is highly influenced by salinity, several study showed that under high salinity level certain element significantly accumulated in plant, for example Na^+ , Cl, K and Ca. The absorption of nutrient from soil depends on chemical composition of soil and pH. In this study Na^+ and Cl was the main sources for salinity; as a result the accumulation of Sodium was higher in shoot more than root with increased amount of accumulation as the level of salinity increase while in plant inoculated with bacteria the Sodium accumulation levels was lower in shoot and root. This could be attributed the concentration of Na^+ in the soil cause the plant to absorb more Na^+ . In addition the three varieties responds differently to salinity and bacterial inoculation treatments, for example the level of Ca^+ , Na, and K^+ in Reehan was higher at higher level of salinity with trends shifted to higher accumulation in shoot. The same trend was observed for Nabawi and Icarda in relative to K and Ca, but the opposite for Na as its accumulation reduce with response to bacterial inoculation and salinity.

Increase Sodium accumulation and Chloride has the ability to influence on plant enzymes and cause cell swelling, resulting in reduced energy production and other physiological changes (Larcher., 1980) and Cl

accumulation damage photosynthetic function through the inhibition the activity of nitrate reductase (Xu et al. 2000), Sodium accumulation destroy soil structure, increase soil pH and decrease water infiltration and aeration of soil, and leading to soil compaction.

Tester and Davenport (2003) reported that under increase accumulation of Na^+ and Cl^- rhizosphere concentration, there are competitive interactions with other nutrient ions (K^+ , NO_3^- and H_2PO_4^-) for binding sites and transport proteins in root cells, and thereafter for translocation, deposition and partitioning within the plant. Abdennaceur et al., (2014) suggested that increase the level of Sodium ions in the plant leaves results in ion toxicity.

In our study low Sodium accumulation in plant inoculated with bacteria was observed at 50-100 mM NaCl especially in the shoot and root of Reehan and Nabawi, in contrast the accumulation of Sodium in shoot of Nabawi and Icarda 5 was increased as salinity level increase in plant without bacterial inoculation at higher salinity level 200 mM compared to inoculated plants. Sodium Chloride is more likely to affect rhizosphere microbial community structure indirectly through root exudates quantity and quality than directly through microbial toxicity and that plant health is a major determinant in rhizosphere microbial community structure (Nelson and Mele, 2007) this funding could be used to explain the reduction in the effect of bacterial inoculation at higher salinity level.

PGPR can influence in host physiology and in the foliar reduction of Na^+ and Cl^- ions accumulation by increasing K^+ and Ca^{+2} . Wheat plants separately inoculated with *Pseudomonas putida*, *Enterobacter cloacae*,

Serratia ficaria and *Pseudomonas fluorescens* have been reported to increase the K^+/Na^+ ratio by increasing K^+ effectively influencing salinity tolerance (Nadeem et al., 2013). Inoculation with *Pseudomonas sp.* on eggplant (*Solanum melongena* L.) significantly increased K^+ and Ca^{+2} , and decreased Na^+ shoot concentrations under saline conditions but not under non stress conditions (Fu et al., 2010). Similar results in cotton by inoculation of *Pseudomonas putida* Rs-198 increased K^+ and Ca^{+2} , and decreased Na^+ in leaves and roots (Yao et al., 2010).

As an effect of salinity the availability, transport and mobility of Ca^{+2} and K^+ are affected in growing parts of plants. Potassium can act as a cationic solute responsible for stomatal movements as a response to changes in water status on bulk leaf (Caravaca et al., 2004) and Ca^{+2} regulates early signaling processes at the onset of salt stress (Kawasaki and Moritsugu, 1978; Muhammed et al., 1987).

Interactions of Ca^{+2} with other ions at high salinity are also known to occur and low Ca^{+2}/Na^+ concentration ratios result in reduced growth and in some cases tissue Ca^{+2} deficiencies (Grieve and Maas, 1988; Kent and Leuchli, 1985; Maas and Grieve, 1987; Muhammed et al., 1987).

Salinity reduced transpiration in barley, but supplemental Ca^{+2} did not alter transpiration rates (Cramer et al., 1989). In wheat (Hawkins and Lewis, 1993a) and cotton (Leidi et al., 1991), no significant effects of supplemental Ca were observed in salt-stressed plants on stomatal conductance, transpiration, and photosynthesis.

Lynch et al., 1988 demonstrated that Na: Ca⁺² ratios in expanding leaf tissue increased with increasing salinity and leaf growth was reduced significantly in barley, salinity reduced total phospholipid content of the membrane (Cachorro et al., 1993b; Yu et al., 1998). One cause of reduced Ca⁺² availability is that Na replaces Ca in the leaf apoplast (Zid and Grignon, 1985). According to this study the salinity showed that increased in Calcium concentration in root of plant that not treated with bacteria in 3 different varieties of barley. Bacteria demonstrated positive effect on Calcium accumulation in shoot of plant variety at high level of salinity.

Potassium plays a major role in enhancing tolerance of plants to drought by increasing translocation and maintaining water balance. The presence of Na⁺ in the treatment solutions decreased total K⁺ accumulation (Amtmann and Sanders, 1999) suggested that Na⁺ at low levels not only harmless but can be very useful, especially in low K⁺ conditions. This is because, in hydrated form, Na⁺ and K⁺ are chemically and structurally very similar. In barley photosynthesis decreased when the K⁺ concentration in the growing medium increased this is the negative effects of salt (Degl'Innocenti et al., 2009).

Potassium uptake is mainly based on ion diffusion, which is a mechanism that is sensitive to environmental factors such as soil type, precipitation, and temperature (Véry and Sentenac 2003). Greatest K⁺ reduces the number of tillers per plant, Potassium application increased the thickening and lignification of outermost epidermal cell wall and the cell walls of the sclerenchymatous cell layer under the epidermis (Pirj et al., 2012).

Andersen, Jensen, and Lösch (1992b) reported an increase in the number of spike-bearing tillers in barley resulting from increased rates of K^+ application, although the total number of tillers increased under field conditions as the rate of K^+ applied increased. Pirj et al., (2012) demonstrated that entire plant leaf area increased in plants grown under the greatest K^+ application rates. Successive increases in K^+ levels led to a significant increase in dry matter accumulation (Shivay et al., 2003).

In regards to Potassium accumulation highly increased at 100mM in response to bacterial inoculation. The partitioning of Potassium accumulation between shoot and root in the three varieties was slightly different. K^+ was very low at high level of salinity in the root of Reehan and high accumulation at highly level of salinity in the shoot of Reehan plant. High Potassium accumulation in the root at 100mM and in the shoot at 100mM and 150mM salinity level with bacterial inoculation for Nabawi was observed. Icarda 5 showed increase in K^+ accumulation at high salinity level in root of plant which was treated with bacteria. Depletion of Potassium ions by plants reduces the ability of rhizobacteria to use Potassium ions as a primary osmoregulator (Jofre et al. 1998).

Ehsan Tavakkol et al., (2011) reported that the high Cl^- and Na^+ concentrations of saline soils can be a major cause of a reduction in barley growth. In this study the general trends showed increase level of Cl^- compared to Na^+ . This outcome is in agreement with Ehsan Tavakkol et al., (2011) as they concluded that the concentrations of Na^+ and Cl^- increased in barley plants exposed to salt, with tissue Cl concentrations of

soil-grown plants generally exceeding those of Na^+ . Increases in plant ion concentrations occur with all plants exposed to NaCl. Barley plants responded positively to Na^+ and Cl^- at very low concentrations (up to 40 mM in external solution), which is the range found in the non-saline soil. However, at high salinity level the plant growth and reproduction reduce and accumulation of Na^+ and Cl^- increase. Moreover, the inoculation with *B. megatrium* reduce the effect of salinity and the accumulation of Na^+ and Cl^- which indicate the positive effect of this bacteria to increase salinity tolerance in barley. Several researcher suggested that reduced Na^+ loading into the xylem is one of the main mechanisms of salinity tolerance and it is often considered one of the most crucial features of restricting Na^+ accumulation in plant tissues (Tester and Davenport, 2003; Munns and Tester, 2008). In contrast a significant correlation between reduced leaf chlorophyll content and the parameters of chlorophyll fluorescence with increasing Cl^- concentration but not Na^+ was found (Ehsan Tavakkol et al., 2011)

The greater reduction in growth under NaCl treatment compared with Na^+ and Cl^- separately, suggests that high concentrations of Na^+ and Cl^- have an additive and/or interactive effect. High Na^+ interferes with K^+ and Ca^{2+} nutrition and stomatal regulation, while high Cl^- concentration reduces photosynthetic capacity due to chlorophyll degradation.

Bacteria can alter root uptake of toxic ions and nutrients by altering host physiology or by directly reducing foliar accumulation of toxic ions (Na^+ and Cl^-) while improving the nutritional status of both macronutrient (N, P

and K). The Chloride accumulation in the shoot and root in our study was high at higher level of salinity without bacterial inoculation in Reehan and Nabawi bacterial inoculation reduce the accumulation of Cl^- . However, the level of Cl^- increased in shoots and roots in Icarda 5 when inoculated with bacteria at higher salinity level (200 mM).

Nitrogen is an essential element for plants, it utilizes Nitrogen (N) in the form of NO_3^- and NH_4^+ (Shah Jahan et al., 2016) essential constituent of protein (build from amino acids that involves in catalization of chemical responses and transportation of electrons) and chlorophyll (enable the process of photosynthesis) present in many major parts of the plant (Bloom, A.J., 2015 and Hemerly, A., 2016).

Nitrogen plays important role in different physiological processes. It imparts dark-green color in plants, promotes leaves, stem and other vegetative part's growth and development, and stimulates root growth (Tschoep et al., 2009; Lima et al., 2010). Nitrogen affect on early growth, improve fruit quality, increases protein content of fodder crops; it encourages the uptake and utilization of other nutrients including Potassium, phosphorous and controls growth of plant (Bloom, A.J., 2015 and Hemerly, A., 2016).

The higher rate of Nitrogen accumulation improved Potassium uptake and increased proline content in leaf of plant. Nitrogen rate increases photosynthetic processes, leaf area production, leaf area duration as well as net assimilation rate (Ahmad et al., 2009). The plants also displayed higher photosynthetic rates and more efficient N metabolism resulting in improved

plant growth and seed yield (Siddiqui et al., 2010). Nitrogen improves plant growth and yield regardless of whether the crop is salt-stressed or not. Despite the lack of evidence indicating that N applied to saline soils or media above a level considered optimal under non-saline conditions improves plant growth or yield, a number of laboratory and greenhouse studies have shown that salinity can reduce N accumulation in plants (Cram, 1973; Pessarakli and Tucker, 1988; Feigin et al., 1991; Pessarakli, 1991; Al-Rawahy et al., 1992). This is not surprising since an increase in Cl uptake and accumulation is often accompanied by a decrease in shoot-NO₃ concentration.

Increase in N levels enhanced stomatal conductance and chlorophyll, malondialdehyde, and water content in the plant tissues (Tavori et al., 2004). In this experiment the results showed that the root of plants inoculated with bacteria at high level of salinity have low Nitrogen percentage in root but high in the shoot for Reehan, Nabawi and ICARDA 5.

Plant growth promoting rizobacteria increased the accumulation of P and N in Reehan, Icarda5 and Nabawi at moderate to high salinity. This results in consensus with other researchers who have been reported that PGPR (*Klebsiella oxytoca* Rs-5 and *Pseudomonas putida* Rs-198) inoculation influence positively plant biomass, increase of N, P, K⁺, and Ca⁺² absorption and decrease of Na⁺ absorption in cotton under salt stress (Yue et al., 2007; Yao et al., 2010).

Phosphorus is an essential macronutrient for plant growth and reproduction, it found in every living plant cell. The higher concentrations

of nutrient accumulate in the plant cells more than that presents the soil solution surrounding plants. This allows roots to extract nutrients from the soil solution where they are present in very low concentrations (Better. 1999), bacteria increase the available P and K in the soil and the concentration of Phosphorus in shoot reflected the accumulation of shoot dry weight.

Inoculation increases organic matter content of soils and improves physical, chemical, and biological characteristics of soils, and consequently enriches the availability and intake of plant nutrients (Singh and Prasad, 2011). Phosphorus essential nutrients for plant growth and development, it can be present in soil as Ca, Al, or Fe precipitates, or it can be adsorbed to soil minerals. However, only soluble, inorganic phosphate is readily available to bacteria. Not only bacteria but also plants utilize phosphate around roots (Jungk et al 1993) and plants therefore compete with microorganisms for available phosphate in the rhizosphere. Our study showed positive effect of bacterial inoculation on Phosphorus accumulated in root of barley at 100mM salinity level in two varieties Reehan and ICARDA 5, while the Phosphorus in shoot was low at high salinity level. The positive effect of bacterial inoculation on total Phosphorus accumulation was observed at 100mM salinity level.

Conclusion

The outcome of this study revealed that the bacterial strains *B. megaterium* has played an important role in alleviate salinity stress effect on barley and have a positive effect on agronomic trait and chemical composition.

- 1) The bacterial inoculation significantly reduces the effect of salinity on chlorophyll content in the three landrace.
- 2) chlorophyll concentration was high in plant which inoculated with Bacteria
- 3) In ICARDA 5 and Nabawi at 100-150 mM bacterial inoculation have positive impact by reducing the impact of salinity on leaf length, width and number, root weight, shoot weight and plant height which are growth parameters.
- 4) Low Sodium accumulation in plant inoculated with bacteria was observed at 200 mM NaCl especially in the shoot and root of ICARDA 5 and Nabawi and in Reehan at 200 mM NaCl.
- 5) Plant Growth Promoting Rhizobacteria increased the accumulation of P and N in Reehan, Icarda5 and Nabawi at moderate to high salinity.

Based on our results it is recommended to use *B. megaterium* at moderate salinity levels for barley production.

Recommendations

- 1) Uses of *Bacillus megaterium* to alleviate salinity effect on plant growth & development.
- 2) Test the Bacteria on other plant variety such as Cereal & Vegetables Crops (Tomato, Cucumber, Maize, Wheat and Tobacco).

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Appendixes

Data Tables of Experiments

Appendix (1) Standard Errors of Means for chlorophyll content in Reehan

	Bac	Salinity	Bac with Salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.934	1.476	2.088
L.S.D.			6.160
CV%			40.2
d.f.=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation			

Appendix (2) Analysis of Variance for awn softness in Reehan

Source of variation	d.f.	M.S.	F pr.
Bac	1	0.0480	0.693
Salinity	4	7.8287	<.001
Bac. Salinity	4	0.2580	0.502
Residual	20	0.2987	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (3) Standard Errors of Means for awn softness in Reehan

	Bac	Salinity	Bac with Salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.1411	0.2231	0.3155
L.S.D.	0.9308		
CV%	41.4		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (4) Analysis of Variance for leaf number in Reehan

Source of variation	d.f.	M.S.	F pr.
Bac	1	3.482	0.138
Salinity	4	2.657	0.165
Bac. Salinity	4	0.210	0.964
Residual	20	1.460	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (5) Standard Errors of Means for leaf number in Reehan

	Bac	Salinity	Bac with Salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.312	0.493	0.698
L.S.D	2.058		
CV%	24.1		
d.f=degree of freedom e.s.e= Estimated Standard Error l.s.d= Least significant differences			
CV=Coefficient of Variation			

Appendix (6) Analysis of Variance for leaf area length in Reehan

Source of variation	d.f.	M.S.	F pr.
Bac	1	1.5778	0.164
Salinity	4	1.5514	0.126
Bac. Salinity	4	0.6779	0.485
Residual	20	0.7574	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (7) Standard Errors of Means for leaf area length in Reehan

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.225	0.355	0.502
L.S.D.	1.482		
CV%	22.7		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (8) Analysis of Variance for leaf area width in Reehan

Source of variation	d.f	M.S.	F pr.
Bac	1	0.000120	0.845
Salinity	4	0.034967	<.001
Bac. Salinity	4	0.000487	0.957
Residual	20	0.003067	
Total	29		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (9) Standard Errors of Means for leaf area width in Reehan

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.0143	0.0226	0.0320
L.S.D.	0.0943		
CV%	16.1		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (10) Analysis of variance for peduncle length in Reehan

Source of variation	d.f.	M.S.	F pr.
Bac	1	8.8563	0.001
Salinity	4	8.4314	<.001
Bac. Salinity	4	1.1754	0.163
Residual	20	0.6421	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (11) Standard errors of means for peduncle length in Reehan

	Bac	Salinity	Bacteria with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.207	0.327	0.463
l.s.d			1.365
CV%			41.5
d.f=degree of freedom e.s.e= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (12) Analysis of variance for plant height in Reehan

Source of variation	d.f.	M.S.	F pr.
Bact	1	35.035	0.049
Salinity	4	30.185	0.021
Bac. Salinity	4	14.616	0.172
Residual	20	8.204	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (13) Standard Errors of Means for plant height in Reehan

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.740	1.169	1.654
L.S.D.	4.878		
CV%	15.5		
d.f.=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (14) Analysis of Variance for root weight in Reehan

Source of variation	d.f. (m.v.)	M.S.	F pr.
Bac	1	1.89	0.869
Salinity	4	132.03	0.143
Bac. Salinity	4	72.15	0.401
Residual	19(1)	67.69	
Total	28(1)		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (15) Standard Errors of Means for root weight in Reehan

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	19	19	19
E.S.E.	2.12	3.36	4.75
L.S.D	14.06		
CV%	54.6		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (16) Analysis of Variance for spike length in Reehan

Source of variation	d.f.	M.S.	F pr.
Bac	1	0.0203	0.693
Salinity	4	2.8074	<.001
Bac. Salinity	4	0.5118	0.015
Residual	20	0.1266	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (17) Standard Errors of Means for spike length in Reehan

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.0919	0.1452	0.2054
l.s.d	0.6059		
CV%	39.6		
d.f=degree of freedom	E.S.E= Estimated Standard Error	L.S.D= Least significant differences	CV=Coefficient of Variation

Appendix (18) Analysis of Variance for tiller in Reehan

Source of variation	d.f.	M.S	F pr.
Bac	1	0.03008	0.334
Salinity	4	0.23958	<.001
Bac. Salinity	4	0.19258	0.002
Residual	20	0.03075	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (19) Standard Errors of Means for tiller in Reehan

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	0.0453	0.0716	0.1012
L.S.D.			0.2987
CV%			140.3
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation			

Appendix (20) Analysis of Variance for chlorophyll content in Nabawi

Source of variation	d.f.	M.S.	F pr.
Bacteria	1	0.17	0.934
Salinity	4	273.90	<.001
Bac. Salinity	4	1.62	0.991
Residual	20	23.55	
Total	29		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (21) Standard Errors of Means for chlorophyll content in Nabawi

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E.	1.253	1.981	2.802
L.S.D.	8.266		
CV%	51.0		
d.f=degree of freedom	E.S.E= Estimated Standard Error	L.S.D= Least significant differences	CV=Coefficient of Variation

Appendix (22) Analysis of Variance for awn softness in Nabawi

Source of variation	d.f.	M.S	F pr.
Bac	1	2.1333	0.016
Salinity	4	4.0767	<.001
Bac. Salinity	4	0.3767	0.333
Residual	20	0.3080	
Total	29		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (23) Standard Errors of Means for softness in Nabawi

	Bac	Salinity	bac with salinity	
Rep.	15	6	3	
d.f.	20	20	20	
E.S.E.	0.143	0.227	0.320	
L.S.D.	0.945			
CV%	75.7			
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation				

Appendix (24) Analysis of Variance for leaf number in Nabawi

Source of variation	d.f.	M.S.	F pr.
Bac	1	33.5668	<.001
Salinity_	4	15.1613	<.001
Bac. Salinity	4	3.4635	<.001
Residual	20	0.4184	
Total	29		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (25) Standard Errors of Means for leaf number in Nabawi

	Bac	Salinity	bacteria with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.167	0.264	0.373
L.S.D.	1.102		
CV%	22.1		
d.f.=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation			

Appendix (26) Analysis of Variance for leaf area length in Nabawi

Source of variation	d.f.	M.S.	Fpr.
Bac	1	25.6133	<.001
Salinity	4	15.8034	<.001
Bac. Salinity	4	2.2383	0.004
Residual	20	0.4187	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (27) Standard Errors of Means for leaf area length in Nabawi

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.167	0.264	0.374
L.S.D.	1.102		
CV%	36.0		
d.f=degree of freedom	E.S.E= Estimated Standard Error	l.s.d= Least significant differences	CV=Coefficient of Variation

Appendix (28) Analysis of Variance for leaf area width in Nabawi

Source of variation	d.f.	M.S.	F pr.
Bac	1	0.128053	<.001
Salinity	4	0.097913	<.001
Bac. Salinity	4	0.011620	0.005
Residual	20	0.002267	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (29) Standard Errors of Means for leaf area width in Nabawi

	Bac	Salinity	Bac with Salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.01229	0.01944	0.02749
l.s.d.	0.08109		
cv%	35.0		
d.f=degree of freedom e.s.e= Estimated Standard Error l.s.d= Least significant differences CV=Coefficient of Variation			

Appendix (30) Analysis of Variance for peduncle length in Nabawi

Source of variation	d.f.	M.S	F pr.
Bac	1	7.6205	0.003
Salinity	4	8.8648	<.001
Bac. Salinity treat	4	1.7287	0.050
Residual	20	0.6640	
Total	29		
d.f.=degree of freedom m.s= Mean Square Fpr= F test			

Appendix (31) Standard Errors of Means for peduncle length in Nabawi

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.210	0.333	0.470
l.s.d.			1.388
cv%			77.5
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation			

Appendix (32) Analysis of Variance for plant height in Nabawi

Source of variation	d.f.	M.S	F pr.
Bac	1	390.241	<.001
Salinity	4	317.839	<.001
Bac. Salinity	4	39.867	0.002
Residual	20	6.478	
Total	29		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (33) Standard Errors of Means for plant height in Nabawi

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.657	1.039	1.470
L.S.D.	4.335		
CV%	22.9		
d.f=degree of freedom E.S.E = Estimated Standard Error L.S.D = Least significant differences			
CV=Coefficient of Variation			

Appendix (34) Analysis of Variance for root weight in Nabawi

Source of variation	d.f. (m.v.)	M.S	F pr.
Bac	1	61.32	0.276
Salinity	4	89.91	0.168
Bac. Salinity	4	65.05	0.296
Residual	15(5)	47.99	
Total	24(5)		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (35) Standard Errors of Means for weight in Nabawi

	Bac	Salinity	bacteria with salinity
Rep.	15	6	3
d.f.	15	15	15
E.S.E	1.79	2.83	4.00
L.S.D	12.06		
CV%	40.7		
d.f=degree of freedom E.S.E = Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation			

Appendix (36) Analysis of Variance for spike length in Nabawi

Source of variation	d.f.	M.S.	F pr.
Bac	1	2.8244	0.002
Salinity	4	3.2354	<.001
Bac. Salinity	4	0.4880	0.095
Residual	20	0.2125	
Total	29		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (37) Standard Errors of Means for spike length in Nabawi

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.1190	0.1882	0.26610.095
L.S.D.	0.7851		
CV%	67.4		
d.f=degree of freedom	E.S.E= Estimated Standard Error	l.s.d= Least significant differences	CV=Coefficient of Variation

Appendix (38) Analysis of Variance for tiller in Nabawi

Source of variation	d.f.	M.S.	F pr.
Bacteria	1	1.0453	0.004
Salinity	4	0.7820	<.001
Bac. Salinity	4	0.7953	<.001
Residual	20	0.1000	
Total	29		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (39) Standard Errors of Means for tiller in Nabawi

	Bac	Salinity	Bac with salinity
Rep.	15	6	3
d.f.	20	20	20
E.S.E	0.0816	0.1291	0.1826
L.S.D.	0.5386		
CV%	148.2		
d.f.=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences CV=Coefficient of Variation			

Table (40) Analysis of Variance for chlorophyll content in Icarda5

Source of variation	d.f.	M.S.	F pr.
Bacteria	1	59.0	0.461
salinity	4	6428.4	<.001
Bac. Salinity	4	262.0	0.051
Residual	140	108.1	
Total	149		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (41) Standard Errors of Means for chlorophyll content in Icarda 5

Table	bacteria	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E.	1.200	1.898	2.684
L.S.D.	7.505		
CV%	61.0		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D = Least significant differences			
CV=Coefficient of Variation			

Appendix (43) Analysis of Variance for awn softness in Icarda 5

Source of variation	d.f.	m.s.	F pr.
Bacteria	1	4.5067	0.008
salinity	4	36.6767	<.001
Bac. Salinity	4	2.1900	0.008
Residual	140	0.6143	
Total	149		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (44) Standard Errors of Means for awn softness in Icarda 5

	bacteria	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
e.s.e.	0.0905	0.1431	0.2024
l.s.d.	0.5658		
cv%	77.3		
d.f=degree of freedom e.s.e= Estimated Standard Error l.s.d= Least significant differences CV=Coefficient of Variation			

Appendix (45) Analysis of Variance for leaf number in Icarda 5

Source of variation	d.f.	M.S.	F pr.
Bacteria	1	39.527	<.001
salinity	4	90.877	<.001
Bac. Salinity	4	14.577	0.003
Residual	140	3.429	
Total	149		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (46) Standard Errors of Means for leaf number in Icarda 5

	bacteria	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E.	0.214	0.338	0.478
L.S.D.	1.337		
CV%	54.8		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (47) Analysis of Variance for leaf area length in Icarda 5

Source of variation	d.f.	M.S.	F pr.
bacteria	1	17.750	<.001
salinity	4	45.836	<.001
Bac. Salinity	4	1.607	0.361
Residual	140	1.466	
Total	149		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (48) Standard Errors of Means for leaf area length in Icarda 5

	bac	salinity	bacteria and salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E.	0.1398	0.2211	0.3127
L.S.D	0.8742		
CV%	67.9		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (49) Analysis of Variance for leaf area width in Icarda 5

Source of variation	d.f.	M.S.	F pr.
bacteria	1	0.05607	0.043
salinity	4	0.42193	<.001
Bac. Salinity	4	0.01707	0.286
Residual	140	0.01347	
Total	149		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (50) Standard Errors of Means for leaf area width in Icarda 5

	bac	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E	0.01340	0.02119	0.02996
l.s.d.	0.03747	0.05924	0.08378
cv%	65.2		
d.f=degree of freedom E.S.E = Estimated Standard Error l.s.d= Least significant differences CV=Coefficient of Variation			

Appendix (51) Analysis of Variance for peduncle length in Icarda 5

Source of variation	d.f.	M.S.	F pr.
bac	1	28.340	0.011
salinity	4	118.607	<.001
Bac. Salinity	4	8.865	0.088
Residual	140	4.280	
Total	149		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (52) Standard Errors of Means for peduncle length in Icarda 5

	bac	Salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E	0.239	0.378	0.534
l.s.d.	1.494		
cv%	97.7		
d.f=degree of freedom E.S.E= Estimated Standard Error l.s.d= Least significant differences CV=Coefficient of Variation			

Appendix (53) Analysis of Variance for plant height in Icarda 5

Source of variation	d.f.	M.S	F pr.
bac	1	750.85	<.001
salinity	4	2116.44	<.001
Bac. Salinity	4	30.58	0.615
Residual	140	45.75	
Total	149		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (54) Standard Errors of Means for plant height in Icarda 5

	bacteria	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E	0.781	1.235	1.746
L.S.D.	4.883		
cv%	51.7		
d.f=degree of freedom ESE=Estimated Standard Error LSD=Least significant differences CV=Coefficient of Variation			

Appendix (55) Analysis of Variance for root weight in Icarda 5

Source of variation	d.f. (m.v.)	M.S.	F pr.
bac	1	484.3	0.109
salinity	4	279.9	0.207
Bac. Salinity	4	285.9	0.199
Residual	15(125)	166.7	
Total	24(125)		
d.f=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (56) Standard Errors of Means for root weight in Icarda 5

Table	bac	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	15	15	15
e.s.e.	1.49	2.36	3.33
l.s.d.	4.49	7.11	10.05
cv%	60.2		
d.f=degree of freedom E.S.E= Estimated Standard Error L.S.D= Least significant differences			
CV=Coefficient of Variation			

Appendix (57) Analysis of Variance for spike length in Icarda 5

Source of variation	d.f.	M.S.	F pr.
Bacteria	1	10.935	<.001
Salinity	4	20.688	<.001
Bac. Salinity	4	1.7418	0.024
Residual	140	0.6006	
Total	149		
d.f.=degree of freedom M.S= Mean Square Fpr= F test			

Appendix (58) Standard Errors of Means for spike length in Icarda 5

	Bacteria	salinity	bacteria with salinity
Rep.	75	30	15
d.f.	140	140	140
E.S.E	0.0895	0.1415	0.2001
L.S.D.	0.5595		
CV%	88.5		
d.f=degree of freedom E.S.E= Estimated Standard Error l.s.d= Least significant differences CV=Coefficient of Variation			

جامعة النجاح الوطنية

كلية الدراسات العليا

تأثير بكتيريا *Bacillus megaterium* على تحمل الشعير للملوحة

اعداد

هناء محمد محمود جردانه

إشراف

د. هبة الفارس

د. عبدالله العمري

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الإنتاج النباتي والوقائي، كلية الدراسات العليا، جامعة النجاح الوطنية، نابلس، فلسطين.

2018

ب

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الملخص

الشعير من أهم محاصيل الحبوب حيث يحتل المركز الرابع على مستوى العالم من حيث الأهمية، والملوحة هي أكثر العوامل المحددة لإنتاجية المحاصيل الزراعية.

يهدف هذا البحث إلى دراسة تأثير استخدام بكتيريا *Bacillus megaterium* على ثلاثة أصناف من الشعير تم زراعتها على تراكيز من الملوحة (0.50.100.150.200).

أظهرت الدراسة تأثيراً إيجابياً للبكتيريا على الموصفات الشكلية للشعير: طول الأوراق، عرض الأوراق، عدد الأوراق، وزن الجذور، وزن الجزء العلوي من النبات، ارتفاع النبات وأيضاً حصل زيادة في محتوى الكلوروفيل في النبات. وأمن الناحية الكيميائية كانت استجابة النبات لبكتيريا *B. megaterium* إيجابية عند مستوى متوسط من الملوحة حيث أن البكتيريا تعمل على تحسين امتصاص النبات للمواد والعناصر الغذائية حيث عملت البكتيريا زيادة تراكم البوتاسيم والكالسيوم والنيتروجين والفسفور.

عمل البكتيريا على تحسين نمو صنف الشعير (إيكاردا 5 ونبوي) في ظل نموها بمستوى عالي من تراكيز الملوحة وقللت من تراكم الكلور والصوديوم مقارنة في النباتات التي لم يتم معاملتها بالبكتيريا.