An-Najah National University

Faculty of Graduate Studies

Phytoremediation for Treatment of Brackish Water from Reverse Osmosis Plant

By

Rinad Jalal Yahya Hamed

Supervisors

Dr.Shehdah Jodeh

Co- Supervisors

Dr. Raed Alkowni

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Chemistry, Faculty of Graduate Studies, An-Najah National University, Nablus- Palestine.

2014

Phytoremediation for Treatment of Brackish Water from Reverse Osmosis Plant

By

Rinad Jalal Yahya Hamed

This Thesis Was Defended Successfully on 21/8/2014,and approved by:

Defense Committee Members	**Signature**
1. Dr. Shehdah Jodeh/ Supervisor	
2. Dr. Raed Alkowni/ Co-Supervisor	
3. Dr. Subhi Samhan / External Examiner	
4. Dr. Ghaleb Adwan / Internal Examiner	

Dedication

After thank Allah for my graduation. My special thanks for my Dad and my Mom and whole of my family and every one for everything in this life and for this moment to let me stand with proud.

Finally my special thanks for Nagham and Anees both of you make me strong in this life with ambition towards the best for you and for the reconstruction of our beloved country.

Acknowledgements

After thank Allah for my graduation, I would like to thank Dr. Shehdah Jodeh for his supervision and continued support, Dr. Raed Alkowni for his help, supervision and continued support, Prof. Glick from Waterloo University – Canada for his bacteria supplement.

My special thanks to Palestinian Water Authority and MEDRC (Middle East Desalination Research Center) for their foundation ,and this wonderful opportunity to work on this topic, especially the environmental issues are within my concern, Moreover the cooperation for analysis of plant photosynthesis in different labs.

الاقرار

أنا الموقع ادناه مقدم الرسالة التي تحمل عنوان:

Phytoremediation for Treatment of Brackish Water from Reverse Osmosis Plant

اقر بان ما اشتملت عليه هذه الرسالة انما هو نتاج جهدي الخاص, باستثناء ما تمت الاشارة اليه حيثما ورد وان هذه الرسالة ككل من او جزء منها لم يقدم من قبل لنيل أي درجة او بحث علمي او بحثي لدى أي مؤسسة تعليمية او بحثية اخرى.

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 الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

Table of Contents

List of Tables

List of Figures

List of Schemes

List of Abbreviations

ACC	1-Amino Cyclopropane-1-Carboxylate
AL	Actinic Light used in PAM fluorometry
CEC	Cation Exchange Capacity
dd H_2O	De-ionized and Distilled water
EC	Electrical Conductivity
$EC_{1:2}$	Electrical Conductivity of a soil extract with 1 part of soil to 2 parts of water (w/v)
ECe	Electrical Conductivity of a saturated soil paste extract
FR	Far Red light used in PAM fluorometry
IAA	Indole-3-Acetic Acid
LHCI	Light Harvesting Chlorophyll Protein Complex I
LHCII	Light Harvesting Chlorophyll Protein Complex II
ML	Modulated Measuring Light used in PAM fluorometry
OEC	Oxygen Evolving Complex
PPM	Part Per Million
PAM	Pulse Amplitude Modulated
Pheo	Pheophytin
PGPR	Plant Growth Promoting Rhizobacteria isolated based on ACC deaminase activity
PSI	Photosystem I
PSII	Photosystem II
PQ	Oxidized Plasto Quinol pool
PQH	Reduced Plasto Quinol pool
ROS	Reactive Oxygen Species
SAM	S-Adenosyl-Methionine
SAR	Sodium Adsorption Ratio

SP	Saturating Pulse used in PAM fluorometry
TSB	Tryptic Soy Broth
RO	Reverse Osmosis
UW3	*Pseudomonas putida*
UW4	*Pseudomonas putida*
C^3	Cubic Meter
mM	Mile Molarity
R.P.M	Round Per Minute
PPT	Part Per Thousand
Kc	Crop Coefficient
ARF	Auxin Responses Factor
MAPK	Nitrogen – Activated Protein
QACS	Quaternary Ammonium Compounds
ICP	Inductive Coupled Plasma
OD	Optical Density
IAA	Indo-3-Acetic Acid
Ado Met	S- Adenosyl Methionie

Chlorophyll Fluorescence Nomenclature

F	Actual fluorescence intensity at any given time.
F’	Fluorescence at any light level and induction state. Some PSII closed ($0 \leq qP \leq 1$, $0 \leq qN \leq 1$), some ΔpH
F_o	Minimal fluorescence in dark-adapted tissue; fluorescence intensity with all PSII reaction centers open while the photosynthetic membrane is in the non-energized state (qP = 1 and qN = 0); ΔpH. It can also be used for the O level in Kautsky nomenclature.
F_m	Maximal fluorescence in dark-adapted tissue; fluorescence intensity with all PSII reaction centers closed (qP = 0), all non-photochemical quenching processes are at a minimum (qN = 0); no ΔpH
F_v	Variable fluorescence in dark-adapted tissue; maximum variable fluorescence in the state when all non-photochemical processes are at a minimum ($qP = 1 \rightarrow 0, qN = 0$), i.e. Fm-Fo
F_s	Fluorescence in steady states; defined by an author as a period within which the fluorescence intensity does not change while the external circumstances remain constant
F_s’	Steady-state fluorescence at any light level. Some PSII closed ($0 \leq qP \leq 1$, $0 \leq qN \leq 1$), some ΔpH
F_v/F_m	Exciton transfer efficiency in dark-adapted tissue; (Fm-Fo)/Fm
F_o’	Minimal fluorescence in light-adapted tissue (quick application of Far-Red PSI light); fluorescence intensity with all PSII reaction centers open in any light adapted state (qP = 1 and $qN \geq 0$), some ΔpH
F_m’	Maximal fluorescence in light-adapted tissue; fluorescence intensity with all PSII reaction centers closed in any light adapted state (qP = 0 and $qN \geq 0$)
F_v’	Variable fluorescence in light-adapted tissue; maximum variable fluorescence in any light adapted state, i.e Fm’ – Fo’, caused by closure of PSII in the light ($qP = x \rightarrow 0$, $0 < qN \leq 1$)
F_v’/F_m’	Exciton transfer efficiency in light-adapted tissue; (Fm’ – Fo’)/Fm’
qP	Photochemical quenching; (Fm’ –F)/(Fm’ – Fo’)
qN	Non-photochemical quenching; 1-(Fm’-Fo’)/(Fm-Fo)
Yield	Effective quantum yield of PSII; (Fm’-Fs)/Fm’

Phytoremediation for Treatment of Brackish Water from Reverse Osmosis Plant

By

Rinad Jalal Yahya Hamed

Supervisors

Dr. Shehdah Jodeh

Co- Supervisors

Dr. Raed Alkowni

Abstract

Brackish water as byproduct from Reverse Osmosis plant (RO) after desalination process, this considered as environmentally impact from RO usage. It contains significant concentrations of dissolved salts ions such as Na^{+}, Cl^{-}, Ca^{2+}, Mg^{2}, K^{1+}, SO^{2-}, and CO_3^{2-} as major ions. Total Dissolved Salts (TDS) of these ions ranged from (5000 mg/L -10000 mg/L). Depletion of brackish water in unfriendly environmental ways causes plant growth inhibition due to osmotic stress caused to plant and soil; also will limiting the fields for agricultural use in the country. Phytoremediation are one of the methods can be used for water and land salt remediation.

In phytoremediation techniques, plants are used to extract, immobilize and degrade contaminants. The phytoextraction of salts relies on the uptake of ions into plants biomass during brackish water irrigation process. Salts ions are up taken by plants, sequestered and harvested as a plant biomass. This method removes the salts from soil and/or brackish water and leaving the environment clean.

As high salt concentrations inhibit plant growth, Plant Growth Promoting Rhizobacteria (PGPR) were found to improve plant growth by lowering production of stress ethylene compound within plants, thereby increasing the biomass and photosynthetic activity.

In this research, PGPRs were implemented to investigate the efficiency of phytoremediation techniques for treatment of generated brackish water. Two strains of PGPR (*Pseudomonas putida* UW3 and *Pseudomonas putida UW4*) were isolated from natural compound and obtained from Prof. Glick, Waterloo University - Canada, had been selected to be used with two plants: Barley (*Hordeum valgare* L.) and Malt plants (*Panicum maximum Jacq*.). Trials include treatment of these plants with PGPR and without PGPR in order to study the effects of PGPRs on the plant responses toward brackish water irrigation. All trials were carried in a designed green house.

The results showed that PGPRs had significant effects on plant growth (biomass), photosynthetic activity, membrane stability, and root and shoot lengths increase under salt stress compared to control trials treated without PGPRs and irrigated with fresh water and brackish water.

Greenhouse studies showed that plants treated with PGPRs and irrigated with brackish water increased significantly in biomass percentage for trails treated with fresh ware, 6000 mg/L of brackish water, 10000 mg/L of brackish water related for treated Barley seeds with UW3 (237.31%, 249.40%, 156.11%) and for treated Barley seeds with UW4 (156.11%,

237.31%, 288.83%) and for trials treated with UW3 and UW4 (128.12%, 267.67%, 288.56%) compared to control trials without PGPR irrigated either with fresh water (dd H_2O) (100%), or 6000 mg/L (8.98 %) and 10000 mg/L of brackish water (150.08%). It was noticed that the PGPRs treated plants had (283%), increase in their root and shoot length (respectively). Salt ions accumulation was found to be increased in shoots (159.09 mmol, 179.73 mmol) /0.114m^2 of pots. TDS for decant water decreased to reach (0.101 mg/L). Electrolyte leakage assay showed that plant treated with PGPRs resulted in same values for trials treated with fresh water, less electrolyte leakage from membrane equal to 304 mg/L.

In addition, the several chlorophyll a fluorescence parameters, Fv/Fm, Y (II) and QN obtained from Pulse Amplitude Modulation (PAM) fluorometry showed that treated plants with PGPRs resulted in improvement in their photosynthesis under brackish water.

The novel results of this research study that carried for the first time where PGPRs (UW3, UW4) had been used for improving the phytoremediation activities of two salt tolerant plants: Barley (*Hordeum valgare* L.) and Malt plants (*Panicum maximum Jacq*.) had showed a very clear and significant improvements of high salt uptake and thus high phytoremediation activities of these plants once they were treated with PGPRs. The results of this research will be considered as an outbreak in the phytoremediation science and future applications.

Chapter One

Introduction

Background

1.1 Importance of Water:

Water is considered to be basic and vital component of the social, economic, political fabric of Palestine. Its sector represents the basic foundation for sovereignty and attachment to our land, there is limited source, classified into surface and ground water. Depletion of water resources recently and deterioration of it becomes the key of environmental challenges; it requires urgent action to treat water to an appropriate quality and quantity for meeting disposal and beneficial reuses [Marie and Vengosh, 2001; Yasser, 2006].

Many techniques and operations have been implemented to treat wastewater and saline water in Palestine. Four reverse osmosis plants exist in Jericho for treatment brackish and brackish water. This operation has side product such as generated brackish water. Disposal of it cause salinity of soil, and inhibit plant growth. To minimize effect of brackish water disposal into environment, many researchers have been put into finding economical and effective methods for treatments of it through many feasible processes [Assaf, 2004].

Phytoremediation technique it's a technique uses of plants to take up ions into their biomass, then above ground biomass can be harvested, Still until

now days Phytoremediation process didn’t use widely due to high salinity inhibit plant growth even tolerant plant species [USEPA, 2000].

In this study, phytoremediation technique implemented for treatment of generated brackish water from RO plant using Barley plant (*Hordeum vulgare L.*) and Malt plant(*Panicum maximum Jacq.*), these plants germinated with PGPR. Some of trials with PGPR imbibed with hydrogen peroxide to study the effect of antioxidant resistance damage cause by production of ROS under salt stress.

1.2 Literature Review:

No Large scale mentioned about reverse osmosis method in treatment generated brackish water; reviewed paper only handled refinement of pores of membrane for distillation. Amount of fresh water added to lower the ions concentration in water. In (2003) Tchobanoglous et al. provided about brackish management and examined broader context of brackish treatment. The treatment technologies include membrane filtration process such as RO; Ion exchange process such as electrolysis or weak acid cation; and exchange or evaporation process such as brackish concentrators.

Mac neilll (2011) mentioned remediation methods for salt impacted soils include excavation, leaching, electronic restoration and phytoremendaition. Phytoremediation enhanced with PGPR shown satisfactory results in infiltration of soils salinity by sequester ions by biomass of plant.

As outlined in by Glick and Penrose, in (1998) PGPR improved plant growth under stressful condition by lowering the ethylene stress hormone, and in (2009, 2014) other researches handled germination of seed with (PGPR) [Munees and Mulugeta, 2014; Wu, 2009]. Their researches applied on field trial with many different species of tall wheat, rice and Barley plants in saline soil. In (2009) Shan and Mac neilll in (2011) determined effect of H_2O_2 seed imbibitions on rate of germination under saline condition, both alone and in combination with PGPR treatment for Barley and tall wheat grass.

1.3 Objective:

1- Study the effect of Plant Growth Promoting Rhizobacteria (PGPR) on plants in terms of biomass production and photosynthetic activity under salt stress will be examined.

2- Study the effect of PGPR on plants cells integrity, salt ions entry damage cell membrane, and increase its permeability studied.

3- Measure NaCl accumulations in plants and compared it with control plants trials.

4- Study the effect of antioxidant H_2O_2 on seed germination rate under brackish water examined.

1.4 Justification:

In our country large amount of generated brackish water (about 10-12 million m^3) produced yearly from five stations of reverse osmosis plants in Jericho districts. Brackish water were disposed in unfriendly environmental ways by spilled them out in soils and/or streams which created further to environmental problems [Palestinian Water Authority, 2013].

Moreover, brackish water from ground water at Jericho area wells and ranged for TDS according to Table 1.1.

Recently some researches proved the effective of Phytoremediation technique in soil salinity treatment. In this research, Phytoremediation will be implemented as a method for treatment of generated brackish water by using selected tolerant plants species germinated with PGPRs at Palestine. The results of these experiments will be used for successful treatments of brackish water field.

Table 1.1: Classification of water categories according to TDS in mg/L (www.who.int/en).

TDS of water in mg/L			
Fresh water	**Brackish water**	**Sea water**	**Brine**
0-1500	1500-10000	10000-35000	> 50000

Chapter Two

Background

2.1 Reverse Osmosis plants:

For any natural process between two solvents differ in concentration with semi membrane located between them, the solvents start to move from an area of low solute concentration (high water potential) through membrane to an area of high solute concentration (low water potential), This process named as osmotic process [Arnot et al., 2011].

Any applied external forces such as pressure to reverse this natural flow become a new process named as reverse osmotic process which is defined as a process of forcing a solvent from a region of high solute concentration through membrane to a region of low solute concentration [Al Agha et al., 2005 and Arnot, 2011].

This reverse osmotic process depends on manufacturing reverse osmosis plant for water purification where reverse osmosis takes place through denser layer polymer matrix- membrane; either of interfacial polymerized layer or natural skin differs in size of pores, according to type of molecules and ions needed to be removed to produce portable water.

Pure solvent produced from the plant and the other solute which contains higher concentration of salt ions retained into the pressurized side of

membrane, named as generated brackish water. It's by product for this process.

This process cannot be considered as economical process. It requires high pressure usually (2-17 bar) for fresh water and brackish water, and (40 – 82 bar) for sea water [Marie and Vengosh, 2001]. One of the most disadvantages is a large quantity of brackish water produced (10-12 million m^3) produced yearly from five stationeries in Jericho district.

2.2 Definitions of brackish water and generated brackish water:

Brackish water term is similar to generated brackish water term in salt ions contents. These differ in terms only to distinguish the latter term as industrial waste generated from reverse osmosis plant.

Brackish water defined as a solution contains significant concentrations of dissolved salts ions. Typically it contain high levels of free ions such as Na^{+}, Cl^{-},Ca^{+2}, Mg^{+2} ,K^{+1},SO^{-2}, andCO_3^{-2} as major ions. These concentration usually expressed as total dissolved salts per liter in units of parts per thousand (per mille) or parts per million (mg/L) [Al Agha et al., 2005; Arnot et al., 2011].

TDS parameter for generated brackish water produced from RO plants in Jericho districts range from 1500- 10000 mg/L [Marie and Vengosh, 2011].

2.3 Measurement of brackish water parameters:

Electrical conductivity is an instrument used for electrolysis of brackish water measurements which measures total amount of minerals salts present in water. The mineral salts constitute of a mixture of electrolytes. These constituents are usually reported in units of TDS (mg/L) or (ds/L) [Al Agha et al., 2005; Arnot et al., 2011]. Table 2.1 shows water salinity based on TDS in water.

Table 2.1: Classification of water categories according to TDS in mg/L. (www.who.int/en)

TDS of water in mg/L			
Fresh water	Brackish water	Sea water	Brine
0-1500	1500-10000	10000-35000	> 50000

The TDS in water between ranges 1500-10000 mg/L consider as highly brackish water.

2.4 Effect of brackish water on environment:

Disposal of brackish water into environment cause problems issues to soils and plants.

2.4.1 Impacts of brackish water on soil quality:

Brackish affects soil structure and increases salinity of soil, especially Na^+ and Cl^-, according to amount of ions impact soil, the soil classified from saline to sodic depend on (conductivity of a saturated paste) EC_e, and high Sodium Adsorption Ratio (SAR) [Bohn et al., 1985].

Sodium is particular concern for soil quality. Where negatively charged particles from soil structure, these negatively charged particles typically matched with divalent cations which they are calcium and magnesium. This composition connects clay particles into large flocs. These flocs don't pack tightly to allow for air, water and roots to pass through it easily, additions of sodium ions as monovalent cations result in exchange between monovalent and divalent cations at negative charges in soil particles. These exchange results in variation in soil structure cause disruption on flocculation of soil, where flocs disperse and soil particles pack more tightly [Bohn et al., 1985; Cramer, 2002].

For measurement of soil salinity EC, TDS, SAR parameters are used for determination of salinity of soil and its quality where:

EC term abbreviated for Electrical Conductivity for soil solution extract. Total concentration of ionized solutes report in units of (ds/m) or (mg/L) [Alva et al., 1991 and Walton et al., 1989].

$EC_e = K \times EC_{x:y}$ ……..……..…..Equation (1)

Where:

EC_e defined as soil sample with deionized water added just to the saturation points.

$EC_{x:y}$ where x mass of soil and y is volume of water used to make the saturation point.

K it's an empirically determined conversion factor between two formulas shown above, usually the k value for the equation below is typically between 2 and 4, and it based on the ionic content [Alva et al., 1991].

TDS is another parameter refers to total dissolved solids. This is less common measurement for ions salts, report amount of dissolved ions in any solution with units of mg/L, by weighing precipitated minerals of filtered brackish water after dried of known volume for total sample. TDS can be related to electrical conductivity by following equation [Alva et al., 1991].

TDS = k x EC …….Equation (2)

On the other hand, SAR term refers to sodium adsorption ratio which determines risk of damage happen to soil structure by sodium ion related to calcium and magnesium cations as shown below:

$$SAR = \frac{[Na^+]}{\sqrt{\frac{([Ca^{2+}] + [Mg^{2+}])}{2}}}$$ ……..……..……Equation (3)

This equation presents a comparison of concentration of sodium ion to calcium and magnesium ion, typically these divalent cations act as counter ions in soil flocculation [Alva et al., 1991].

These parameters help for determination of salinity in soils; Table2.2 shows ranges of reference measurement value of soil salinity indicate best soil can be used for cultivation.

Table2.2: Classification of Soils by EC and SAR [Mac neilll, 2011]

Criteria	Unconditional Use	Moderately Saline	Saline	Highly Saline
EC(dS/m)	< 2	3-5	6-8	> 8
SAR	< 5	6-8	9-12	> 12

The EC and SAR are parameters show the levels of salinity of soils as shown in Table 2.2, where best condition for plant growth for salinity below a value of 2 ds/m and for sodicity as measured by the SAR are below 4 or 5.

These references values reported in Table 2.2 used in study for determination of salinity of soil.

2.4.2 Impact of brackish water on plants:

Impact of brackish water is the most severe environmental stress on plants. The common ions stress and inhibit plant growth are sodium and chloride. When these ions enter the soil and surround the rhizosphere(part of root). It causes differences between water potential in roots above water potential in soils. This change lowers the movement of water from soil into rhizosphere, limiting water and nutrient uptake [Aard, 2007; Ashraf, 2004; Das and Parida, 2005].

2.4.2.1 Ion specific damage:

2.4.2.1.1 Na^+ ion toxicity:

Na^+ is the primary causes of disorder from enzyme activation to protein synthesis. It considered more toxic than Cl^- ion.

Once high concentration of Na^+ enters rhizosphere, it rapidly translocate to shoots via the xylem. Then it does accumulate in leaves result in necrosis and short of lifetimes of individual leaves.

Moreover, sodium has numerous physiological effects. It causes deficiencies of other nutrients by interfering with ion transporters K^+. K^+ is essential to activate more than 50 enzymes and synthesis of protein which play role in cellular functions. This interfering happen due to Na^+ is similar to ionic radius to K^+ this similarity allow for competition between these two ions. This competition results in an overabundance of sodium in tissue compared to potassium, and enters in coordination with t-RNA, resulting inhibited protein synthesis, leads disruption these cellular functions[Blaha et al., 2000; Blumwald and Aharon, 2000; Carden et al., 2003].

The same competitive is found with displacement with calcium ion by sodium ion, where it lowered calcium concentration within plant. This competitive impair gas exchange rate for photosynthesis. Even deficiencies of magnesium due to sodium entrance inhibit photosynthetic rates in plants, further chlorophyll synthesis and functions [Parida and Das, 2005].

2.4.2.1.2 Cl^- ion toxicity:

Chloride ion requires in plants to some limited levels as vital ions inside plants. It's involved in photosynthesis mechanisms in adjusting osmotic potential and maintains electrical charge through membrane [Naidoo and Somaru, 2008].

Excess levels than required for plants process causes toxicity and inhibition of photosynthesis process. Its accumulation causes toxicity to leaves [James et al., 2006; Naidoo and Somaru, 2008].

2.5 Salt tolerance level in plant and its mechanisms:

Plants are divided into two groups according to their ability to tolerate salt which they are Halophytes and Glycophytes. Halophytes are more adapted to salt stress than Glycophytes. Differences between these groups are in the stability of their enzymes and physiological process; even Halophytes are inhibited at some point of high concentration of salts [Das and Parida, 2005].

Some examples of salt tolerance plant as: Oats, Barley and Wheat, also for tolerant grass include: Tall Wheatgrass and alkali grass [Ashraf, 2004; Niazi et al., 1991].

Tolerance mechanism of Halophytes can be classified into avoidance or adaptation or accumulation as shown in Table 2.3 [Munns and Tester, 2008].

Table 2.3: Tolerance mechanisms of Halophytes [Munns and Tester, 2008].

Avoidance	1- Grow only during favorable seasons 2- Grow only in favorable areas. 3- Limitation of root growth to select soil horizons
Adaptation Process	1-Selectivity against Na and Cl. 2- Exclusion of salt from shoots. 3- Diversion of salt out of assimilating tissues. 4- Compartmentalization of salts with in plant, tissue, and cells
Tolerance	1- Increase salt tolerance of tissue, cells and organelles. 2- Increase in halo –succulence: a) Increase in leaf –succulence. b) Increase in stem –succulence relation of leaves.

2.5.1 Osmotic stress:

Osmotic results when these ions enter the soil and surround the rhizosphere (part of root). It causes differences between water potential in roots above water potential in soils. This change lowers the movement of water from soil into rhizosphere, limiting water and nutrient uptake [Aard, 2007; Ashraf, 2004; Das and Parida, 2005].

In order to overcome osmotic stress it should counteract its action by continuously pump sodium and chloride ions to above ground tissue. This process has been effectively employed by Halophytes, it considers as key to distinguish it from Glycophytes, another mechanism includes biosynthesis a serious of organic compounds called: compatible osmolytes. Compatible osmolytes compounds are usually molecular weight, high-

water soluble and non-toxic at higher cellular content, such as sugars, acids, Quaternary Ammonium Compounds (QACS). These compatible osmolytes can counteract negative effects of high osmotic pressures in plant tissues. Proline is another synthesized compound also wildly used in plants cytosol, under salts stress. The precursor for Proline biosynthesis is glutamic acid and bifunctional enzymes pyrroline-5-carboxylate synthases reductase (P5CR). It acts as correlation with salt stress tolerance [Munees and Mulugeta, 2014; Munns, 1993; Munns and Tester, 2008].

2.5.2 Ion selectivity stress:

To cope brackish water effect plants tend to be selectivity of ions, by taken up ions into plants and exclude those are toxic. It is stored in vacuoles within plant cells to maintain osmotic potential in the vacuole and cytoplasm. This translocation of Na^+ is achieved via Na^+ diffusion channels, Na^+ pumps and Na^+/H^+ antiporters, when Na^+ accumulates in vacuole, osmotic potential balanced between the cytoplasm and vacuole, moreover the stress can be resolved by synthesis and accumulation of organic solutes that do not inhibit biochemical reactions in plants such as Proline and Sucrose [Apse et al., 2011; Carden et al., 2003; Karely et al., 2000].

2.5.3 Oxidative stress:

Under non- stressed conditions, the photo system process inside chloro plastes run naturally with production of byproduct which is (R O S). R O S

represented as: Singlet oxygen (O_2), superoxide ($O_2^{\cdot -}$), hydroxyl group ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2), these byproducts produced in rate of 240 $mMs^{-1}$for superoxide and 0.5 mM for hydrogen peroxide under non stressed conditions [Apel and Hirt, 2011].

While under salt stress, plants need to maintain turgor pressure and compartmentalization, so induced osmotic pressure leads to stomata closure, cause immediately decrease in CO_2 diffusion rate and photosynthetic fixation of CO_2, this increases rate of superoxide to 240-720 mMs^{-1} and for hydrogen peroxide 5-15 mM. This rapid increase of ROS in cells is called “oxidative burst”, where it distrust the cellular metabolisms [Apel and Hirt, 2011; Babu et al., 2001 and Wahid et al., 2007].

According to studies done by [Apel and Hirt, 2011; Babu et al., 2001; Wahid et al., 2007] other reasons causes increasing rate of ROS under salt stress are:

(1) Closure of stomata to prevent water evaporates. This closure leads restriction in supply of CO_2, lead to underperformance of Calvin cycle which fixes carbon and $NADP^+$ (electron acceptor). Under this salt stress, less amount of $NADP^+$ produces. Thus electron transfer to reduced molecular oxygen is reduced to superoxide by ferredoxin in photo system I (PSI).

(2) Enzyme responsible of electron transports systems affected by ion toxicity. Under salt stress when light energy captured by the light

harvesting complex (LHC) exists a triplet (ground state) to singlet oxygen, which is represented of ROS.

(3) Under non stressed condition, 10% of electron leak out from the transport chain, while under stress condition the amount of leakage of electrons increase in photo system (II) reaction center this raise in leakage of electron produces more superoxide and hydrogen peroxide.

2.5.4. Salt stress and photosynthesis:

Photosynthesis is a physiological process in plant uses energy to form O_2, carbohydrates and ATP (adenosine triphosphate). The process starts with absorption of light and convert of photon energy to electron. Then electron excited to higher energy levels through electron transport chain in thylakoid membrane, ended with change $NADP^+$ to NADPH form and adenosine triphosphate (ATP) [Baker, 2008; Flexas et al., 2004].

Salt stress impaired photosynthesis process by restriction availability of CO_2 for carboxylation reaction due to stomata closure. Accumulation of high concentration of salts in photosynthesis tissues result in swelling of thylakoids and distortion of chloroplast membrane; disrupt all process in plant. Measurement of photosynthesis can be used as another indicator of plants under salt stress using PAM fluometry spectroscopy [Beer, 2008; Meloni and Oliva, 2003].

2.6 Remediation techniques:

Remediation of soil affected by brackish is achieved by physical removal of ions from soil. Physical removal techniques include: excavation, leaching and recovery, electro kinetic restoration and photo remediation [Qadir et al., 2007; Zhang et al., 2005].

2.6.1 Phytoremediation:

Phytoremediation is a physical removal technique, which is implemented in this research. This technique differs from other mentioned techniques by using plants to mitigate organic and inorganic contaminants in soils [USEPA, 2000].

Advantages of phytoremediation techniques over other mentioned remediation techniques depend on cost effective; economical easily applied [Su et al., 2008].

Phytoremediation has different mechanisms based on contaminates fates. These mechanisms are: degradation, extraction, volatilization, transformation, filtration or combinations of these. The mechanism carried in this study is phytoextraction mechanisms in which plants take up salts ions during irrigation with brackish water and accumulate it in above ground portions of plant, after biomass reached its crop coefficient (K_c) it can be harvested lead to clean soil, even there is limitation to its advantages. Phytoremediation consider as time consuming, it requires several growing seasons to lower levels of salts or unwanted contaminants

as mentioned by study of Shan (2009), beside high levels of salts inhibited plant growth and germinations, even for salt tolerant plants species [James et al., 2006; Munns and Tester, 2008; Shan, 2009].

2.7 Plant Growth Promoting Rhizobacteria (PGPR):

PGPR is naturally occurring bacteria. Rhizosphere refers to narrow zone of soil direct surround around the root system of plant.

These microbes naturally motivated plant growth promotion through direct and indirect mechanisms shown in Table 2.4 and Table 2.5 show examples of some of these strains via its functions [Munees and Mulugeta, 2014].

Table 2.4: PGPR mechanisms [Munees and Mulugeta, 2014].

PGPR action through directly and indirectly mechanism	1- Nitrogen Fixation
	2- Hormone Production
	3- Helps in Nodulation
	4- Nutrient Uptake
	5- Siderphores production bio control

Phytoextraction depends on ability of plant to grow and extract contaminants of salt in its biomass. High concentration of salts above effectiveness of remediation process cause to produce ethylene hormone stress, this hormone lowers rates of germination and biomass production

One way to enhance plants growth under stress is to lower hormone stress in plants, this can be done by PGPR [Glick and Penrose, 1998; Kende, 1993; Qadir et al., 1996; Wu, 2009].

Direct mechanisms include: production enhancement substances, facilitate acquisition of nitrogen, phosphorous and motivation plant hormone concentration levels.

Indirect mechanisms involve decreasing inhibitory effects of many pathways limit plant growth or effect photosynthesis process [Glick and Penrose, 1998; Munees and Mulugeta, 2014].

Table 2.5: PGPR strains [Munees and Mulugeta, 2014].

PGPR strains	PGPR strains mechanisms
Pseudomonas putida	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Pseudomonas aeruginosa	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Klebsiella sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Enterobacter asburiae	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization
Pseudomonas sp. A3R3	IAA, siderophores
Psychrobacter sp. SRS8	Heavy metal mobilization
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides
Pseudomonas aeruginosa 4EA	Siderophores
Bradyrhizobium sp. 750, *Pseudomonas*sp., *Ochrobact rum cytisi*	Heavy metal mobilization
Bacillus species PSB10	IAA, siderophores, HCN, ammonia
Paenibacillus polymyxa	IAA, siderophores
Rhizobium phaseoli	IAA
Stenotrophomonas Maltophilia	Nitrogenase activity, phosphate solubilization, IAA, ACC Deaminase

For this study chosen strains which are *P. putida* UW3 and *P. putida* UW4 implement indirectly mechanism which explained in details in next section.

2.7.1. PGPR and brackish water:

In presence of up to 172 mM NaCl Glick (1998) reported that PGPR strains had high ACC deaminase activity, enhanced to more resistance under saline condition which is observed increase yields, with enhancement of nitrogen fixation as shown in Figure 2.1 [Shan, 2009].

2.7.2. Ethylene and ACC deaminase:

Naturally produced ethylene is necessary components for many plants for seed germination, but high levels of it can impede plant growth. PGPR are able to inhibit production of high concentration of ethylene through hydrolyzed ethylene precursor ACC [Glick, 1995].

ACC deaminase defines as amino cyclopropane-1-carboxylate (ACC) deaminase produced by some strains of PGPR. Under salt stress inside plant root ACC synthesis converts S- adenosyl methionie (AdoMet) into ACC which convert after that to ethylene by oxidation of ACC, where high concentration of ethylene cause stress to plant and growth inhabitation, so existence of PGPR on the rhizosphere of roots exuded ACC and by the enzyme ACC deaminase its hydrolyzed to ammonia and α-ketobutyrate, this lead to take another pathway in the reaction result in decrease in amount of ethylene and thereby alleviates ethylene induced stress and prevent inhabitation of root elongation. [Glick, 2004; Munees and Mulugeta, 2014; Mac neilll, 2011; Wu, 2009]. The path ways are shown in Figure 2.1.

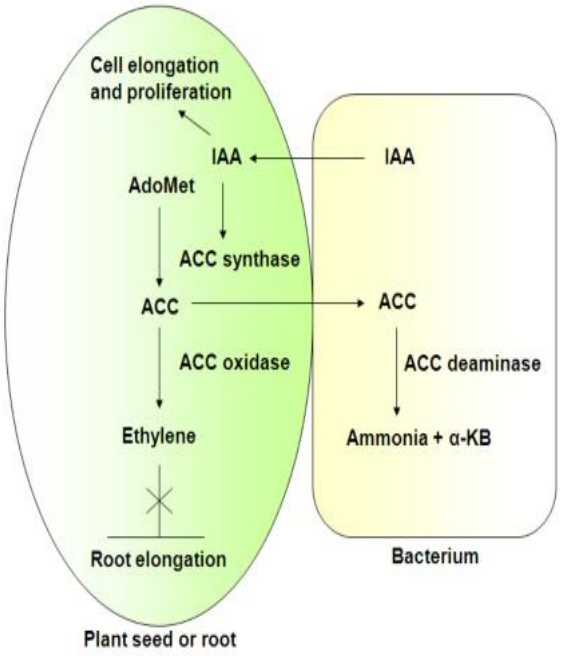


Figure 2.1: Schematic diagram of PGPR containing ACC deaminase lower the ethylene hormone [Shan, 2009].

In 1995, 1997 and 1998 Glick and coworker had showed that ACC deaminase producing bacteria have been promoted plant growth under different environmental stress include: salt stress, water logging, heavy metals drought, petroleum exposure, metal organic contaminants. Consequently, PGPR effect on plant appear in longer root length and shoot length [Gilck, 2004; Glick, 1995; Glick and Bashan, 1997; Glick and Penrose, 1998].

2.7.3. Auxin production by ACC deaminase producing PGPR:

Some strains of PGPR such as UW3 and UW4 secrete Indo-3-Acetic Acid (IAA), which consider as regulator for plant growth and it enter plant cells to stimulate root growth; also it stimulates ACC synthesis, as consequence.

The concentration on ethylene depends on the balance of the IAA and ACC deaminase [Glick and Penrose, 1998; Munees and Mulugeta, 2014].

In 2004 Glick et al., proposed a model to explain how ethylene and IAA interact as feedback loop; decrease in levels of ethylene by ACC deaminase not only regulates plant stress responses, also relieves ethylene repressed Auxin Responses Factor (ARF) synthesis lead to plant growth promotion resulted from both stress alleviation and growth stimulation [Glick, 2004].

2.8. Effects of ROS on seed germination plant:

Under stress production of ethylene hormone in high concentration and plants resort to closure its stomata to limit water loss by evaporation. This closure procedure halts gas exchange between plants and atmosphere where this halts increase in content of oxygen species compared to carbon dioxide concentration, where carbon dioxide consider more necessary than oxygen species for carbon fixation and acceptance of electron from PSI and PSII. Oxygen species convert to ROS as mentioned in pervious section and disrupt plant physiological [Apel and Hirt, 2011; Babu et al., 2001; Wahid et al., 2007].

Figure 2.2 shows transfer of light through PS II and PSI in plants. Diagram (A) shows the normal movement of electrons, resulting in CO_2 as terminal electron acceptor and fixation of carbon into sugars. Diagram B shows exposure to osmotic stress resulting in closure of stomata, resulting in reactive oxygen species as terminal electron acceptor [Mac Neill, 2011].

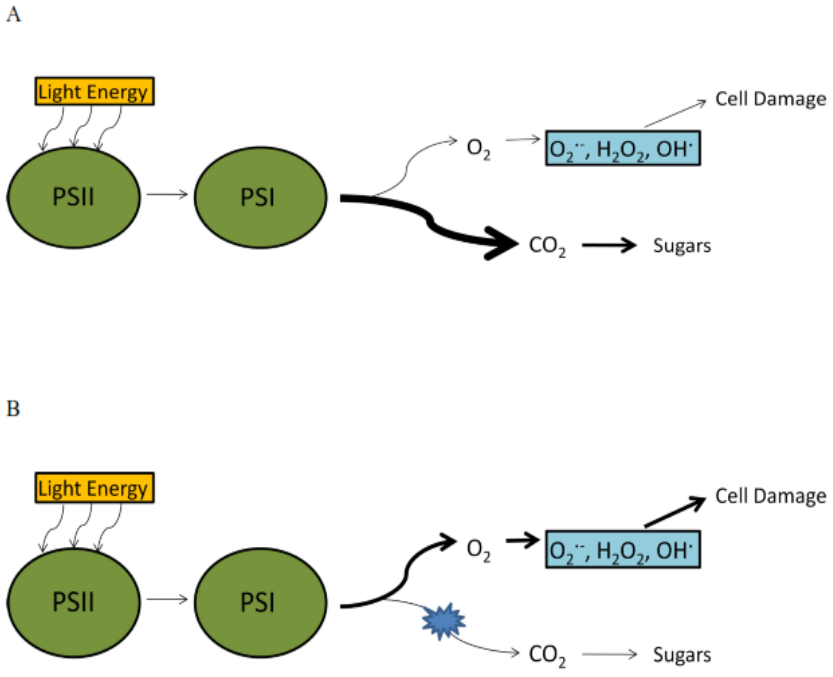


Figure 2.2: Two paths one for open stomata represented in A and second one for closed stomata represented in B [Apel and Hirt, 2011; Babu et al., 2001].

Notice electron movements for closed stomata pathway, if O_2 is final electron acceptor, it will result in ROS . This species interact with DNA, pigments, protein, lipids and other essential cellular components leading to a series of random destructive process. For DNA and protein include denaturation, also loss of membrane integrity [Apel and Hirt, 2011; Babu et al., 2001; Wahid et al., 2007].

Meanwhile, for open stomata 20-25% of electrons diverted to formation of ROS, these little amounts of it participate in cell signaling. It represent as antioxidant as H_2O_2 which activate several nitrogen – activated protein (MAPK). MAPK represents central for mediating cellular responses to multiple stress [Mac Neill, 2011; Miller et al., 2010; Mittler, 2002; Wahid et al., 2007].

In this experiment exogenously imbibing of H_2O_2 solution to seed at concentration of 60 mM as recommended from previous study by Mac neilll, 2011 to study antioxidants activation under brackish water effect [Mac neill, 2011; Meloni and Oliva, 2003; Miller et al., 2010].

2.9 Pulse Amplitude Modulated (PAM) Fluorometry:

Photosynthetic performance of plants evaluated through the chlorophyll fluorescence measurement. Biophysical process carries through three main protein complexes PSII. The cytochrome b6/f complex and PSI are shown in Figure 2.3; PSII located on the membrane of plants and consists of light – harvesting center II (LHCII), Oxygen –evolving complex (OEC), Reaction center (P680), Primary electron acceptor pheophytin (Pheo) and secondary acceptor Q_A and $Q_{B.}$ PSI contains light harvesting center I (LHCI) and reactions center p700 number of electron acceptor [Beer, 2008].

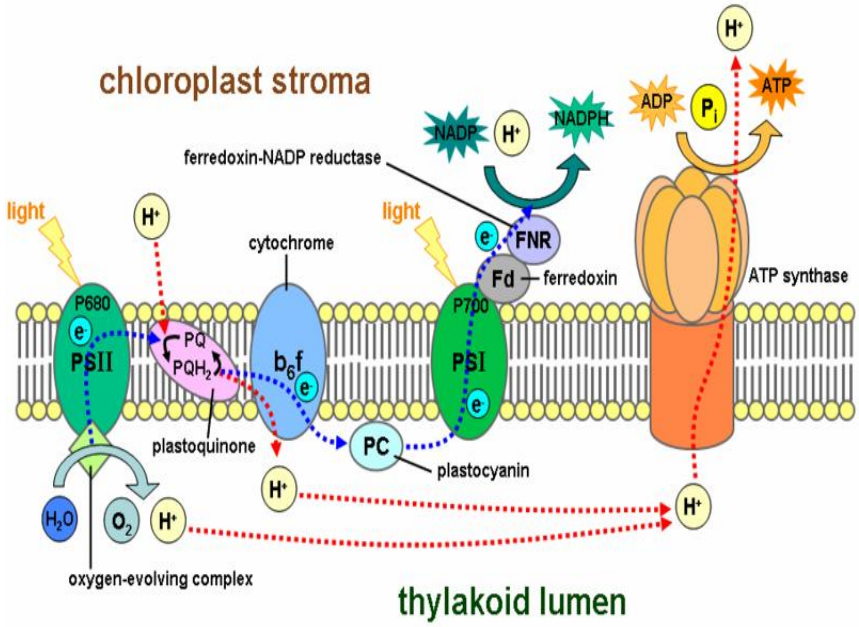


Figure2.3: Schematic of the thylakoid membrane showing the components of photosynthetic electron transport chain [Beer, 2008].

When light absorbed by chlorophyll it passes one of these following ways:

1- Dissipation as heat.

2- Remission as light.

3- Energy to drive photosynthesis.

In this research, (PAM) fluorometry measured chlorophyll a fluorescence, Recoding information from instrument indicates functionally of PSII as flow of electron, rate of photosynthesis by emitted light from the pulse and measured light, heat dissipation is relatively constant during measurements. The following charts indicate several chlorophyll fluorescence parameters, as: Fv/Fm, yield, Qp, Qn, are shown in Figure 2.4 [Mac neill, 2011].

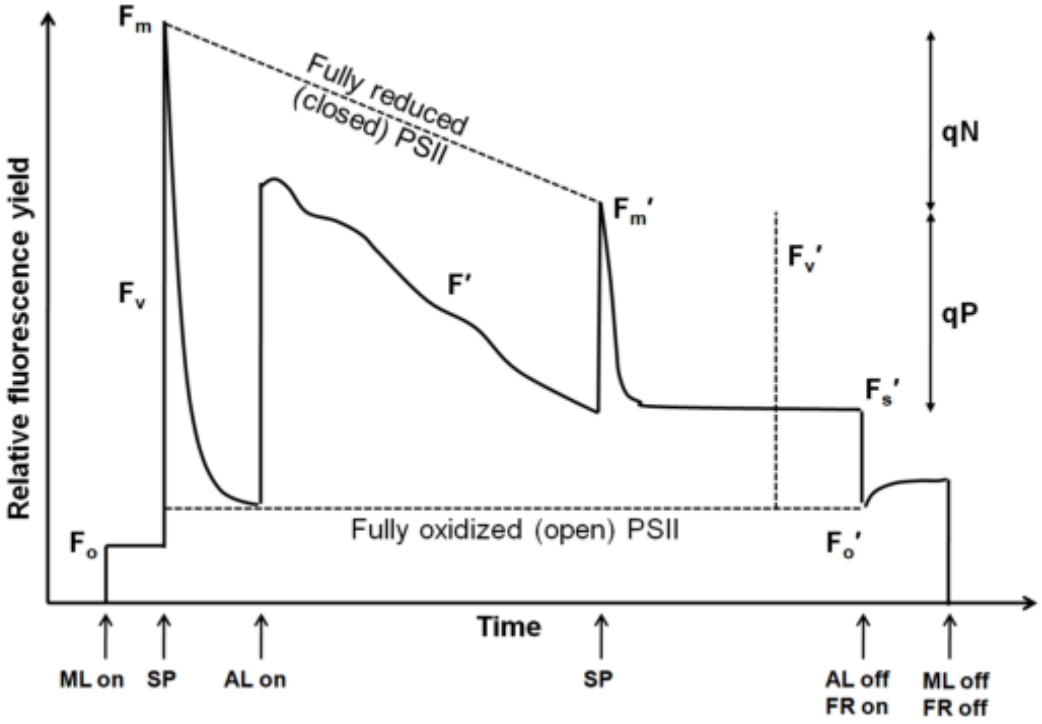


Figure 2.4: Nomenclature of PAM fluorescence parameters for dark-adapted leaf [Mac neill, 2011].

These parameters used to assess the effective of photochemistry in plants, beside in this study they are as indication effect of salinity on photosynthetic electron transports.

Each term abbreviated as followed:

ML term: Modulated measuring Light.

SP term: Saturating Pulse.

AL term: Ancident Light.

FR term: Far Red light.

Fv term: is the variable difference Fluorescence between Fm and F_0.

Fm term: Maximal Fluorescence of dark adapted tissue.

Fm ′ term: Maximal Fluorescence of light adapted tissue.

Fo term: Minimal Fluorescence.

Fs term: Stead state Fluorescence.

Yield parameter equal to:

Yield = Fv / Fm..…… Equation 4

Fv =Fm -Fo…….....…… Equation 5

It represent maximum quantum yield of PSII center when it's open.

Y is another calculation of yield at steady state photosynthesis and represented by:

Y = [Fm – Fs / Fm]……...……..Equation 7

Optimal values for yield ranges between 0.5 to 0.75, lowered value indicates that plant is stressed.

q_p term is : Photochemical Quenching represented as

q_p =[(Fm‘ –Fs) /(Fm‘-Fo)] ……...……Equation 8

q_n term is : non-Photochemical Quenching of fluorescence which is represented by:

[1- (Fm‘-Fo) / (Fm- Fo)]……...…….Equation 9

Value of qp indicate PSII reaction center that are open and equal the approximate oxidation of PSII, while qn parameter related to the dissipation of energy as heat and photo inhabitation [Shan, 2009].

Chapter Three
Material and Methods

3.1. Selecting and culturing PGPR:

In this research two salt tolerant plants species selected [Barley plant (Hordeum vulgare L.), and Malt plant (Panicum maximum Jacq.) and used for phytoremediation. In order to increase their liability and tolerance to salty conditions, trials tested by incorporating them with (PGPR):UW3 and UW4.These strains will be used in coating seeds separately, or in combination.

These two bacterial strains: UW3 and UW4; had been selected and brought from Prof. Glick lab; at Waterloo University Canada, were grown in Troptic Soy Growth (TSB) media. The media for UW3 growth was the only one that contained 100 mg/L of Ampicillin antibiotic (AMP). Solid media had been prepared by addition of 7.5 g of agar for preparation of solid plates. Bacterial strains were cultured on solid and liquid media for each strain at 30 ^{0}C for overnight. Some of these prepared bacteria were transferred to sterile falcon tubes with addition of glycerol layer (1:1) volume and stored at -80 ^{0}C as stock liquid solutions.

For liquid cultures preparations, bacterial inoculums had been transferred to 50 mL falcon tubes containing proper TSB media and incubated at 30 ºC with shaking at 200 r.p.m in rotatory shaker (orbital shaking incubator, labtech, LSI-3016 A) for 26 hour.

3.2. Seed treatment with PGPR:

Cultures for each strain were transferred to two 50 mL falcon tubes separately, followed by centrifugation at 2000 r.p.m for 20 minutes using (Universal 320 R). The pellets were resuspended in 10 mL of dd H_2O and the Optical Density (OD) had been measured for each strain at wavelength 600 nm by UV-spectrophotometer (Spectro UV-Vis Dual Beam -8 Auto cell, UVS- 2700) to have 1.5 OD for UW3 which is perfect germination and 2.0 OD for UW4 include for perfect germination [Mac neill, 2011; Shan, 2009].

For adhesion process of bacterial cells to the seeds surfaces, methylcellulose white gel polymer was prepared. Briefly,7g of methylcellulose powder were dissolved in 500 mL of ddH_2O; stirred for one hour until most of clumps had been dissolved, before they were autoclaved for 20 minutes at 110 ^{0}C and 100 psi using auto cleave (EQUS steam sterilization auto cleave). The resulted polymer was white gel and it becomes clear gel upon cooling.

The next step was including the adhesion process by adding of 2.5 volumes of methylcellulose polymers to one volume of bacterial suspension. Then the bacterial-methylcellulose polymers incorporated with (2.5:1) volume for Malt seeds and up to (7:1) volume for Barley seeds. It is worth to mention that plant seeds had been disinfected previously by soaked in bleach sodium hypochlorite (1%M) for 10 minutes, followed by three times washing with ddH_2O.

After seeds treatments with PGPR, they were dried for 5 minutes at room temp before they were transferred into sealed autoclaved plastic bags, and then stored at 4 ^{0}C for one week prior usage.

3.3. H_2O_2 imbibing of seeds:

This exogenously imbibing of H_2O_2 solution to seed to study antioxidants activation under brackish water effect and not depend on ozone in air due the process of coating done to seed before germination.

Seeds had been soaked in prepared solution of 60 mM H_2O_2 for 3 hours as recommended from experiments of Mc niell, 2011 after imbibing process, part of seeds was treated with UW3 strain as mentioned in section 3.2. Others were soaked only in H_2O_2 and both were transferred to autoclaved sealed plastic bags stored at 4 ^{0}C and used within one week of imbibition.

3.4. Measurement of PGPR growth curve at saline condition:

Saline media were prepared to study growth of PGPR at different saline condition for testing their performance to salt especially salt ions (Na^+ and Cl^-) ions. Salt ionic compound concentrations usually found in brackish water were between 5000-10000 mg/L. For that, (TSB) media with different concentration of NaCl (0.05g, 0.08 g, 0.16g, and 0.24 g,) were prepared in 50 mL falcon tubes containing of TSB in 20 mL liquid solution.

After that, UW3 was cultured in each falcon tubes at 30 $\pm$ 1 ^{0}C and shacked at 200 r.p.m for 10 hours by shaker (orbital shaking incubator, lab tech, LSI-3016 A). Then OD read for each falcon tube was at wavelength 600 nm by UV- spectrophotometer (Spectro UV-Vis Dual Beam -8 Auto cell, UVS- 2700) at different time intervals from 1 – 8 hours to study the bacterial growth responses within each range of dissolved salts of brackish water. Each absorbance measurement was performed in triplicate at each time for ensuring the accuracy of readings, and OD at zero time was read, with – ve control.

3.5. Measurement of soil salinity:

Soil samples were selected to be loam soil collected from An-Najah field campus, where they similar in texture to Jericho area soil. The soil samples were filled in bags and autoclaved (EQUS steam sterilization auto cleave) to ensure removal of any bacterial and/or fungi infections. Then soils were allowed to dry to remove moisture, and sieved using 10 mm particle size sieve.

Electrical conductivity was measured for randomly chosen samples. Measurement based upon ECe (soil saturated with water) and $EC_{1:2}$ (1:2 represent ratio of soil to water extract). These measurements were carried out according to published procedure by [Shan, 2009]; measurements for two parameters were performed in triplicate. $EC_{1:2}$measurement done by addition of 15 g of sterile-soil to 30 mL of ddH_2O in 50 mL sterile falcon tube. The mixtures were shaken on rotator shaker (Orbital shaking

incubator, lab tech, LSI-3016 A) at 200 r.p.m for 30 minutes to make them homogenous mixtures, and then centrifuged at 2000r.p.m for 10 minute (Universal 320 R). Then EC was measured for supernatant using electrical conductivity meter instrument (4510 – conductivity meter, Jen way).

For ECe (soil saturated with water) measurements; 50 g of sterile soil was mixed with sufficient ddH_2O in 100 mL beaker till reach saturation. Where saturation, point indicated by shining appearance of the paste. The paste allowed settling down at least 4- hours to ensure the saturation criteria after saturation criteria had been reached, the mixture then centrifuged at 2000 r.p.m for 10 minutes by centrifuge (Universal 320 R). EC of the filtrate and supernatant were measured by electrical conductivity meter (4510 – conductivity meter, Jenway), and K value was determined by ratio between $EC_{1:2}$ to ECe.

After salinity measurements, soil samples were filled in plastic pots of 17* 16*15 cm (length*width* height) with 12 medium holes at bottom for drainage. Then each pot was filled with 350 gram of sieved soil.

3.6. Preparation and measurement of brackish water by using EC:

Two concentration of brackish water were prepared in lab which equal to 6000 and 10000 mg/L, that had been chosen based on daily ranges of generated brackish water obtained from Jericho RO plant. The two prepared concentrations contained four salts which are: (NaCl, $KNO_{3,}$ $MgCl_2$ and $CaCO_3$). For preparation of concentration of 6000 mg/L, 3g of NaCl was added to 1g of each compounds $KNO_{3,}$ $MgCl_2$, $CaCO_3$ separately, in 1 liter of warm distilled water and stirred to make homogeneous solution. On other hand for concentration of 10000 mg/L, 7 g of NaCl were added to 1 g for other compound added separately in 1 liter of warm distilled water, after that electrical conductivity for both solution were measured to ensure total dissolved ions within prepared.

During irrigation period, descended water due to gravity forces (gravitational water) were collected for measurement to detect any contaminant ions that could be leached out. These measurements were included also the determination for how much leaching water could be arrived to ground water and cause salinity.

3.7. Greenhouse plant germination and growth assays:

The two salt tolerant plant species used in this research (Barley and Malt plants) were obtained from National Agriculture Research Center (NARC) Ministry of Agriculture, Jenin.

About 20 seeds of each plant were grown in sterile pots with 100-200cm^3 sterile loamy soils. The seeds were germinated on the top of each pot after covering them with a thin layer of about 5 cm of soil. The total numbers of pots were 36 representing the number of trials that made for this research study as shown in the schemes 3.1-3.6. Each pot was placed on aluminum trays with dimensions (16*10*6) (length *width*height) to collect the gravitational water that will be used later for measurement of soil leaked ions left after each irrigation and seeds for treated trials all of them were grown.

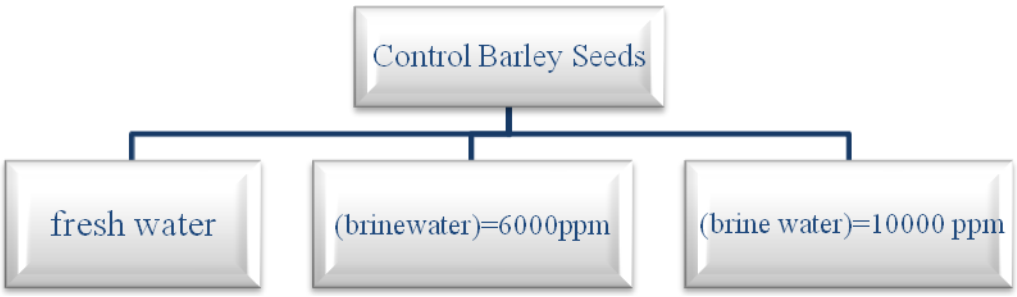
All pots were planted in early February in 2014; and maintained in miniature greenhouse built in backyard of my house. All pots were placed inside in rows to make it easy for irrigation (Figure 3.1). This was to mimic the climate condition in Jericho. Greenhouse temperature was measured twice daily. No human interference for the temperature or light intensity during the period of the experiments.

Before germination all pots were irrigated with fresh water twice daily for five days. Pitchers used with holes to regulate operation of irrigation, after that each pot was irrigated to type of water it was labeled for, once on daily basis.

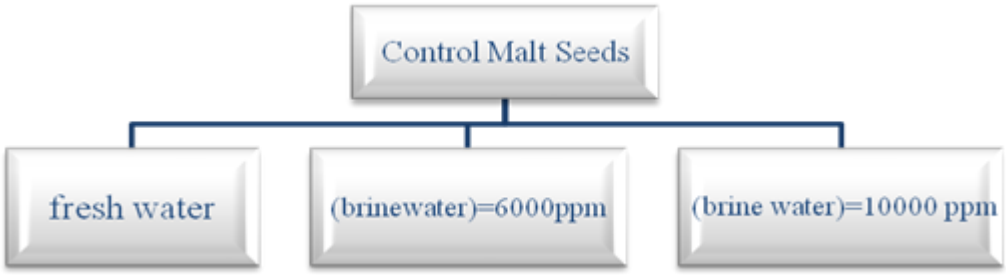
During growth stages plants had been photographed and the length shoot were measured, before it reached crop coefficient (Kc) end cycle of its life. After 30 days all plants were taken from pots and subjected to tests.

Figure 3.1: Greenhouse model, miniature greenhouse built in backyard of my house. All pots were placed inside in rows to make it easy for irrigation, Greenhouse temperature was measured twice daily. No human interference for the temperature or light intensity during the period of the experiments.

Control seeds pots used in this experiments. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt Plants.



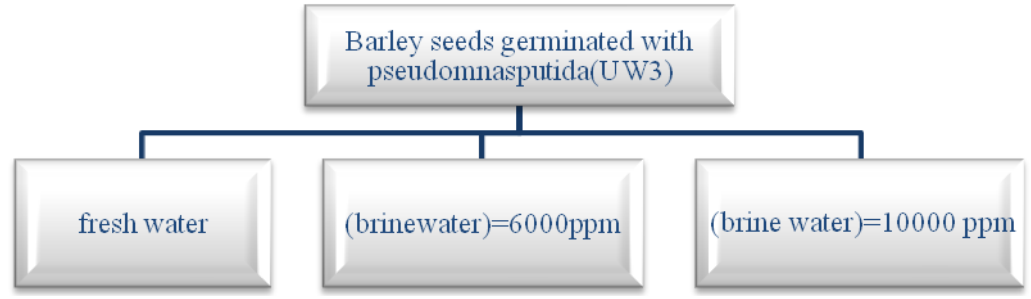
(a)



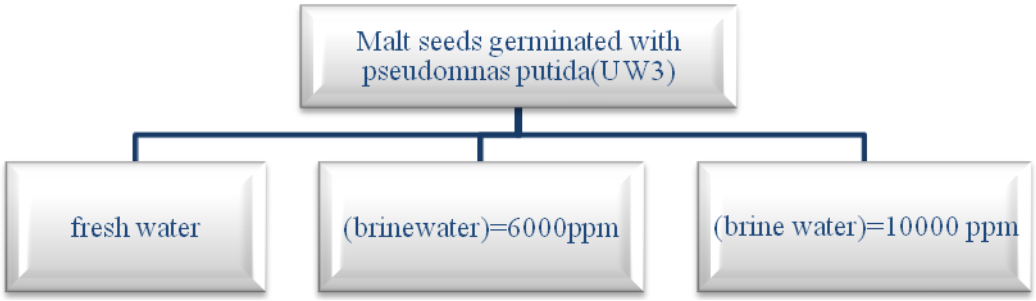
(b)

Scheme 3.1: Control seeds pots used in this experiments. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.

Seeds pots germinated with UW3 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants



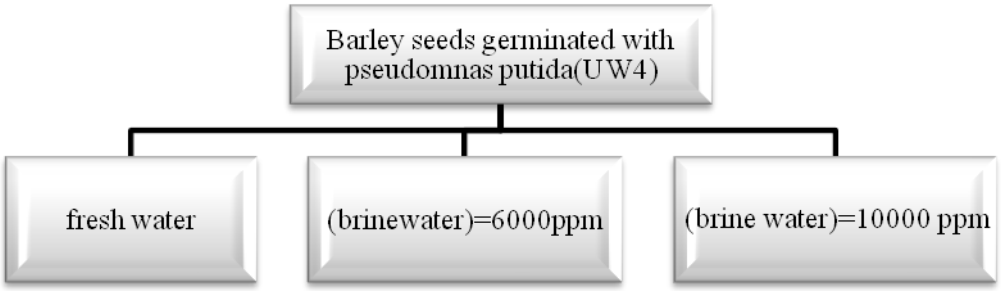
(a)



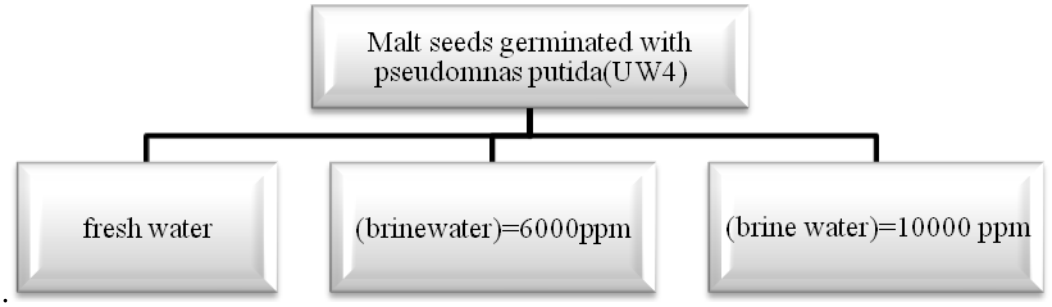
(b)

Scheme 3.2: Seeds pots germinated with UW3 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants

Seeds pots germinated with UW4 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants



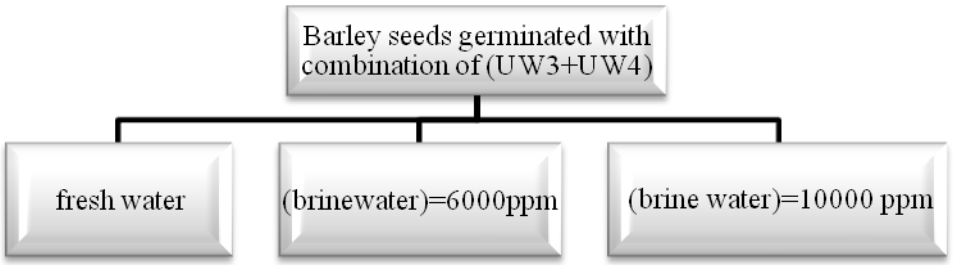
(a)



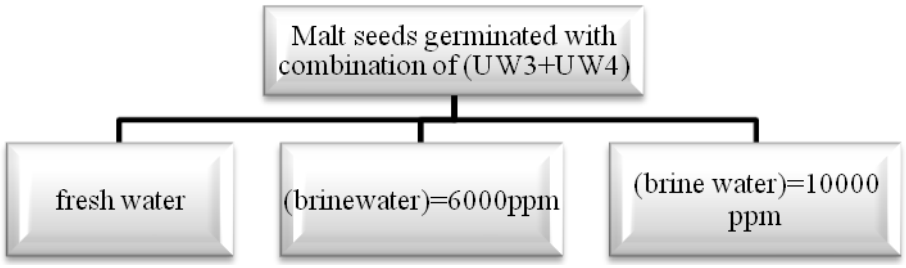
. **(b)**

Scheme 3.3: Seeds pots germinated with UW4 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.

Seeds pots germinated with UW3+UW4 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.



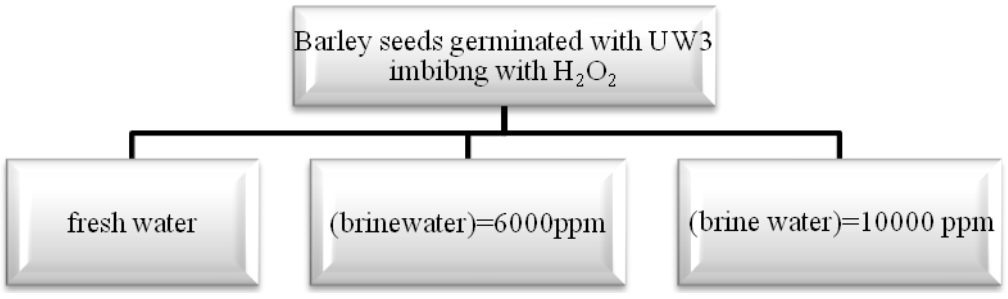
(a)



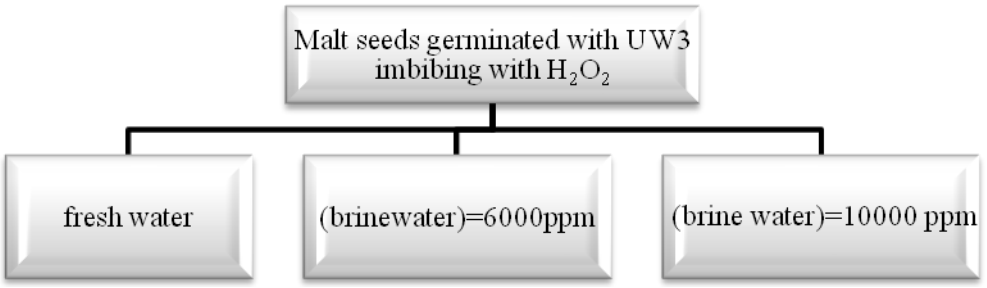
(b)

Scheme 3.4: Seeds pots germinated with UW3+UW4 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.

Seeds pots germinated with UW3+ H_2O_2 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants



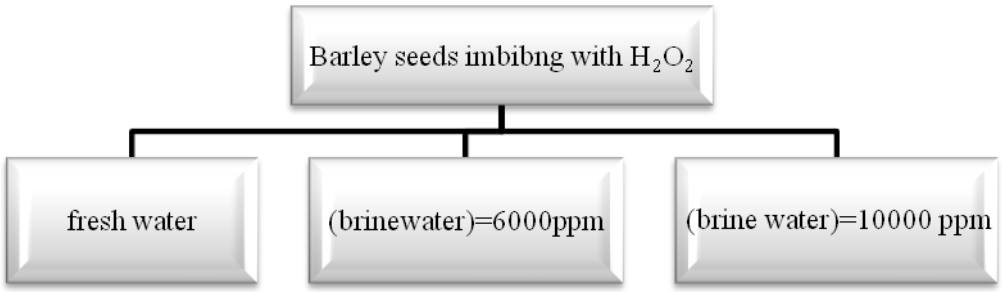
(a)



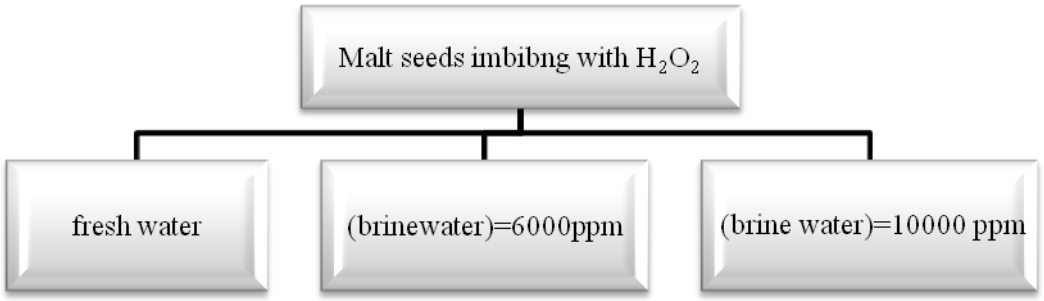
(b)

Scheme 3.5: Seeds pots germinated with UW3+ H_2O_2 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.

Seeds pots germinated with H_2O_2 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.



(a)



(b)

Scheme 3.6: Seeds pots germinated with H_2O_2 used in this experiment. One pot was irrigated with fresh water; the second one was irrigated with brackish water of 6000 mg/L; and the last one was irrigated with brackish water of 10000 mg/L. Each pot contained an average of twenty seeds; (a) for Barley and (b) for Malt plants.

Table3.1: Trials Schemes.

Plant/ Trials	Control trials	Seeds pots germinated with UW3	Seeds pots germinated with UW4	Seeds pots germinated with UW3+UW4	Seeds pots germinated with UW3+ H_2O_2	Seeds pots germinated with H_2O_2
Barely	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water

Malt	Irrigation with : -Fresh water -6000mg/Lof brackish water -10000 mg/Lof brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water	Irrigation with : -Fresh water -6000 mg/L of brackish water -10000 mg/L of brackish water

Measurements include for wet mass in (g) and for dry mass in (g) with differences between wet and dry Length measurements. This procedure was done to compare between trials.

The percentage of wet mass after 30 days = root+ shoot wet mass (g) for each trial / control wet mass for root+ shoot.

Area of pots = $0.114m^2$

% of dry = total dry for any trial /total dry of control barley irrigated with fresh water.

3.8. Salt accumulation in plants:

Salt accumulation test was used in this study to determine the effectiveness of phytoextraction mechanism of the tested plants. It was used to determine how much of salt ions have been eliminated from brackish water. This method was carried for all trials by taking roots and shoots of plants after 30 days, after they were washed with tap H_2O and air dried for 5 days.

Shoot tissues were analyzed for Na^+ concentration by taking 1.0 g of plant shoot tissues into 50 mL Taylor tube. Adding 10 mL of concentrated nitric acid to tube to make decomposition and it was leaved overnight. The tube was heated at 125 ^0C for 4 hours, after that it allowed cooling. then diluted to 12.5 mL with concentrated nitric acid, and 50 mL of distilled water was added to tube, and mixed then aspirated directly into plasma for Inductive Coupled Plasma ICP .

For chloride ion analysis, a titration method with $AgNO_3$ was applied.

3.9. Measurement of Photosynthesis with PAM Fluorometry:

Barley Plant trials were measured for their photosynthesis activities using PAM fluorometry (LUCAM, Fluor cam version 15.1.0). Samples were dark adapted for 20 minutes by turned off all lambs in lab before PAM analysis were carried out to ensure the PSII centers were open. The Fo minimum fluorescence was adjusted to 0.10-12 million ± 0.040 by changing the Florescence rate. Analyses were done for randomly chosen roots from different trials with no other light interference to ensure only fluorescence light were measured.

For the Fm measurements, a single non modulated saturating 0.6 s light pulse was used. Then Fs were measured after 30 second using non modulated 640-700 nm actinic radiation. After this step plants were left for 14 minutes to ensure the fluorescence was reached steady state. A single non modulated saturating 0.6 s light pulse was excited every minute to measure the Fm, in presence of actinic light.

Then all resulted parameters (Fv/Fm, yield, qPN) were measured and marked on graphs.

3.10. Assessment of plant cell membrane stability using the Electrolyte leakage methods:

For each trial fresh shoot samples 1 g fresh weight of similar size were cut into approximately 3 cm long segments, washed with ddH2O, and dried with a Kim wipe. Segments were submerged in 10 mL of ddH_2O in a 20 mL test tube and were placed into vacuum desiccators (Savant, 100). Each sample was subjected to a vacuum at a rate of 100 L/min for 2 hours. Then EC value of the solution was measured at room temperature of 23±1 °C using an electrical-conductivity meter (4510 –conductivity meter, Jenway).

Chapter Four

Results and Discussion

4.1. Measurement of PGPR growth under saline NaCl solutions:

Different concentrations of NaCl- TSB media were prepared to test performance of PGPR salt tolerance on two plant species "Barley and Malt" plant and for testing their performance to salt, especially Na^+ and Cl^- ions are shown in Table 4.1.

Table 4.1: Average absorbance of UW3 grown in NaCl -TSB medium using concentration 0 g, 0.05g, 0.08g, 0.10g, 0.16g, and 0.24g at λ = 600 nm.

	Absorbance at λ = 600 nm				
Weight of NaCl	**1 hour**	**3 hours**	**5 hours**	**7 hours**	**8 hours**
0.05g	0.46	0.47	0.49	0.50	0.56
0.08g	0.43	0.47	0.44	0.49	0.52
0.10g	0.34	0.48	0.41	0.48	0.55
0.16g	0.40	0.41	0.37	0.38	0.47
0.24g	0.38	0.39	0.47	0.53	0.56
0g	0.46	0.49	0.54	0.60	0.70

For % ratio growth= Absorbance of bacteria grown in saline for each weight / Absorbance control (0 g NaCl) at 8 hours. As shown in Table 4.2.

For control (0 g NaCl) = Absorbance of bacteria UW3 grown in control (0 g NaCl) at each time / Absorbance of the bacteria grown in control (0 g NaCl) at 8 hours.

Table 4.2 show % growth of UW3 in saline (0 g, 0.05g, 0.08g, 0.10g, 0.16g, and 0.24g NaCl) / 20 mL TSB medium at **λ** = 600 nm at each time.

Table 4.2: % ratio of UW3 growth.

	% of control -Absorbance at λ = 600 nm				
weight of NaCl	**1 hour**	**3 hours**	**5 hours**	**7 hours**	**8 hours**
0.05g	65%	70%	66%	70%	80%
0.08g	61%	66%	63%	69%	74%
0.10g	48%	67%	57%	68%	78%
0.16g	56%	56%	52%	54%	66%
0.24g	56%	55%	66%	76%	79%
0g	64%	70%	77%	85%	100%

Figure 4.1: % ratio of UW3 growth at λ = 600 nm in NaCl-TSB medium for concentration (0 g, 0.05g, 0.08g, 0.10g, 0.16g, and 0.24g).

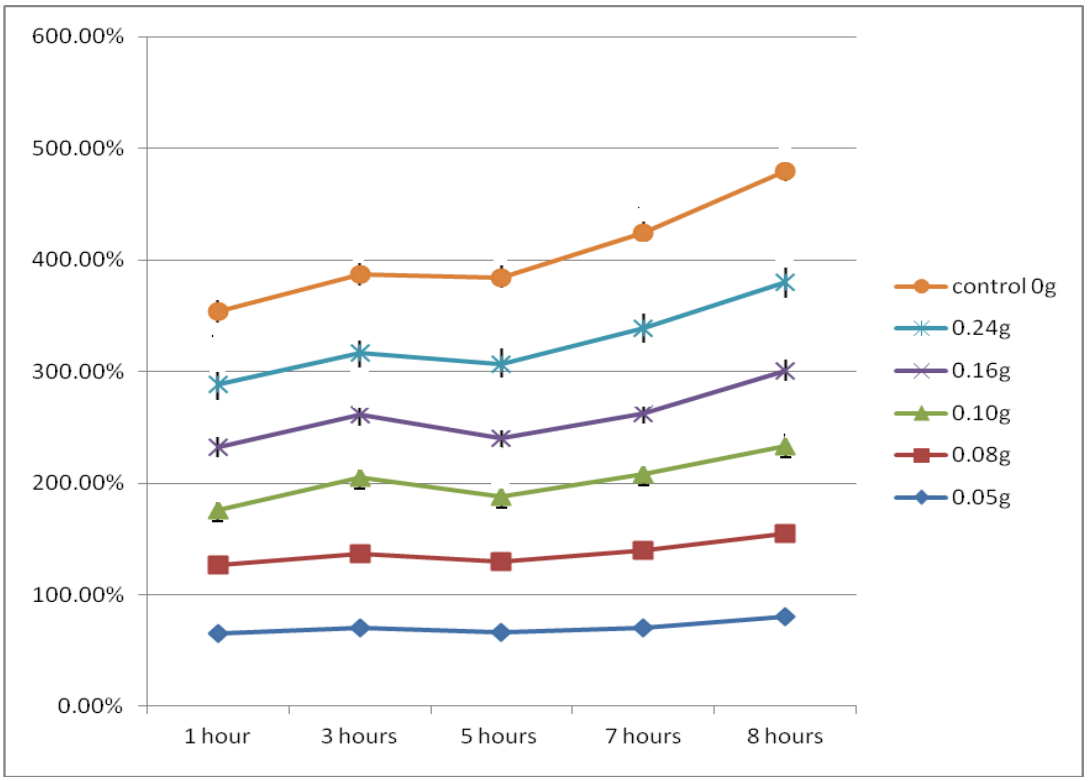


Figure 4.1: % of UW3 growth at λ =600 nm in NaCl -TSB medium for concentration (0 g, 0.05g, 0.08g, 0.10g, 0.16g, and 0.24g) .

The measurements done until 8 hours after that maximum efficiency is reached and become constant, for OD measurements and % of UW3 growth as in (Table 4.1-Table4.3 and Figure 4.1) it showed UW3 germination were increased under saline condition at different time interval, until it reached maximum levels and became constant without any incensement after 8 hours.

This increase indicated that salinity tolerant performances of PGPRs were increased [Shan, 2009]. for TSB medium contained concentration (0.08g, 0.10g, 0.24g) to be as (74.55%, 78.31%, 79.68 %) respectively at 8 hours surprisingly, measurement of bacterial growth was obtained for 0.16g salts

contained media (66.88%), this can be related to some performance of bacteria growth in that tube.

UW3 strains were chosen only for these measurements, since there is no differences UW4 mechanism and UW3 mechanism.

The test can be applied in future researches to study if performance of PGPR can be differentiating with different with time interval, which will indicate more biomass produced.

Shan (2009) study tolerance of UW3, UW4 strains with different concentration of NaCl -TSB medium (0.5%-2.0% g) were observed and noticed there growth increased.

4.2. Soil Electrical Conductivity:

EC measurement for soil done before used to study any changes in its values after irrigation with brackish water. Experimental measurements of (TDS) for random samples of autoclaved Loam soil are shown in Table 4.3.

Table 4.3: TDS measurement for of autoclaved Loam soil random samples, each parameter was performed in triplicate.

Name of parameter	Trial 1 TDS (mg/L)	Trial 2 TDS(mg/L)	Trial 3 TDS(mg/L)	Average	Standard deviation
ECe	70.0	72	67.2	69.7	2.4
EC 1:2	47.7	44.7	46.3	46.2	1.5

According to equation:

TDS (mg/L) =EC (dS/m) × 640……..……Equation (6)

For EC between 0.1 and 5.0 ds/m.

Calculated EC for random samples of autoclaved loam soil are shown in Table 4.4.

Table 4.4: Calculated EC for random samples of autoclaved loam soil, each parameter was performed in triplicate.

Name of parameter	Trial 1 ds/m	Trial 2 ds/m	Trial 3 ds/m	Average	Standard deviation
ECe	0.109	0.112	0.105	0.109	0.003
EC 1:2	0.075d	0.068	0.072	0.0717	0.003

Measurements of EC after 30 days of cultivation period, according to data in Annex 1 are shown in Figure 4.2.

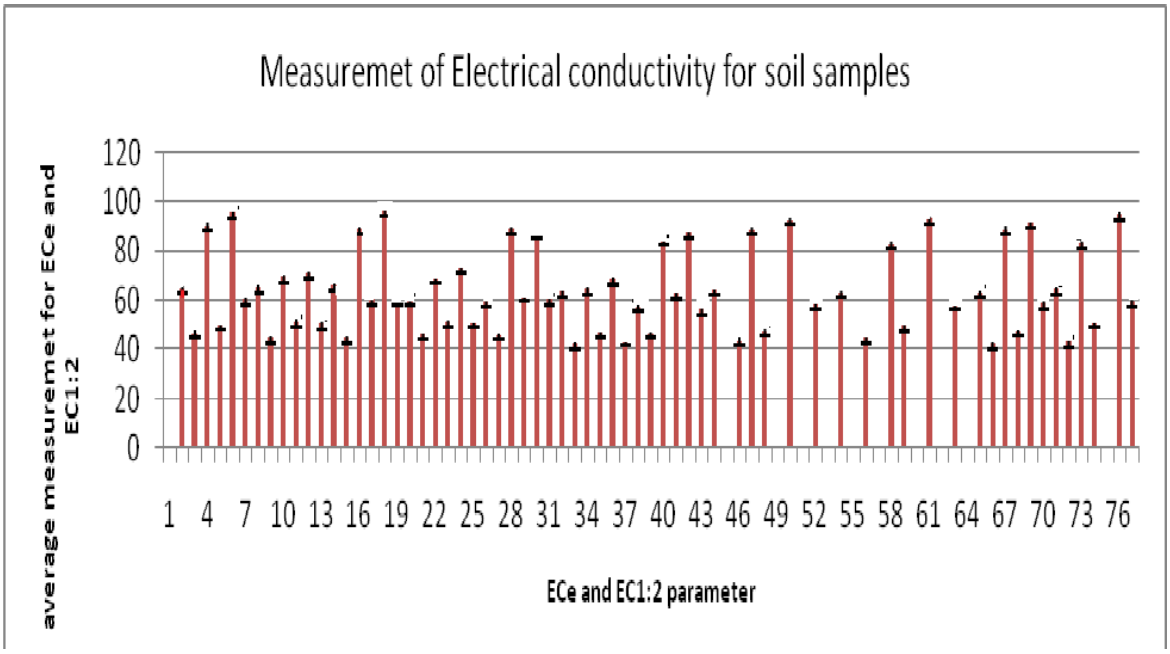


Figure 4.2: Measurements of EC after 30 days of cultivation period, according to data in Annex1.

According to equation:

TDS (mg/L) = EC (ds/m) × 640……Equation (6)

For EC between 0.1 and 5.0 ds/m.

Calculated measurements of EC in unit ds/L after 30 days are shown in Annex 2; each parameter was performed in triplicate.

Texture of soil sample used in this study were similar to texture exist in Jericho area, which is loamy texture in order to be implemented this study in field trial in Jericho area.

According to results in (Annex .1, Annex.2 and Figure 4.2), Barley plant trials treated with PGPRs irrigated with brackish water; their EC and TDS values before and after 30 days showed no obvious changes in their value they were closed to control trial irrigated with fresh water, this indicated accumulation of salts in biomass, furthermore trials treated with H_2O_2; were slightly similar to trials irrigated with brackish water; indicated that PGPR enhance more salt uptake into plant biomass.

In Malt plant trials results were not promising in promoting plant growth, even for trials with PGPRs there values still less than values for Barley plant trial, this can be related to some specific response of plant with these microbes.

4.3. Brackish water parameters measurements:

Annex 3 and Figure 4.3 showed TDS measurement for two synthetic brackish water samples before used in irrigation, and TDS measurements after irrigation which include for decent water (gravitational water) to detect any contaminant ions that could be leached out. These measurements were included for determination of their chance for salts to leach to ground water and cause salinity.

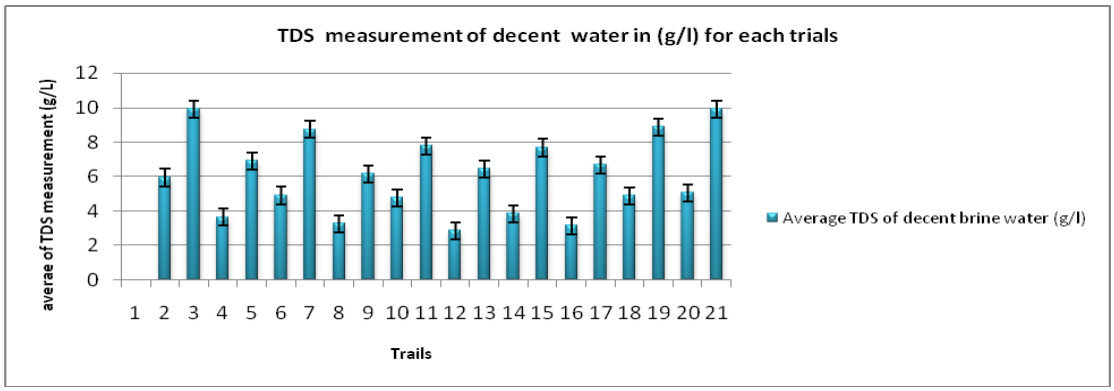


Figure 4.3: TDS Measurements for two synthetic brackish water samples before used in irrigation and after from Annex 3.

TDS measurements for decent water for trails are shown in Annex 3 and Figure 4.3, trials treated with PGPRs their measurements values were less than control trials, this indicate PGPR help in increasingly phytoextraction mechanism for salt uptake into by leaf and stem succulence. Trials included combination UW3 and UW4 shown no significant for their combination over trials treated separately.

TDS for decent brackish water for trials of Barley seeds treated with H_2O_2 and irrigated with 6000 mg/L and 10000 mg/L brackish water gave: (4.89 g/L, 8.87g/L) compared to control (5.94 g/L, 9.92 g/L), this mean only

tolerance mechanisms happened, while hydrogen peroxide aid plant to overcome oxidative stress through participated in cell signaling, (MAPK) [Mac neill, 2011] where this can be separated field study.

TDS measurements for Malt plant trials didn't show obvious significant combination for both strains in salt accumulation of plant over trials treated separately.

Barely plant responded more to PGPR than Malt plant; this can be attributed to large surface area of Barley seeds compared to Malt seeds so more bacteria strains have been adhesion to surface of Barley seeds, another reason may be related to some specie –specific differences in physiology and anatomy as well as specific differences in conditions required for optimal growth for Malt plant differ from Barley plant. This may indicate also that Malt plant may need different PGPR strains other than those UW3, UW4 for their optimal growth condition.

4.4. Measurements of photosynthesis with PAM fluorometry:

Photosynthesis activities of Barley plant trials were measured using PAM. Table 4.6 includes measurement for Fv/Fm for Barley plants trials.

Table 4.5: PAM fluorometry measurements for Fv/Fm for Barley plants, each trial repeated in 4 replicates.

Treatment	Fv/Fm
Control Barley irrigated with fresh water	0.785
Control Barley irrigated with 6000 mg/L of brackish water	0.659
Control Barley irrigated with 10000 mg/L of brackish water	0.594
Treated Barley seeds with UW3 irrigated with fresh water	0.790
Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	0.775
Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	0.788
Treated Barley seeds with UW4 irrigated with fresh water	0.796
Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	0.756
Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	0.778
Treated Barley seeds with UW3 + UW4 irrigated with fresh water	0.736
Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	0.776
Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	0.796
Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water	0.723
Treated Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	0.749
Treated Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	0.769
Treated Barley seeds with H_2O_2 irrigated with fresh water	0.749
Treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	0.686
Treated Barley seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	0.688

According to Eq. (4) and Eq. (5) maximal yield of PSII (Fv/Fm) ratio was calculated, where typical value of it is equal to 0.8 [Mac neill, 2011].

Trails treated with PGPR and irrigated with brackish water their values were closed to 0.8, while control trials their vales were ranged from (0.5 - 0.6) which mean that plant is under stress, and its photosynthesis not proceed as it should.

These indicate the performance of PGPR in increase the photosynthetic activity under salt stress, beside it was obvious in root there color were dark green and taller.

But trials treated only with H_2O_2 their values were closed to control trials which mean there is no significant contribution of peroxide in activating cell signaling.

For trials treated with both strains UW3 +UW4 compared to trials treated with strain separately, maximum yields of PSII of Fv/Fm were not significant higher; these indicate performance of trials with both strains had same effective to tolerate to salinity and same performance of photosynthetic as trials treated separately.

Other photosynthesis parameters measurements, such as (Y (II), NPQ) were measured, as shown below for each trial has its own spectra, include in Tables and Figures below.

1- Control Barley plant irrigated with fresh water:

Replicate of measurements depend upon random selection for each Barley plant trial it include 4 replicate are shown in Figure 4.4.

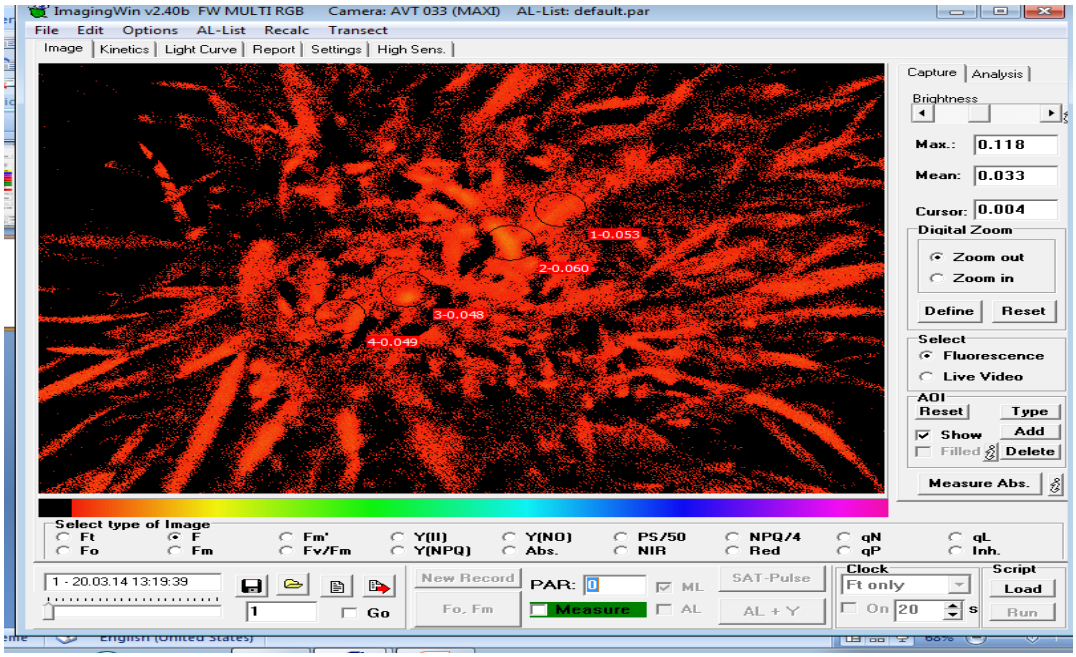


Figure 4.4: photography random selection of Barley plant measurements for Control Barley irrigated with fresh water.

Fs parameter was measured after 30 second using non modulated 640-700 nm actinic radiation, after this step plants were left for 14 minutes to ensure the fluorescence was reached steady state, are shown in spectra 4.6 and same was done for all trials.

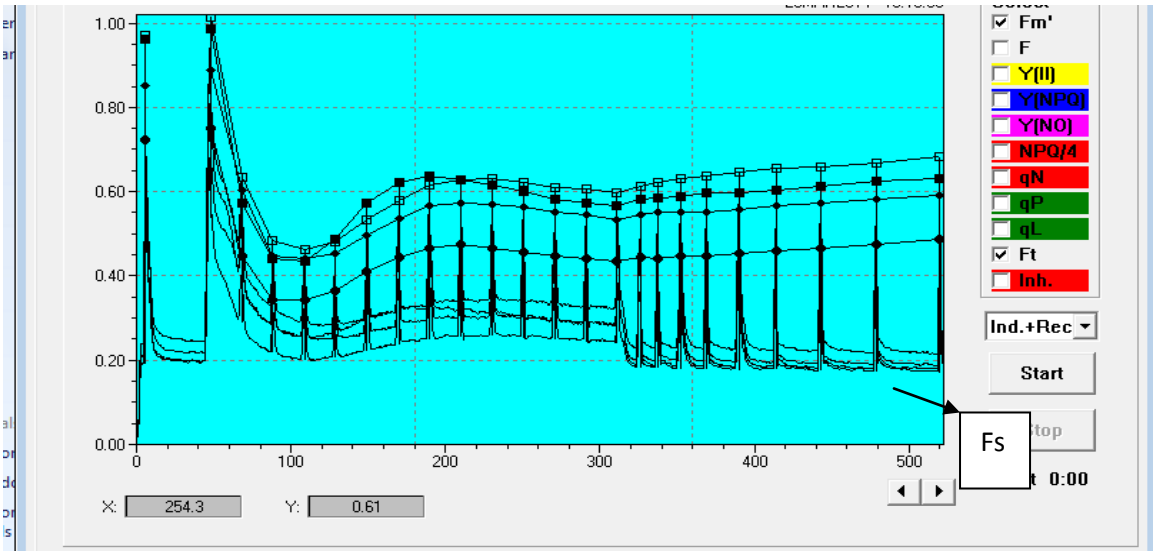


Figure 4.5: PAM fluorometry spectra for control Barley irrigated with fresh water.

Table 4.6 and Figure 4.6 showed PAM fluorometry measurements for yield Y (II) and average NPQ with standard deviation for control Barley trial.

Tables for other trials showed in Annexex.

Table 4.6: PAM fluorometry measurement for control Barley irrigated with fresh water:

Time (min:sec)	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:0	0.7797	0.0192	0.0064	0.0008
0:0:42	0.1595	0.0274	0.0015	0.0008
0:0:62	0.1975	0.0418	0.2513	0.0354
0:0:83	0.2577	0.0466	0.3836	0.0462
0:0:103	0.3083	0.0490	0.3950	0.0532
0:0:123	0.3295	0.0473	0.3925	0.0555
0:0:143	0.3613	0.0443	0.3712	0.0562
0:02:44	0.3727	0.0405	0.3621	0.0555
0:03:04	0.3855	0.0377	0.3505	0.0538
0:03:24	0.3882	0.0382	0.3465	0.0535
0:03:45	0.3880	0.0386	0.3455	0.0521
0:04:05	0.3982	0.0399	0.3377	0.0522
0:04:25	0.4052	0.0354	0.3294	0.0491
0:04:45	0.4165	0.0362	0.3222	0.0489
0:05:06	0.4197	0.0341	0.3192	0.0463
0:05:20	0.6025	0.0368	0.2147	0.0374
0:05:32	0.6137	0.0333	0.1962	0.0305
0:05:46	0.6322	0.0280	0.1757	0.0233
0:06:04	0.6512	0.0234	0.1562	0.0149
0:06:24	0.6633	0.02164	0.1445	0.0113
0:06:48	0.673	0.0171	0.1315	0.0065
0:07:18	0.6865	0.0164	0.1197	0.0047
0:07:53	0.6943	0.0143	0.1117	0.0055
0:08:35	0.7002	0.0128	0.1065	0.0041

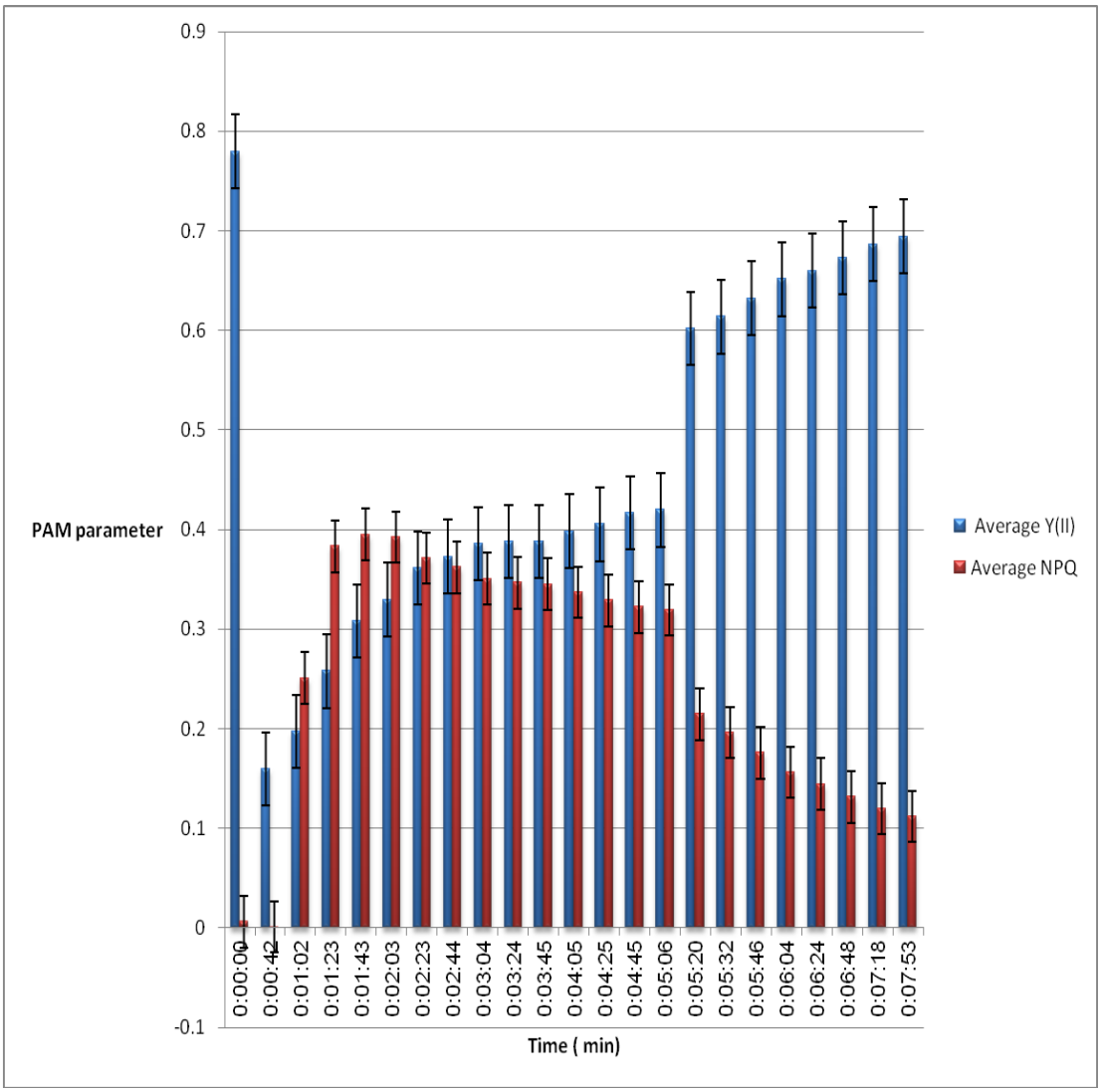


Figure 4.6: PAM fluorometry Chart for control Barley irrigated with fresh water.

2- Barley plant irrigated with 6000 mg/L of Brackish water :

PAM fluorometry spectra and chart for Barley plant irrigated with 6000 mg/L of brackish water are shown in Figure 4.7 and Figure 4.8.

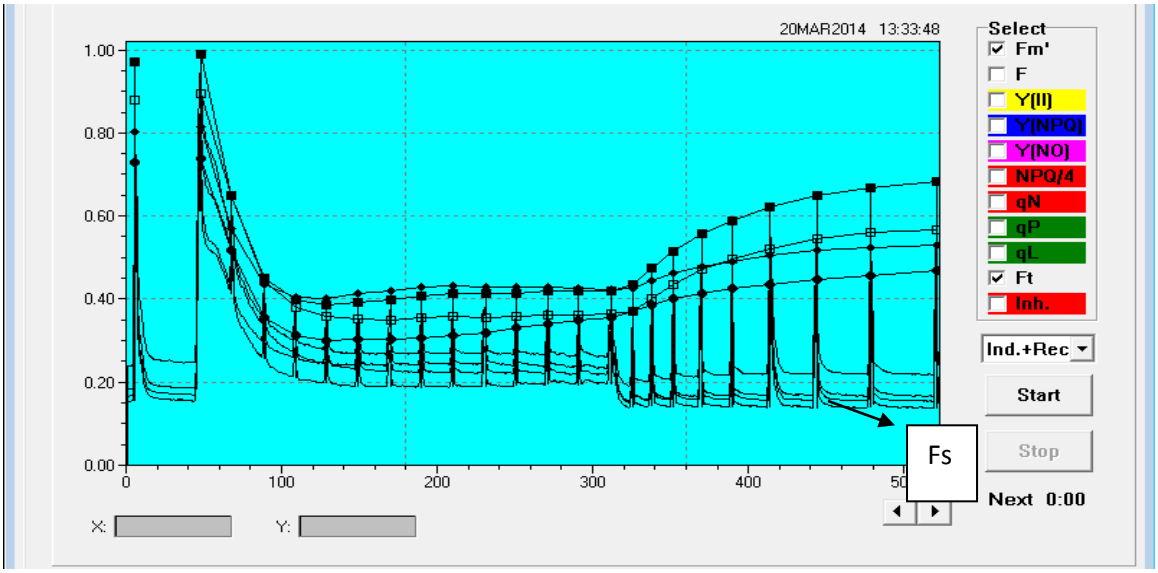


Figure 4.7: PAM fluorometry spectra for Barley Plant irrigated with 6000mg/L of brackish water.

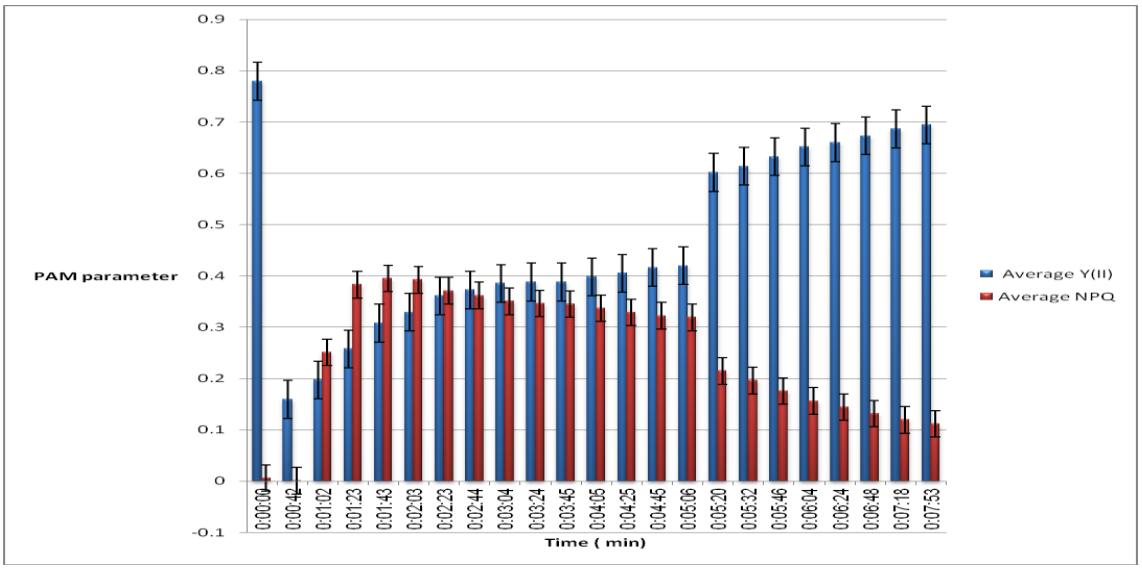


Figure 4.8: PAM fluorometry chart for Barley Plant irrigated with 6000mg/L of brackish water as shown in Annex 5.

3-Barley plant irrigated with 10000 mg/L of brackish water:

PAM fluorometry spectra and chart for Barley plant irrigated with 10000 mg/L brackish water are shown in Figure 4.9 and Figure 4.10.

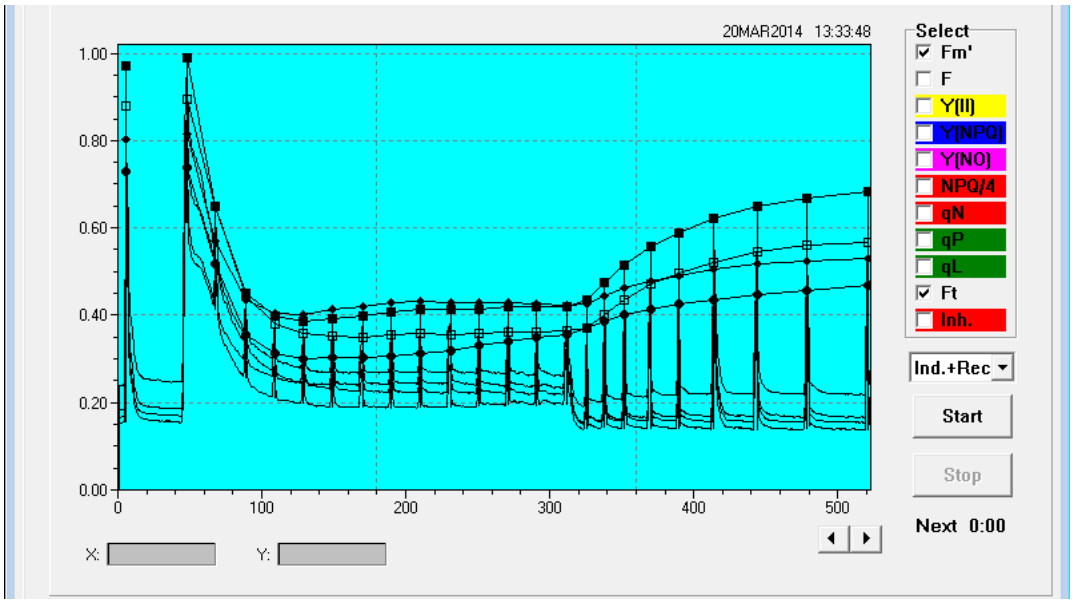


Figure 4.9: PAM fluorometry spectra for Barley plant irrigated with 10000 mg/L of brackish water.

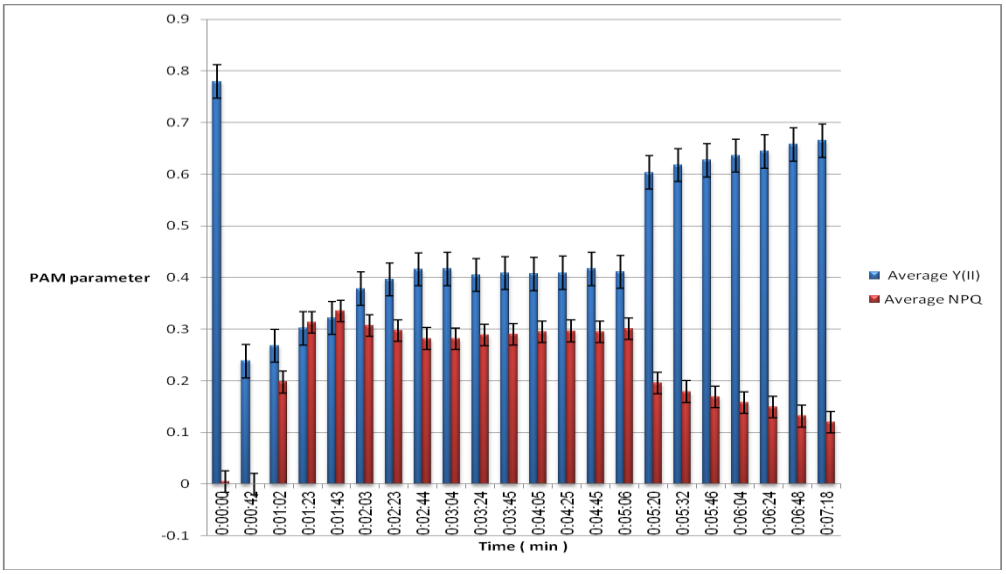


Figure 4.10: PAM fluorometry chart for Barley plant irrigated with 10000 mg/L of brackish water as shown Annex6

4-Treated Barley seeds with UW3 irrigated with fresh water:

PAM fluorometry spectra and chart for treated Barley seeds with UW3 irrigated with fresh water are shown in Figure 4.11 and Figure 4.12.

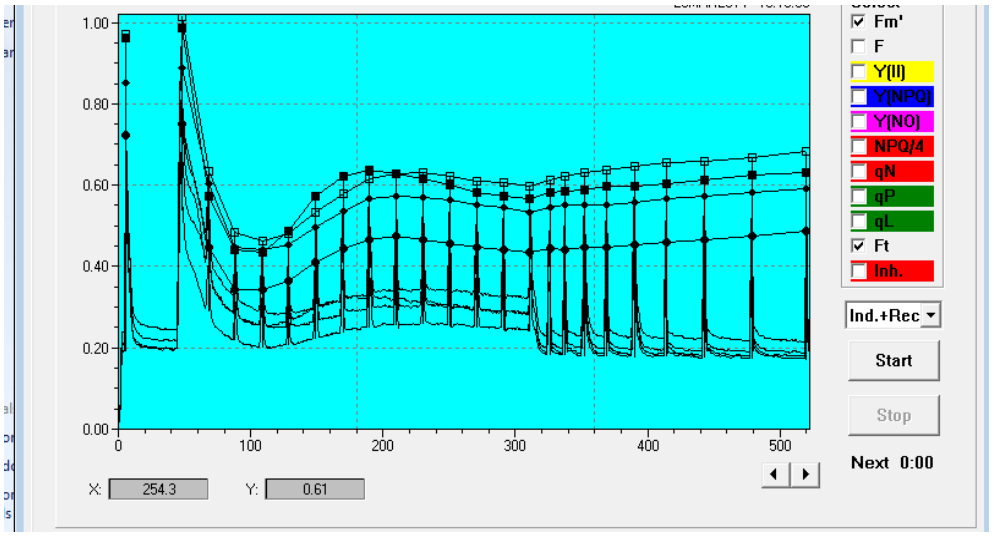


Figure 4.11: PAM fluorometry spectra for treated Barley seeds with UW3 irrigated with fresh water.

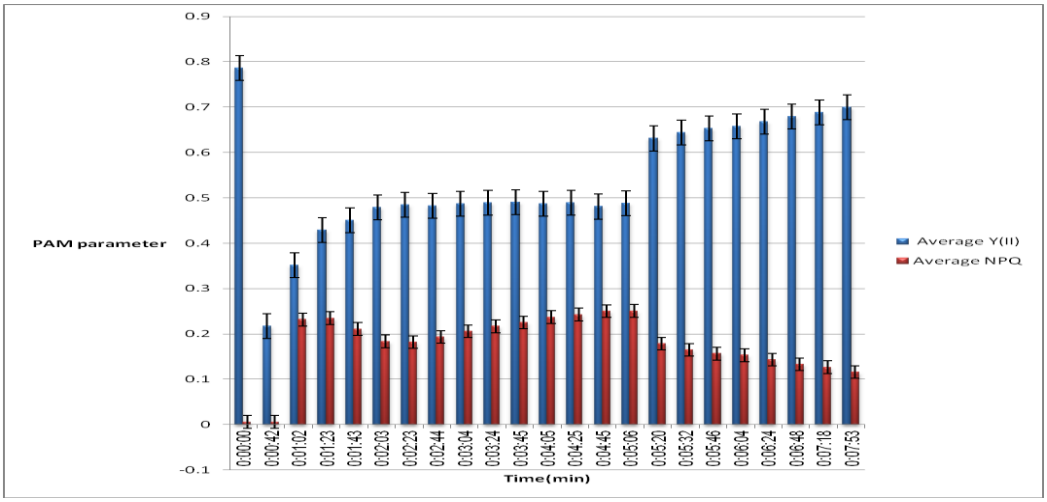


Figure 4.12: PAM fluorometry chart for treated Barley seeds with UW3 irrigated with fresh water as shown in Annex 7.

5-Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water:

PAM fluorometry spectra and chart for treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water are shown in Figure 4.13 and Figure 4.14.

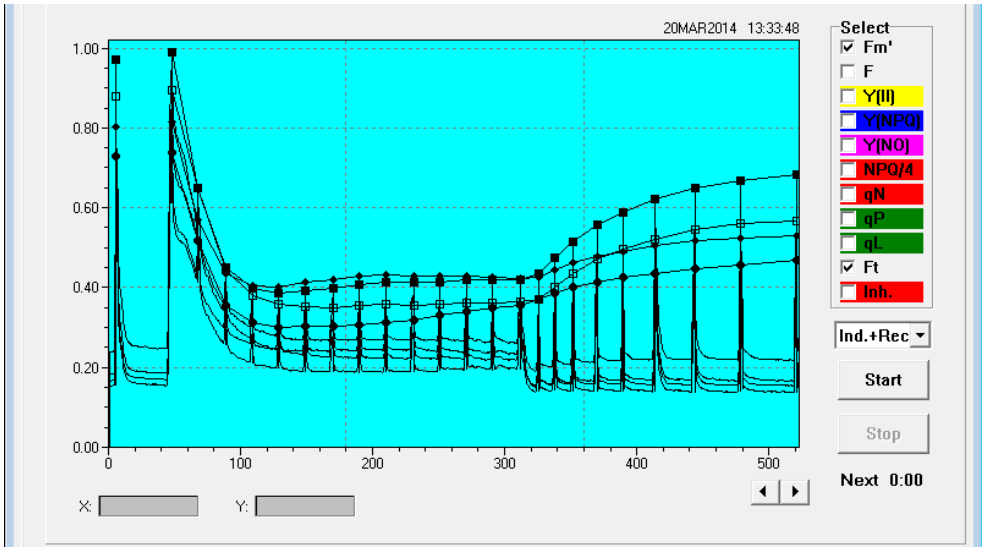


Figure 4.13: PAM fluorometry spectra for treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water.

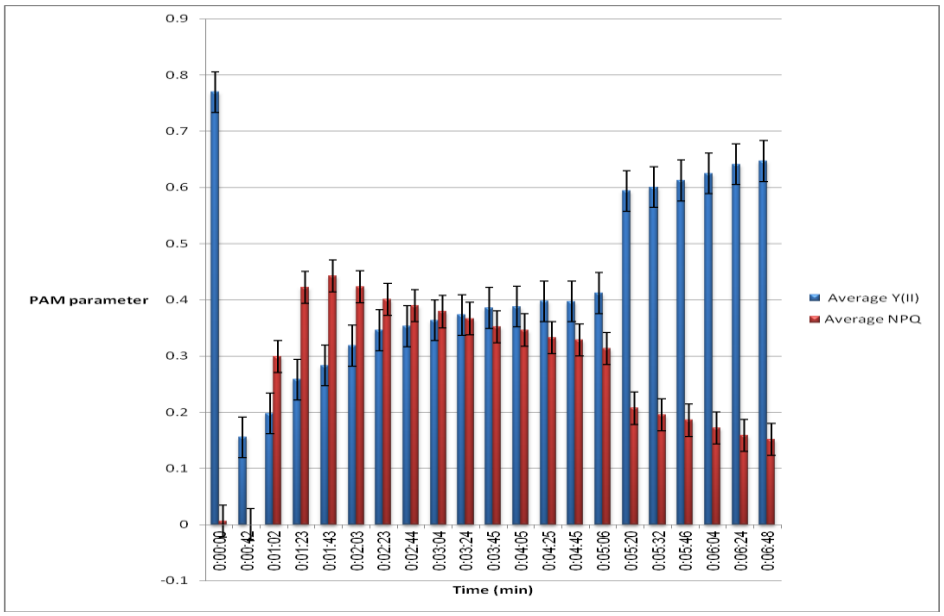


Figure 4.14: PAM fluorometry chart for treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water as shown in Annex 8.

6-Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water:

PAM fluorometry spectra and chart for treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water are shown in Figure 4.15 and Figure 4.16.

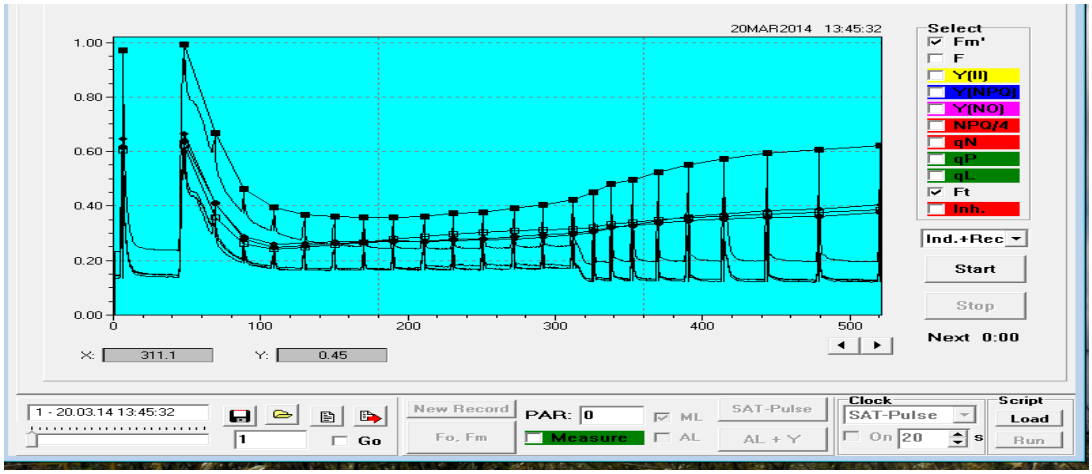


Figure 4.15: PAM fluorometry spectra for treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water.

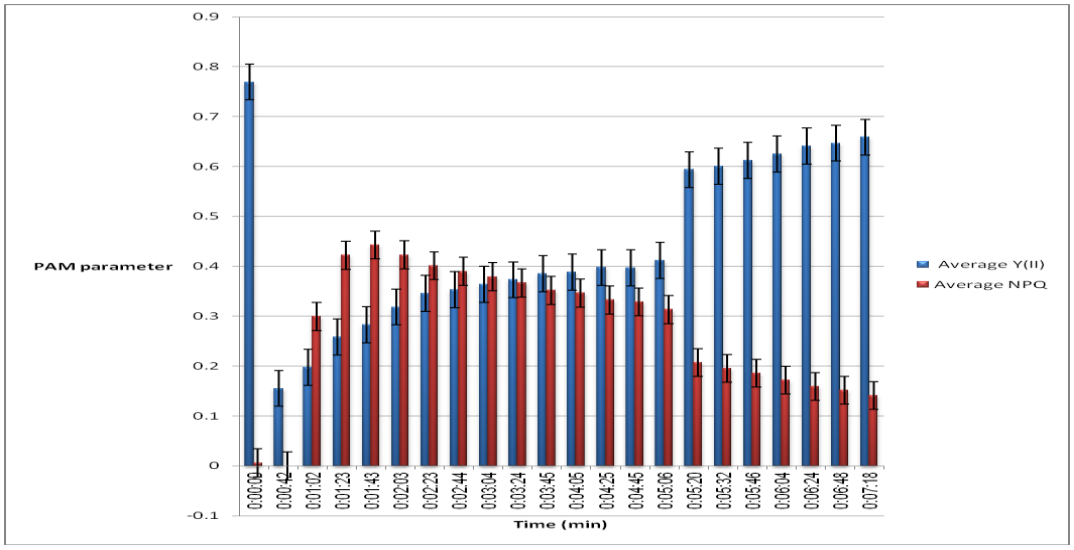


Figure 4.16: PAM fluorometry chart for treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water **as** shown in Annex 9.

7- Treated Barley seeds with UW4 irrigated with fresh water:

PAM fluorometry spectra and chart for treated Barley seeds with UW4 irrigated with fresh water are shown in Figure 4.17 and Figure 4.18.

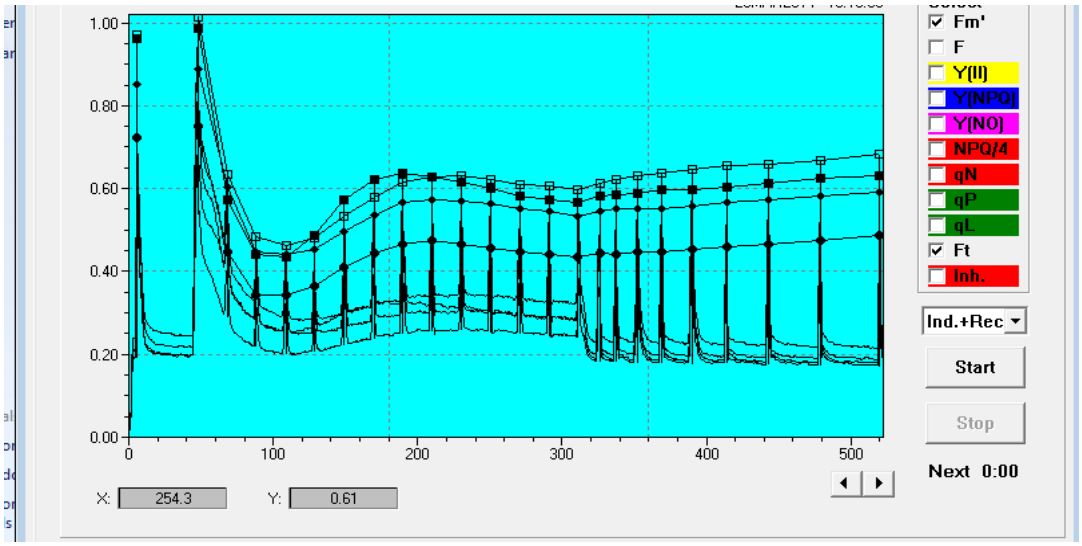


Figure 4.17: PAM fluorometry spectra for treated Barley seeds with UW4 irrigated with fresh water.

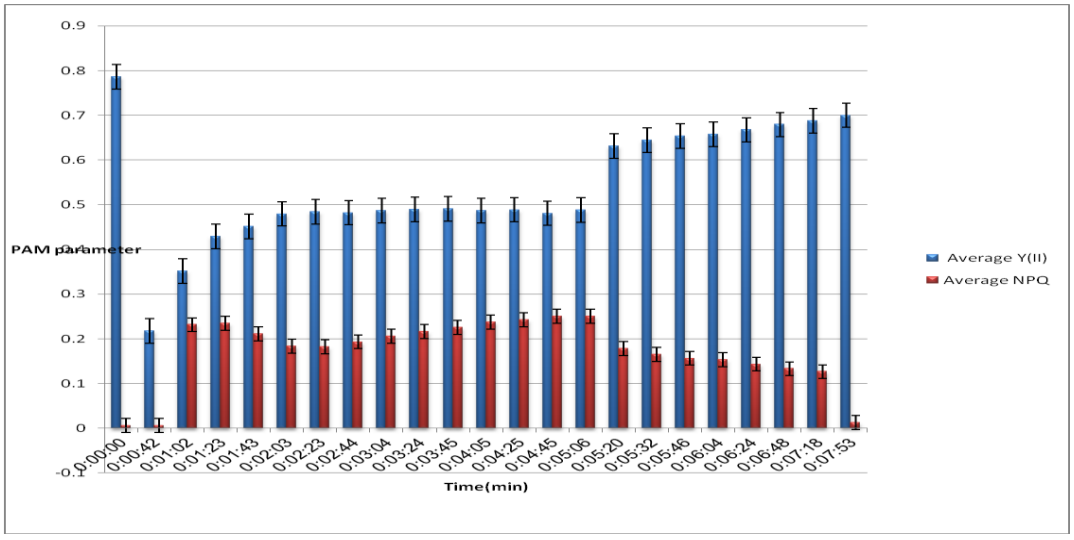


Figure 4.18: PAM fluorometry chart for treated Barley seeds with UW4 irrigated with fresh water for data in Annex10.

8-Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water:

PAM fluorometry spectra and chart for treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water are shown in Figure 4.19 and Figure 4.20.

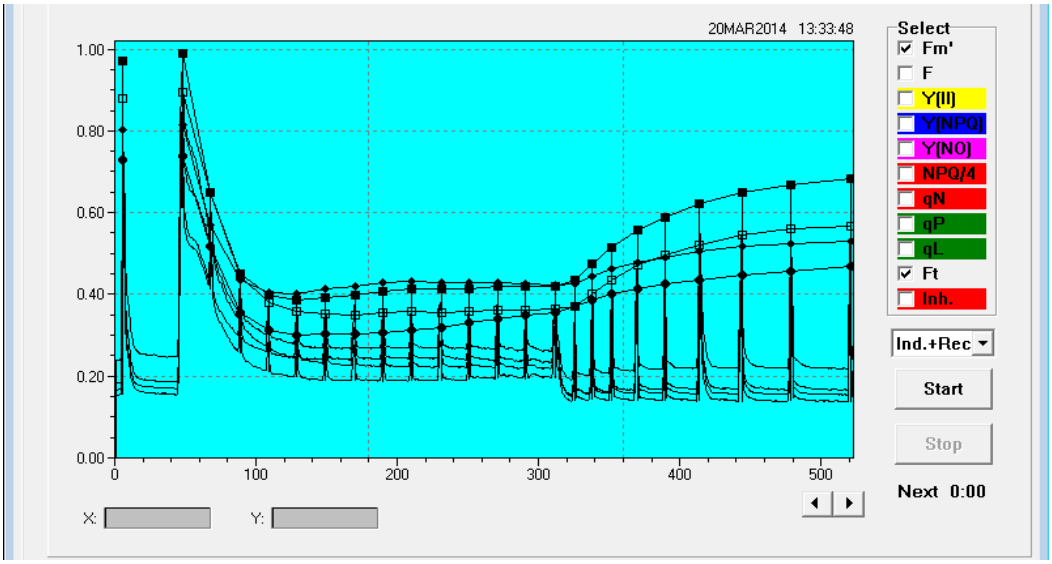


Figure 4.19: PAM fluorometry spectra for treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water.

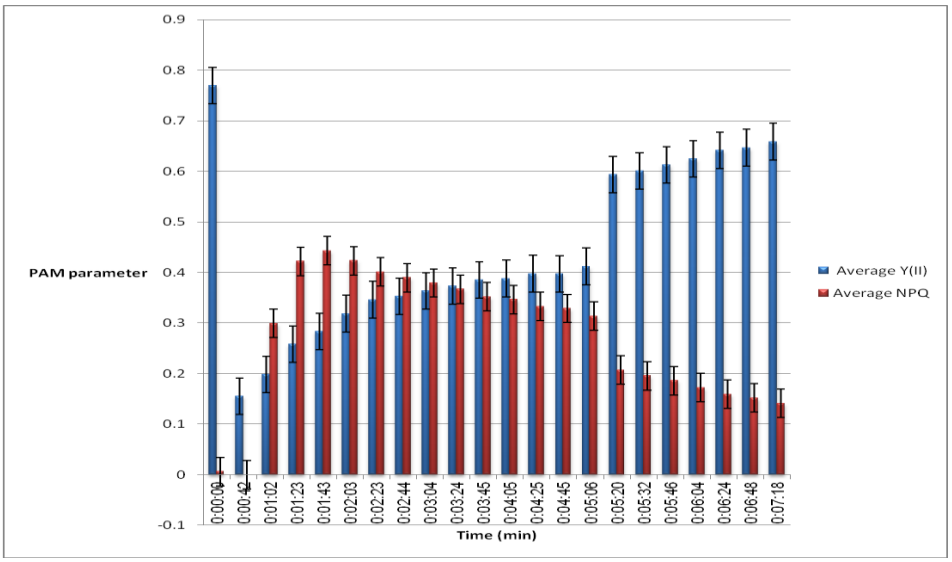


Figure 4.20: PAM fluorometry chart for treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water for data in Annex11.

9-Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water:

PAM fluorometry spectra and chart for treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water are shown in Figure 4.21 and Figure 4.22.

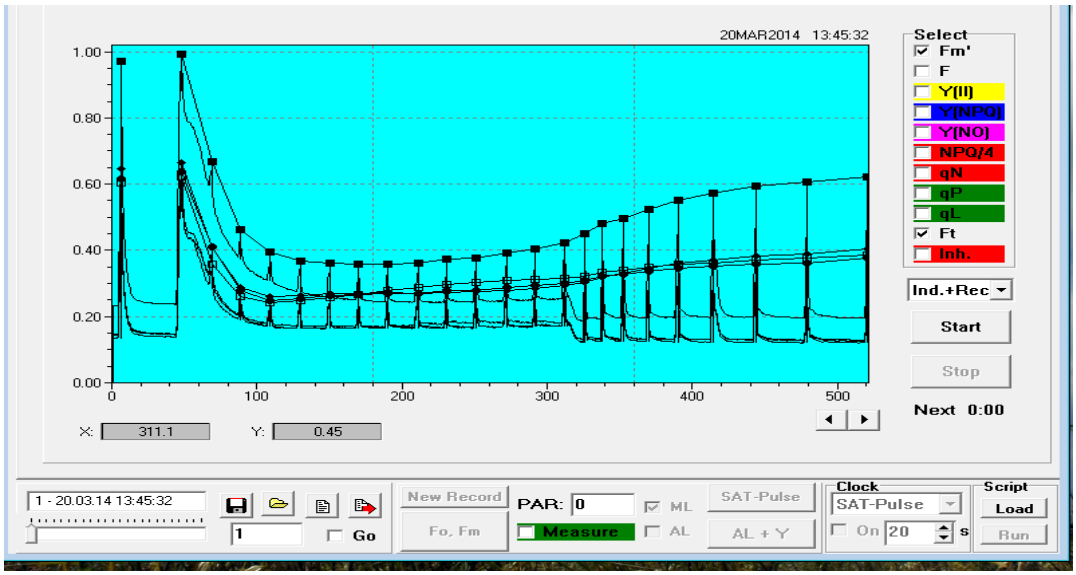


Figure 4.21: PAM fluorometry spectra for treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water.

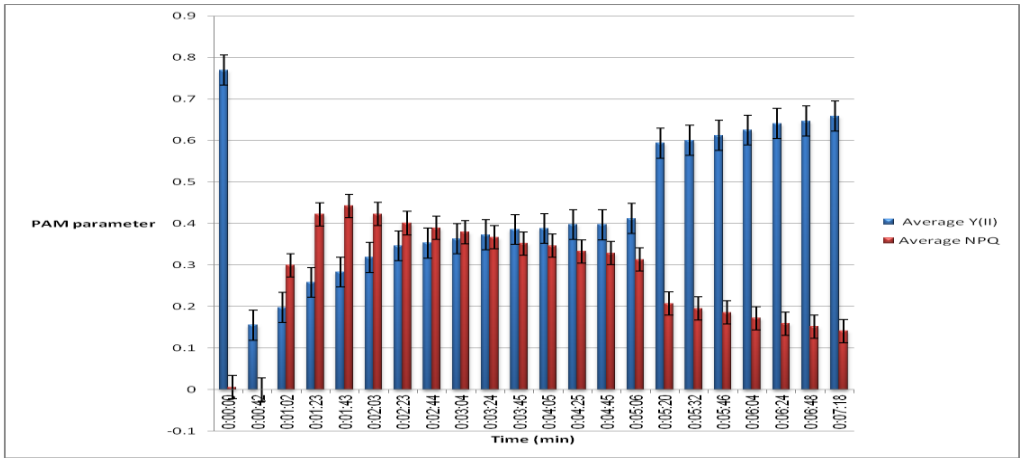


Figure 4.22: PAM fluorometry spectra for treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water for data in Annex12.

10-Treated Barley seeds with UW3+UW4 irrigated with fresh water

PAM fluorometry spectra and chart for treated Barley seeds with UW3+UW4 irrigated with fresh water are shown in Figure 4.23 and Figure 4.24.

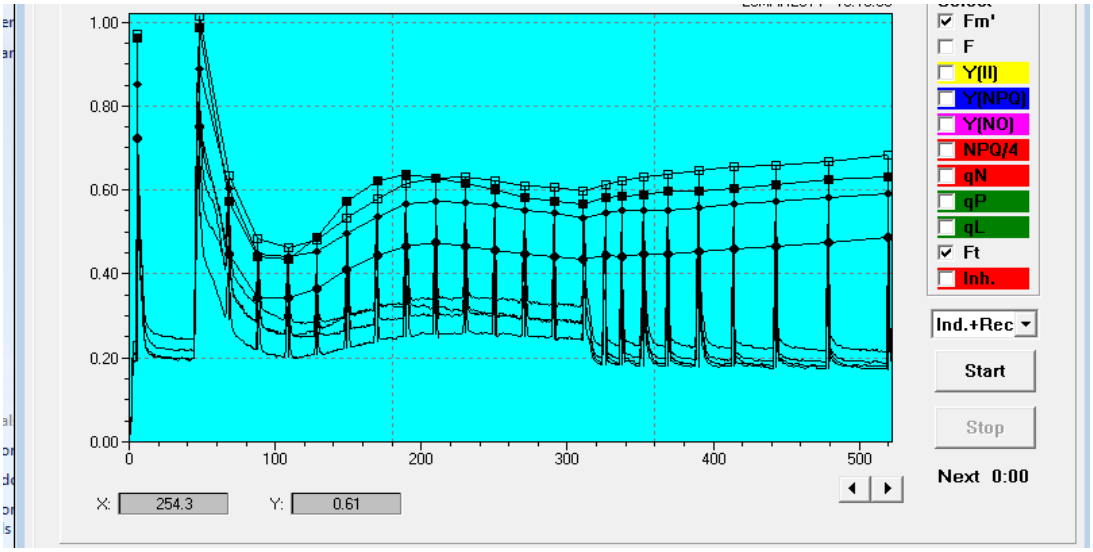


Figure 4.23: PAM fluorometry spectra for treated Barley seeds with UW3+UW4 irrigated with fresh water.

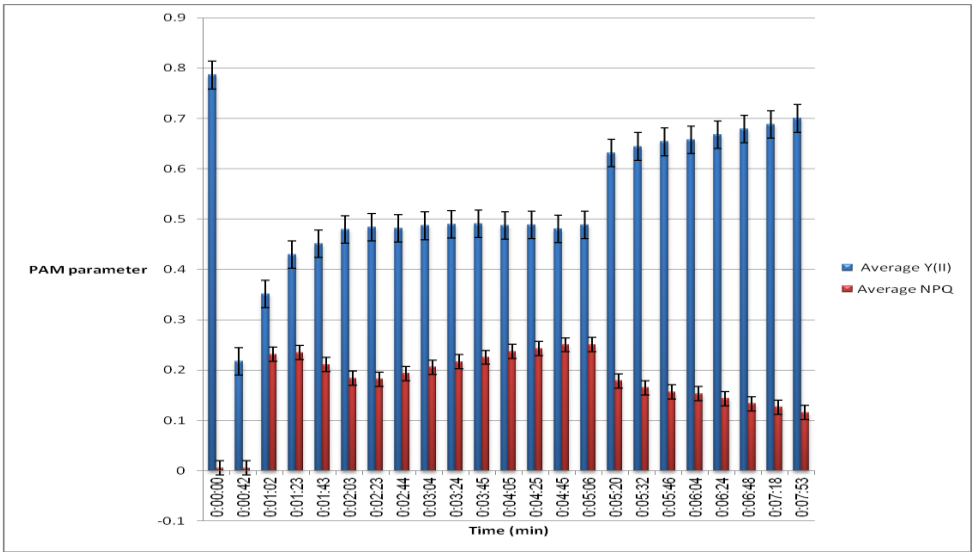


Figure 4.24: PAM fluorometry chart for treated Barley seeds with UW3+UW4 irrigated with fresh water for data in Annex13.

11-Treated Barley seeds with UW3+UW4 irrigated with 6000 mg/L of brackish water:

PAM fluorometry spectra and chart for treated Barley seeds with UW3+UW4 irrigated with 6000 mg/L of brackish water are shown in Figure 4.25and Figure 4.26.

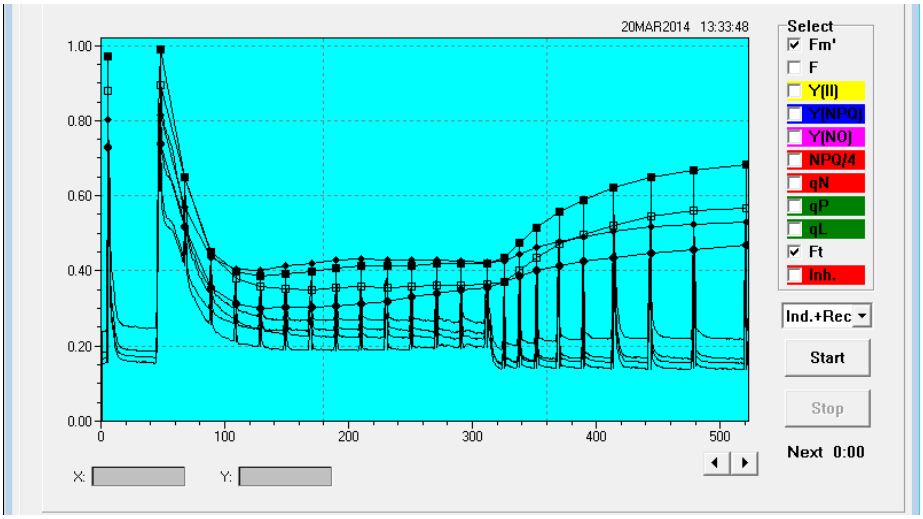


Figure 4.25: PAM fluorometry spectra for treated Barley seeds with UW3+UW4 irrigated with 6000 mg/L of brackish water.

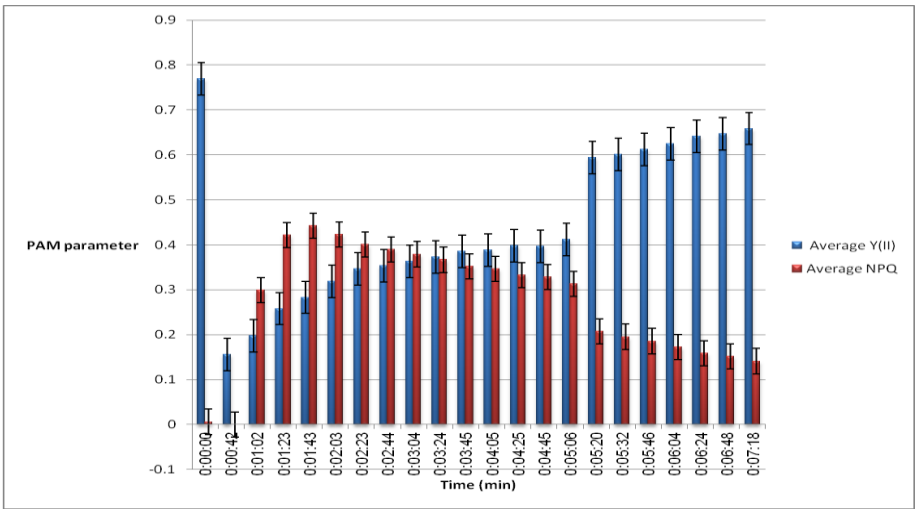


Figure 4.26: PAM fluorometry chart for treated Barley seeds with UW3+UW4 irrigated with 6000 mg/L of brackish water, for data in Annex14.

12-Treated Barley seeds with UW3+ UW4 irrigated with 10000 mg/L of brackish water:

PAM fluorometry spectra for treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water are shown in Figure 4.27 and Figure 4.28.

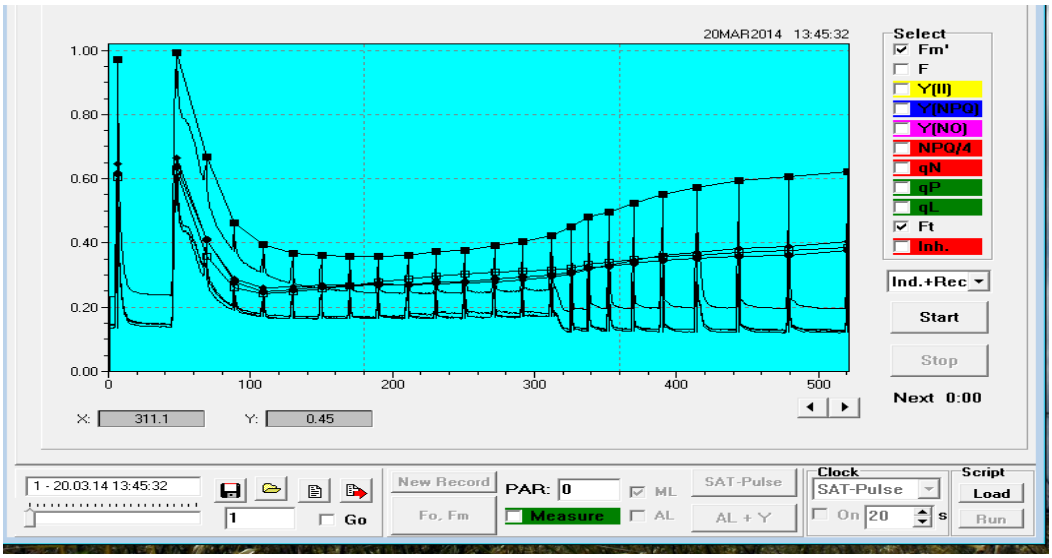


Figure 4.27: PAM fluorometry spectra for treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water.

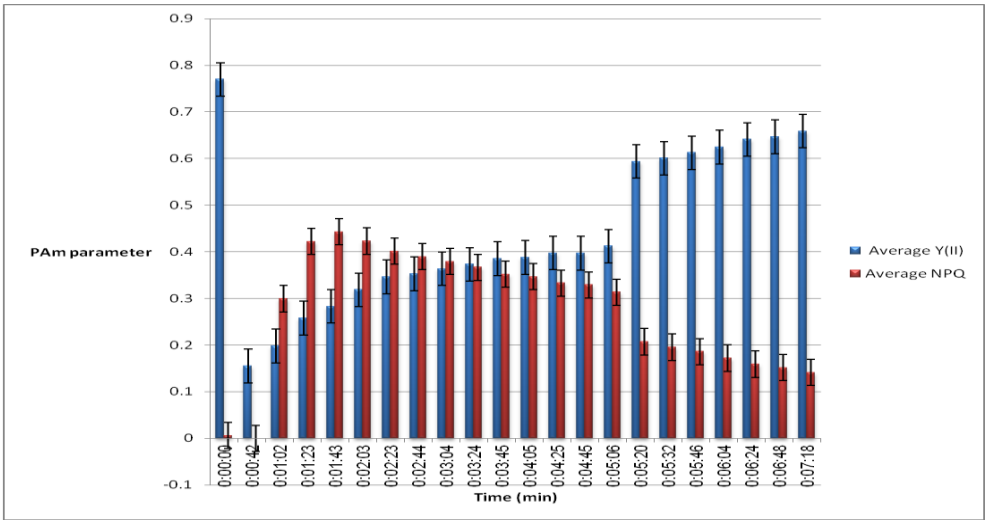


Figure 4.28: PAM fluorometry chart for treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water, for data in Annex15.

13-Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water:

PAM fluorometry spectra for treated Barley seeds with UW3+H_2O_2 irrigated with fresh water are shown in Figure 4.29 and Figure 4.30.

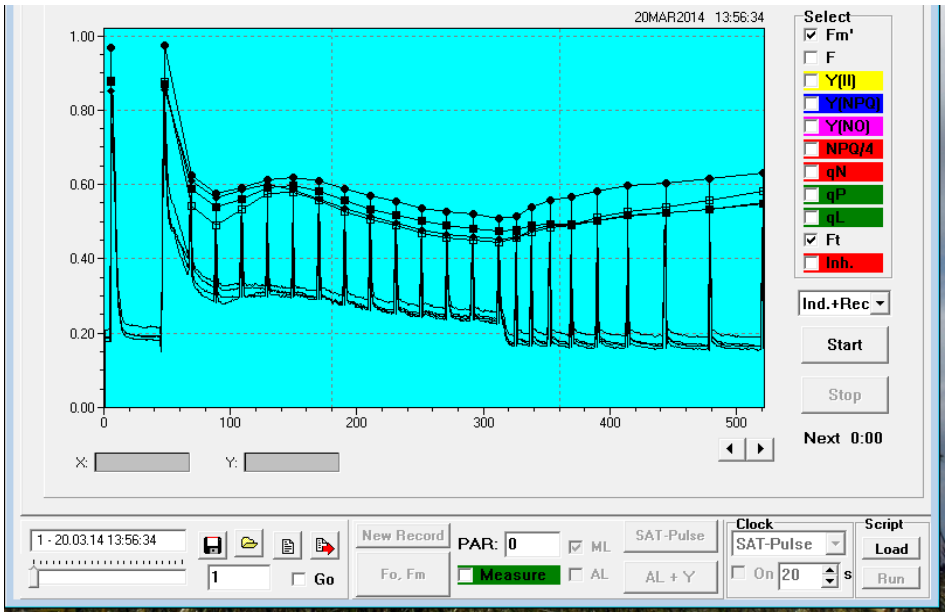


Figure 4.29: PAM fluorometry spectra for treated Barley seeds with UW3+H_2O_2 irrigated with fresh water.

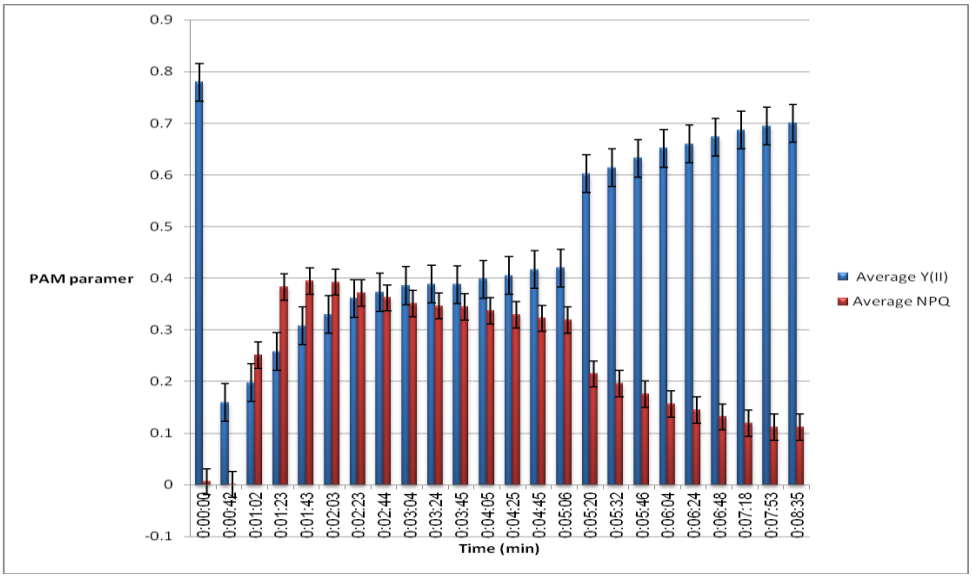


Figure 4.30: PA M fluorometry chart for treated Barley seeds with UW3+H_2O_2 irrigated with fresh water, for data in Annex16

14-Treated Barley seeds with UW3+H_2O_2 irrigated with 6000 mg/L of brackish water:

PAM fluorometry spectra and chart for treated Barley seeds with UW3+H_2O_2 irrigated with 6000 mg/L of brackish water are shown in Figure 4.31 and Figure 4.32.

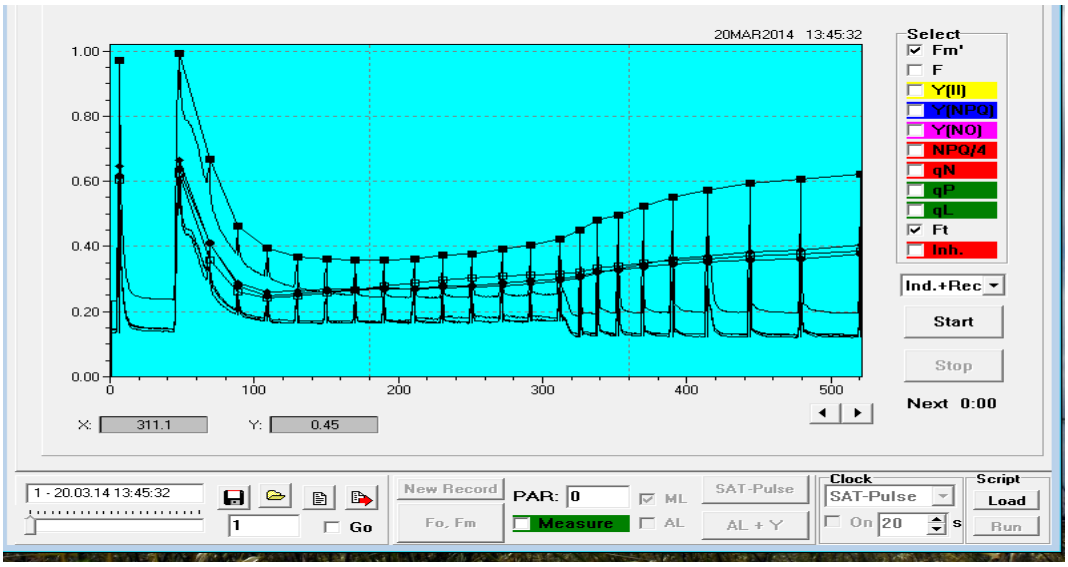


Figure 4.31: PAM fluorometry spectra for treated Barley seeds with UW3+H_2O_2 irrigated with 6000 mg/L of brackish water.

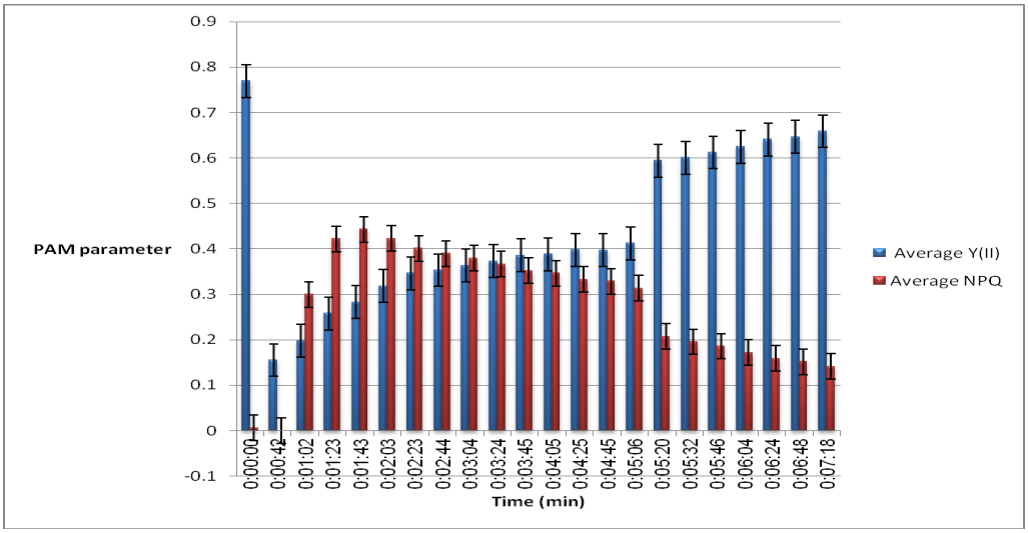


Figure 4.32: PAM fluorometry chart for treated Barley seeds with UW3+H_2O_2 irrigated with 6000 mg/L of brackish water, for data in Annex17.

15- Treated Barley seeds with UW3+H_2O_2 irrigated with 10000 mg/L of brackish water:

Pulse Amplitude modulated fluorometry spectra and chart for treated Barley seeds with UW3+H_2O_2 irrigated with 10000 mg/L of brackish water are shown in Figure 4.33 and Figure 4.34.

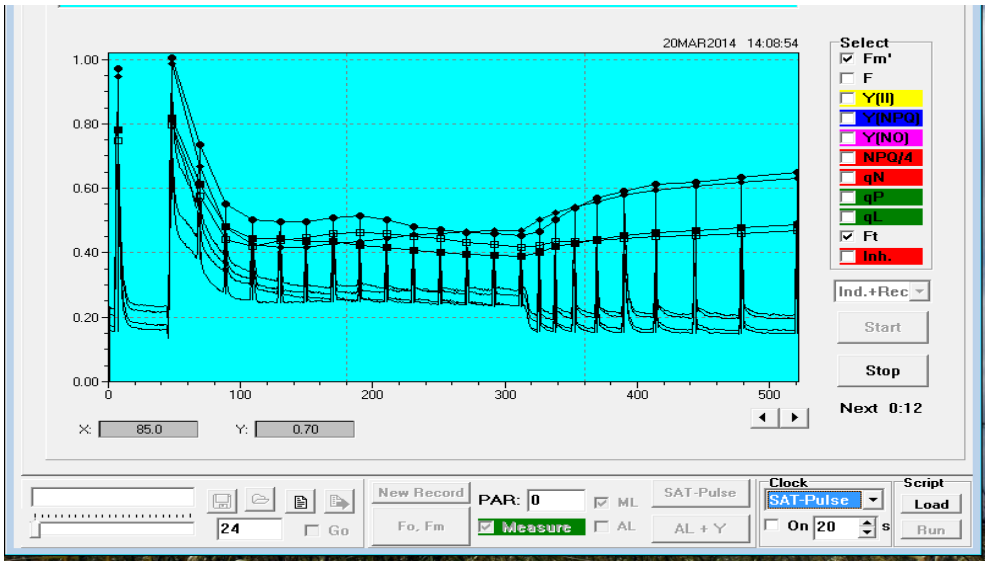


Figure 4.33: PAM fluorometry spectra for treated barley seeds with UW3+H_2O_2 irrigated with 10000 mg/L of brackish water.

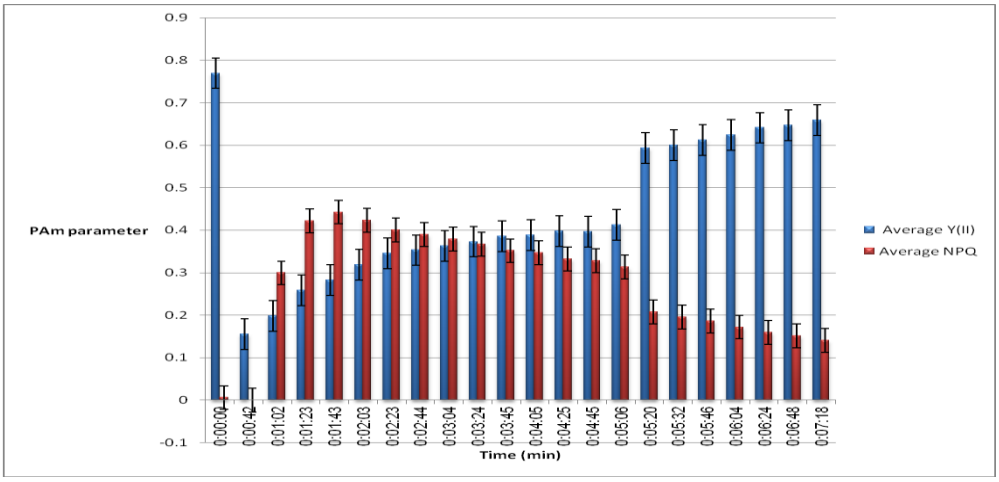


Figure 4.34: PAM fluorometry chart for treated barley seeds with UW3+H_2O_2 irrigated with 10000 mg/L of brackish water, for data in Annex18.

Measurements include function of PSII as flow of electron, rate of photosynthesis by emitted light from the pulse, and measured light. Heat dissipation is relatively constant during measurements.

Measurements showed several chlorophyll fluorescence parameters which are: (Y (II), NPQ). These parameters were measured at minimal fluorescence in dark adapted plant tissue (F_0) and at maximal fluorescence (Fm), steady state fluorescence (Fs) which shown in each spectra, where optimum value ranged between (0.15-0.17) larger than this value mean plant in under stress, and give indication of effect of salt stress on photosynthetic electron transport, [Mac neill, 2011]. Control trials irrigated with brackish water their values were large (0.19-0.23) compared to trials treated with PGPR and irrigated with brackish water (0.15-0.17),these results indicate damaged happen inside cell for trials without PGPR.

Trials treated with PGPR, there photosynthesis measurement (Y (II), NPQ), as in spectra and chart were similar to measurement of control trials treated with fresh water for both trials with or without PGPR, moreover photosynthetic values were shown compared to control values, this mean that PGPRs increase photosynthetic activity inside plant, besides that, it was obvious in root there color was dark green color for shoot and taller leaves [Mac neill, 2011].

For other trials in Figures (4.4-4.34) and Annex (5-18) stress appeared as decrease for values of Average Y (II) and Average NPQ for trials treated without PGPR compared to trials treated with PGPR.

The reason for decrease in photosynthesis in trials without PGPR can be related for accumulation of high concentration of salts in tissue that responsible for photosynthesis process. It could be as a result of swelling of thylakoids, and distortion of chloroplast membrane; which lead to disrupt all process in plant [Mac neill, 2011].

Malt Plant leaves was light green color, this indicated that there were no full photosynthesis processes and didn't show positive response to PGPR treatment as expected. Thus, measurement of photosynthesis by PAM fluorometry instrument include only for Barely plant.

For trials treated with both strains UW3 +UW4 compared to trials treated with strain separately, the maximum yields of PSII were not significant higher. This indicates performance of trials with both strains show same effective to tolerate to salinity and same performance of photosynthetic as trials treated separately.

Shan (2009) study showed some plant species such as Barley plant with PGPR showed high performance of photosynthesis activity in saline soil.

Mc neill (2011) study showed photosynthesis activities for different plants species such as Barley, Oats, and Tall Wheatgrass treated with PGPR and

grown in saline soil field, high performance of their photosynthesis activity.

4.5. Green house studies and dry biomass determination:

Green house studies include measurements of mass for two species plants (Barley, Malt) trials. Measurements include for wet mass in (g) and for dry mass in (g) with differences between wet and dry Length measurements. This procedure was done to compare between trials.

Measurements of root and shoot wet mass (g) for Barley plant trials after 30 days are shown in Table 4.7 and Figure 4.35.

Table 4.7: Measurements of root and shoot wet mass (g) for Barley plant trials after 30 days.

Num	Treatment	Root+ Shoot wet mass (g) After 30 days	% of wet mass	Significant value
1	Control Barley irrigated with fresh water	85.7	100	
2	Control Barley irrigated with 6000 mg/L of brackish water	84.3	98.4	
3	Control Barley irrigated with 10000 mg/L of brackish water	89.4	104.3	Sig
4	Treated Barley seeds with UW3 irrigated with fresh water	165.3	192.9	
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	193.8	226.2	
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	240.8	285.7	Sig
7	Treated Barley seeds with UW4 irrigated with fresh water	176.2	205.6	
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	189.3	220.9	
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	215.3	251.2	Sig
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	203.4	237.3	
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	206.3	240.7	
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	280.8	148.3	
13	Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water	95.3	111.2	
14	Treated Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	202.3	236.1	
15	Treated Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	207.5	242.1	Sig
16	Treated Barley seeds with H_2O_2 irrigated with fresh water	178.6	208.4	
17	Treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	189.6	221.2	
18	Treated Barley seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	189.8	221.5	

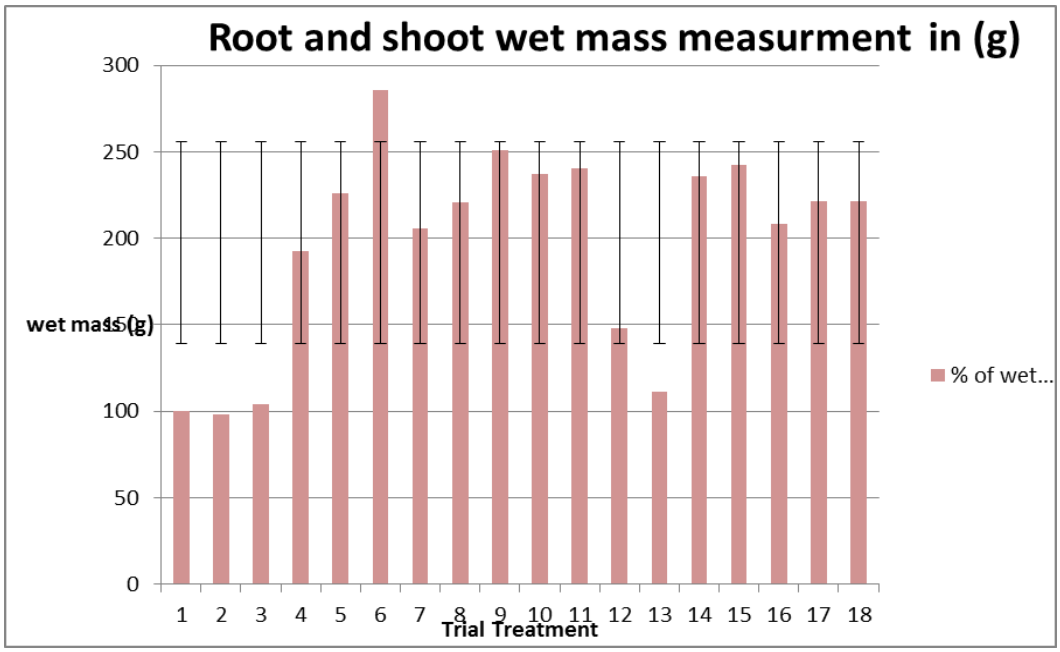


Figure 4.35: Measurements of root and shoot wet mass (g) for Barley plant trials after 30 days**.**

Wet mass measurements for root and shoot after 30 days are shown for trials of Barley seeds treated with UW3, UW4, and (UW3+ UW4) irrigated with 6000 mg/L of brackish water gave higher weights (224.2%, 220.9%, and237.3%) respectively compared with control Barley (98.4%.) subjected to the same salt concentration.

Also trials irrigated with 10000 mg/L of brackish water for trials treated with UW3, UW4, and (UW3+UW4) gave higher values: (285.73%, 251.23% and 148.29%) compared to control trial (104.3%).

These values indicated there is accumulation of salt happen into biomass of trials treated with PGPR, beside PGPR increased phytoremediation mechanisms and salt uptake into biomass, and increase stem –succulence compared to control treatment. Meanwhile control treatment effected by

salinity from brackish water lead to less accumulation of salt in biomass, and only tolerance mechanisms of plant play its role. [Mac neill, 2011].

Compared between combinations treated of trials compared to treat of trials with strains separately, especially trials irrigated with 10000 mg/L the combination didn't show significant results.

Trials include Barley seeds treated with UW3+ H_2O_2 and irrigated with 6000 mg/L of brackish water and with 10000 mg/L of brackish water gives value: (236.1%, 242.1%) compared to control (98.4%, 104.3%) and compared to trials treated with only UW3 irrigated with 6000 mg/L and 10000 mg/L of brackish water had values (220.9%, 251.2 %). These result indicated significant differences and there some contribution of hydrogen peroxide as antioxidant in participating in cell signaling, several nitrogen – activated protein kinase (MAPK) [Mac neill, 2011] this can be separated field study in future.

Trails treated only with H_2O_2 only there were no differences between the wet mas measurement may be this can be related only antioxidant role and tolerance mechanism.

Measurements of root and shoot wet mass (g) for Malt plant trials after 30 days are shown in Table 4.8 and Figure 4.36.

Table 4.8: Measurements of root and shoot wet mass (g) for Malt plant trials after 30 days.

Num	Treatment	Root + Shoot wet mass (g) After 30 days	% of wet mass	Significant value
1	Control Malt irrigated with fresh water	12.4	100	
2	Control Malt irrigated with 6000 mg/L of brackish water	10.04	81.2	
3	Control Malt irrigated with 10000 mg/L of brackish water	7.9	64.2	
4	Treated Malt seeds with UW3 irrigated with fresh water	29.8	240.7	
5	Treated Malt seeds with UW3 irrigated with 6000 mg/L of brackish water	30.6	247.0	
6	Treated Malt seeds with UW3 irrigated with 10000 mg/L of brackish water	31.6	255.8	Sig
7	Treated Malt seeds with UW4 irrigated with fresh water	30.2	244.4	
8	Treated Malt seeds with UW4 irrigated with 6000 mg/L of brackish water	26.2	212.1	
9	Treated Malt seeds with UW4 irrigated with 10000 mg/L of brackish water	34.5	114.1	
10	Treated Malt seeds with UW3 + UW4 irrigated with fresh water	29.2	236.4	
11	Treated Malt seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	32.1	259.4	
12	Treated Malt seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	35.6	288.1	Sig
13	Treated Malt seeds with UW3+H_2O_2 irrigated with fresh water	14.4	102.5	
14	Treated Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	15.7	127.1	
15	Treated Malt seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	14.9	121.1	
16	Treated Malt seeds with H_2O_2 irrigated with fresh water	12.7	103.1	
17	Treated Malt seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	14.3	115.8	Sig
18	Treated Malt seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	13.2	107.0	Sig

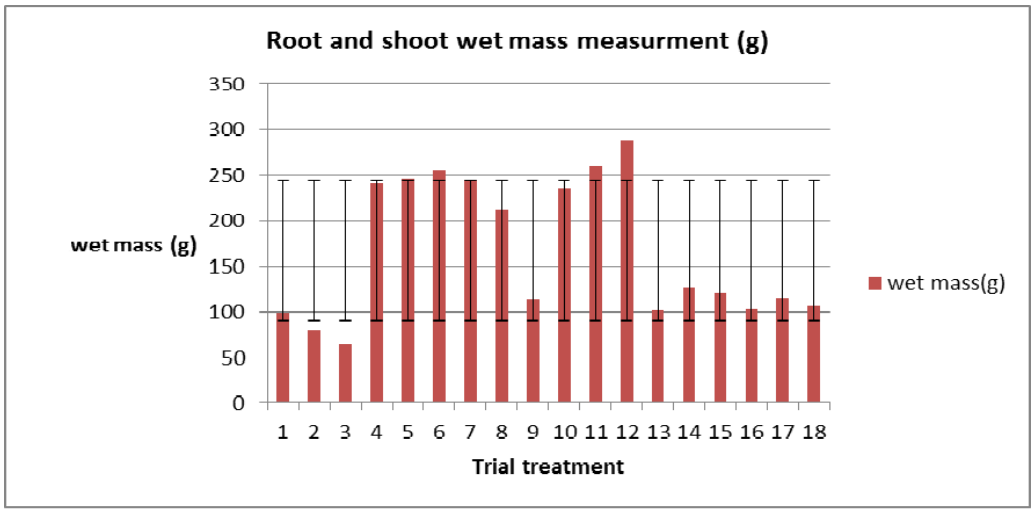


Figure 4.36: Measurements of root and shoot wet mass (g) for Malt Plant trials after 30 days.

Trials of Malt seeds treated with UW3, UW4 and UW3+UW4 irrigated with 6000 mg/L of brackish water (Table4.9 and Figure4.38) gave total biomass values as (247.1%, 212.1%, 259.4%) compared to control ones (81.16%).

Beside for trial of Malt seeds with UW3, UW4 and UW3+UW4 and irrigated with 10000 mg/L of brackish water were given (255.8%, 114.1%, 288.1%) compared to control Malt irrigated with 10000 mg/L of brackish water (64.2%).

It is noticed PGPR increased phytoremediation mechanisms, salt uptake into plant biomass. For those trials treated with PGPR the accumulation of

salt in biomass increase production of biomass compared to controls which were affected by salinity [Mac neill, 2011].

Moreover for trials irrigated with 10000 mg/L accumulated more salt inside biomass which were observed in weights than 6000 mg/L.

Compared between combinations trials and trial treated separately with strains, especially for one irrigated with 10000 mg/L, combination trials didn't show significant results.

Trials include Malt seeds treated with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water and with 10000 mg/L of brackish water gives value : (127.1%, 121.1%) compared to control and compared to trials treated with only UW3 irrigated with 6000 mg/L and 10000 mg/L.

These result indicated significant differences with some contribution of H_2O_2 as antioxidant in participating in cell signaling, (MAPK) [Mac neill, 2011] where this can be as a separated field study in future.

Trails treated only with H_2O_2 only there were no differences between the wet mas measurement may be this can be related only antioxidant role and tolerance mechanism.

For dry mass measurements included root and shoot dry mass (g) for Barley and Malt plant trials after 30 days separately. Differences between measurements were included in order to calculate how much water absorbed by tissue.

Measurements of root and shoot dry mass (g) for Barley plant trials after 30 days, and difference between dry and wet mass are shown in Tables (4.9, 4.10), Figure (4.37, 4.38).

Table 4.9: Measurements of root and shoot dry mass (g) for Barley plant trials after 30 days.

Num	Treatment	Root dry mass (g)	Shoot dry mass (g)	Total dry mass	% of dry mass	Significant value
1	Control Barley irrigated with fresh water	40.5	35.6	76.1	100	
2	Control Barley irrigated with 6000 mg/L of brackish water	6.1	0.713	6.8	8.98	
3	Control Barley irrigated with 10000 mg/L of brackish water	9.3	0.923	10.2	150.08	Sig
4	Treated Barley seeds with UW3 irrigated with fresh water	35.6	83.2	118.8	156.11	
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	93.4	87.2	180.6	237.31	
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	120.5	69.3	189.8	249.40	Sig
7	Treated Barley seeds with UW4 irrigated with fresh water	45.6	73.2	118.8	156.11	
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	103.4	77.2	180.6	237.31	
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	140.5	79.3	219.8	288.83	Sig
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	34.3	63.2	97.5	128.12	
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	115.4	88.3	203.7	267.67	
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	142.3	77.3	219.6	288.56	Sig
13	Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water	45.6	43.2	88.8	116.68	
14	Treated Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	93.4	87.2	180.6	237.31	
15	Treated Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	120.5	69.3	189.8	249.40	Sig
16	Treated Barley seeds with H_2O_2 irrigated with fresh water	43.5	25.6	69.1	90.80	Sig
17	Treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	7.1	0.613	7.735	10.16	
18	Treated Barley seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	62.3	0.892	63.192	83.03	Sig

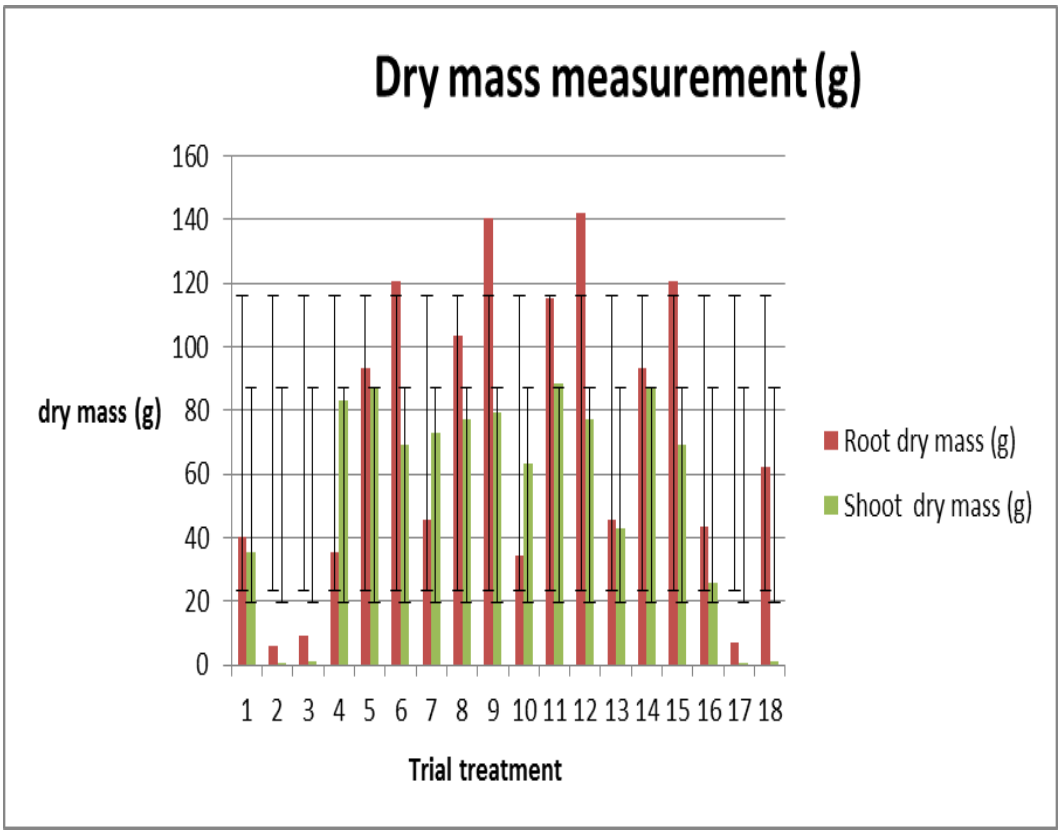


Figure 4.37: Measurement of root and shoot dry mass (g) for Barley plant trials after 30 days.

Table 4.10: Differences between wet biomass and dry biomass for Barley plant trials.

Num	Treatment	Total Dry mass	Total wet \mass	Difference (wet-dry)	Significant
1	Control Barley irrigated with fresh water	76.1	85.7	9.6	
2	Control Barley irrigated with 6000 mg/L of brackish water	6.8	84.3	77.4	
3	Control Barley irrigated with 10000 mg/L of brackish water	10.3	89.4	79.1	Sig
4	Treated Barley seeds with UW3 irrigated with fresh water	118.8	165.3	46.5	
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	180.6	193.8	13.2	
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	189.8	240.8	51.07	Sig
7	Treated Barley seeds with UW4 irrigated with fresh water	118.8	176.2	57.4	Sig
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	180.6	189.3	8.7	
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	219.8	215.3	4.5	
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	97.5	203.4	105.9	Sig
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	203.7	206.3	2.6	
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	219.6	280.8	61.2	
13	Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water	88.8	95.3	6.5	
14	Treated Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	180.6	202.3	21.7	Sig
15	Treated Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	189.8	207.5	17.7	
16	Treated Barley seeds with H_2O_2 irrigated with fresh water	69.1	178.6	109.5	
17	Treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	7.7	189.6	181.8	Sig
18	Treated Barley seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	63.1	189.8	126.6	

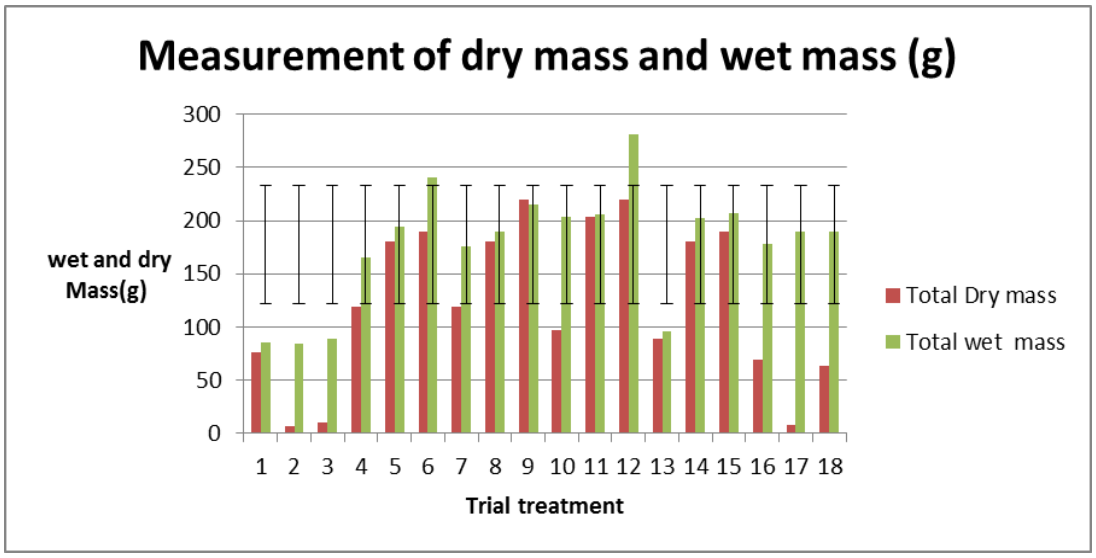


Figure 4.38: Differences between wet biomass and dry biomass for Barley plant trials.

Dry biomass measurement shown (Table 4.10, Figure 4.38 and Figure 4.39) for Barley seeds treated with UW3, UW4 and UW3+ UW4 irrigated with 6000 mg/L of brackish water were: (237.3 %, 237.3%, and 267.7%) compared to control (9.0%).

There were large differences between measurements for those trials with PGPR related to trials without PGPR, furthermore there were increase in root and shoot dry biomass.

Measurement of trials of Barley seeds with UW3, UW4 and UW3+UW4 irrigated with 10000 mg/L of brackish water were: (249.4 %, 288.8%, and 288.6%), compared to control Barley irrigated with 10000 mg/L of brackish water (150.1 %).

Trial with PGPR promote plant more control mechanisms over others trials without PGPR in compartmentalization of salt into vacuoles, synthesis of osomLytes and exclusion of salts ions by roots, promote plant growth to complete their life cycle under stressed condition [Mac neill, 2011].

Trial of Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water its equal (237.3 %), compared to control trial. trials treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water (10.2%), this mean there was accumulation of salts inside biomass and some contribution of hydrogen peroxide as antioxidant in participating in cell signaling, (MAPK) [Mac neill, 2011] and this can be separated field study in future.

Same for measurement of trials of Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water was equal to (249.4%) compared to control, meanwhile for trial with only H_2O_2 its value was (83.0%) which was closed to control one.

These measurements showed that salinity inhibit plant growth for control trials. There was decrease in shoot thickness which attributed to reduced plant cell intercellular space. Less chlorophyll content relative to one treated with PGPR ad one irrigated with fresh water.

Measurements of trials treated with PGPR indicate that ACC deaminase producing by PGPR oxidize ACC to ammonia and α-ketobutyrate. Hence these compounds promote plant growth and lower concentration of ethylene hormone increase plant growth. Furthermore PGPR synthesized IAA compound which stimulate plant growth promotion, which was obvious for trials this study for irrigated with 6000 mg/L and 10000 mg/L [Shan, 2009].

Measurements of root and shoot dry mass (g) for Malt plant trials after 30 days and difference between dry mass and wet mass are shown in Tables (4.11, 4.12) and Figure (4.39, 4.40).

Table 4.11: Measurements of root and shoot dry mass (g) for Malt plant trials after 30 days.

Num	Treatment	Root dry mass (g)	Shoot dry mass (g)	Total Dry mass	% of dry mass	Significant
1	Control Malt irrigated with fresh water	1.234	0.453	1.687	100	
2	Control Malt irrigated with 6000 mg/L of brackish water	0.564	0.311	0.875	51.86	
3	Control Malt irrigated with 10000 mg/L of brackish water	0.234	0.136	0.37	42.28	
4	Treated Malt seeds with UW3 irrigated with fresh water	1.354	0.722	2.076	561.08	Sig
5	Treated Malt seeds with UW3 irrigated with 6000 mg/L of brackish water	2.541	0.731	3.272	157.61	Sig
6	Treated Malt seeds with UW3 irrigated with 10000 mg/L of brackish water	2.785	0.624	3.409	104.18	
7	Treated Malt seeds with UW4 irrigated with fresh water	1.674	0.534	2.208	64.76	
8	Treated Malt seeds with UW4 irrigated with 6000 mg/L of brackish water	1.985	0.604	2.589	117.26	Sig
9	Treated Malt seeds with UW4 irrigated with 10000 mg/L of brackish water	1.967	0.957	2.924	112.94	
10	Treated Malt seeds with UW3 + UW4 irrigated with fresh water	2.497	0.935	3.432	117.37	Sig
11	Treated Malt seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	2.567	0.856	3.423	99.74	
12	Treated Malt seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	1.785	0.277	2.062	60.24	
13	Treated Malt seeds with UW3+H_2O_2 irrigated with fresh water	0.567	0.144	0.711	34.48	
14	Treated Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	0.854	0.670	1.524	214.34	Sig

15	Treated Malt seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	0.875	0.547	1.422	93.31	
16	Treated Malt seeds with H_2O_2 irrigated with fresh water	0.452	0.164	0.616	43.33	
17	Treated Malt seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	0.324	0.054	0.378	61.36	
18	Treated Malt seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	0.275	0.264	0.539	142.59	Sig

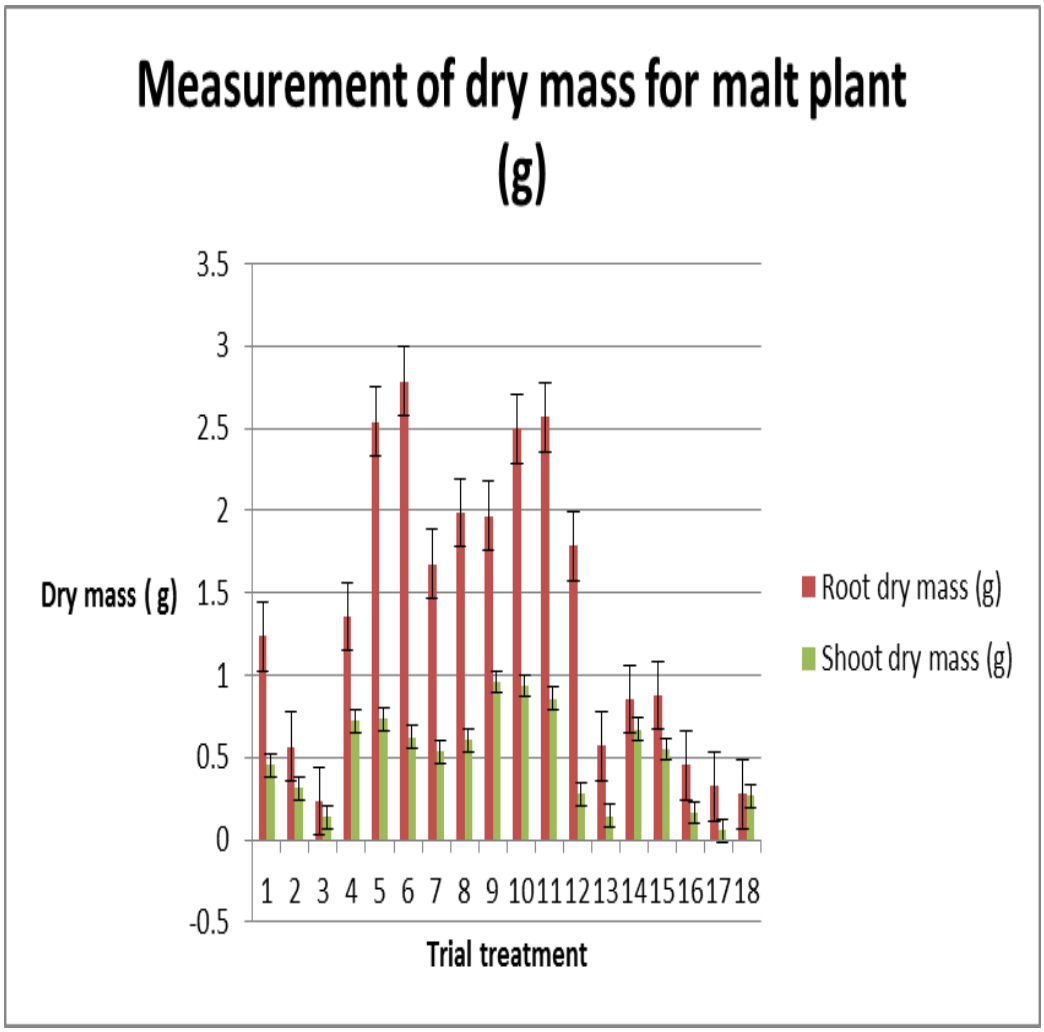


Figure 4.39: Measurements of root and shoot dry mass (g) for Malt plant trials after 30 days.

Table 4.12: Differences between wet biomass and dry biomass for Malt plant trials

Treatment	Total Dry mass(g)	Total wet mass(g)	Difference (wet-dry)	Significant value
Control Malt irrigated with fresh water	1.68	12.37	10.68	Sig
Control Malt irrigated with 6000 mg/L of brackish water	0.87	10.04	9.165	
Control Malt irrigated with 10000 mg/L of brackish water	0.37	7.94	7.57	
Treated Malt seeds with UW3 irrigated with fresh water	2.07	29.78	27.70	
Treated Malt seeds with UW3 irrigated with 6000 mg/L of brackish water	3.27	30.56	27.28	
Treated Malt seeds with UW3 irrigated with 10000 mg/L of brackish water	3.41	31.64	28.23	Sig
Treated Malt seeds with UW4 irrigated with fresh water	2.21	30.23	28.02	Sig
Treated Malt seeds with UW4 irrigated with 6000 mg/L of brackish water	2.58	26.24	23.65	
Treated Malt seeds with UW4 irrigated with 10000 mg/L of brackish water	2.92	34.50	31.57	Sig
Treated Malt seeds with UW3 + UW4 irrigated with fresh water	3.43	29.24	25.81	
Treated Malt seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	3.42	32.09	28.66	
Treated Malt seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	2.06	35.64	33.57	Sig
Treated Malt seeds with UW3+H_2O_2 irrigated with fresh water	0.71	14.35	13.63	
Treated Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	1.52	15.72	14.19	Sig
Treated Malt seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	1.42	14.98	13.55	
Treated Malt seeds with H_2O_2 irrigated with fresh water	0.62	12.75	12.13	
Treated Malt seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	0.38	14.32	13.94	Sig
Treated Malt seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	0.54	13.24	12.70	

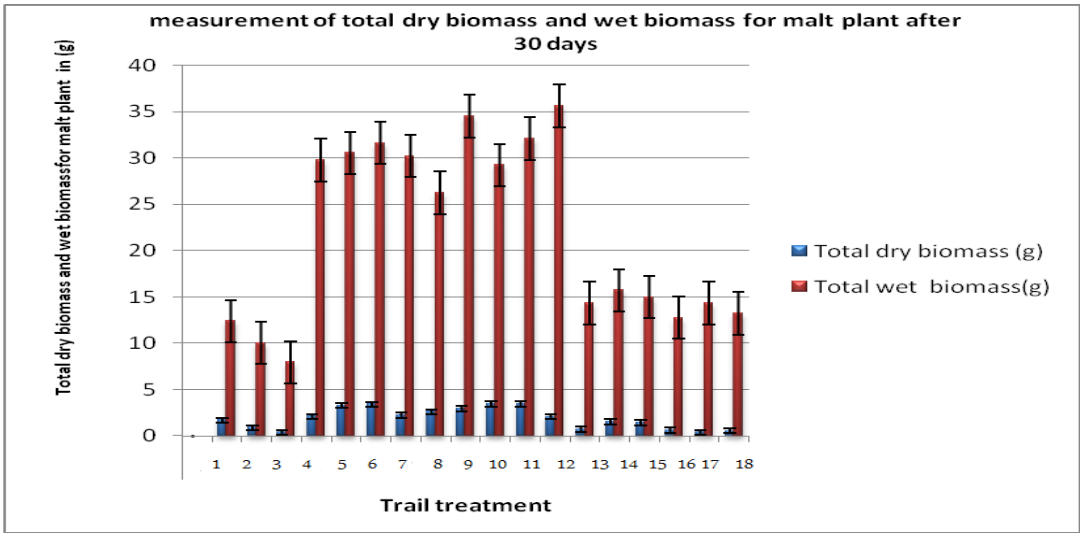


Figure 4.40: Differences between wet biomass and dry biomass for Malt plant trials.

Malt plant seeds as in (Table 4.12, Figure 4.39 and Figure 4.40) treated with UW3, UW4 and UW3+ UW4 irrigated with 6000 mg/L of brackish water were: (157.6 %, 117.3 %, and 99.7%) compared to control (51.9%).

Measurements of Malt plant trial treated with UW3, UW4 and UW3+UW4 irrigated with 10000 mg/L of brackish water were: (104.2%; 112.9%; 60.2%) compared (42.3%).

Measurements for trials treated Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water and 10000 mg/L of brackish water were: (14.2 %, 13.6 %) compared to control the values were closed to it.

Trials of Malt seeds with H_2O_2 irrigated with 6000 mg/L of brackish water, and 10000 mg/L of brackish water were: (13.9 %, 12.7%).

Final Measurements include measurement lengths for shoot (cm) after 14 days, 30 days and for root lengths (cm) for Barley plant after 30 days as shown in Tables 4.13 and Figure 4.41.

Table 4.13: Measurements of Barley plant lengths for shoot (cm) after 14 days and 30 days and root lengths (cm) after 30 days.

Barley Plant num	Treatment	Length of Shoot after 14 days	Length of Shoot after 30 days	Length of root after 30 days	Significant value
1	Control Barley irrigated with fresh water	2-3 cm	6-9 cm	13-15 cm	Sig
2	Control Barley irrigated with 6000 mg/L of brackish water	3-4 cm	5-7 cm	10-13 cm	
3	Control Barley irrigated with 10000 mg/L of brackish water	4-5 cm	5-8 cm	11-13 cm	
4	Treated Barley seeds with UW3 irrigated with fresh water	3-4 cm	7-9 cm	20-23 cm	
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	6-9 cm	10-13 cm	27-29 cm	Sig
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	7-11 cm	11-13 cm	29-32 cm	Sig
7	Treated Barley seeds with UW4 irrigated with fresh water	2-4 cm	7-9 cm	21-25 cm	
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	7-12 cm	11-14 cm	26-30 cm	Sig
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	9-13 cm	12-14 cm	27-32 cm	Sig
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	6-11 cm	9-10 cm	23-26 cm	
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	11-15 cm	11-15 cm	29-32 cm	Sig
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	11-16 cm	12-15 cm	30-36 cm	Sig
13	Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water	6-9 cm	8-10 cm	19-24 cm	
14	Treated Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	7-9 cm	12-13 cm	25-28 cm	Sig
15	Treated Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	8-12 cm	11-14 cm	27-29 cm	Sig
16	Treated Barley seeds with H_2O_2 irrigated with fresh water	4-5 cm	6-8 cm	9-13 cm	
17	Treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	5-8 cm	9-11 cm	15-17 cm	Sig
18	Treated Barley seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	4-7 cm	10-12 cm	14-16 cm	

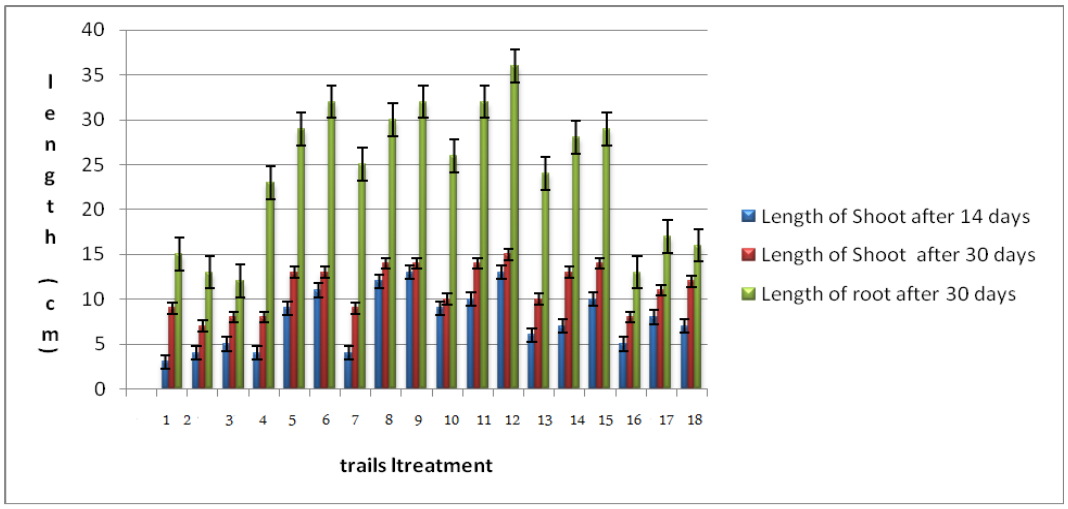


Figure 4.41: Measurements of Barley plant. Lengths for shoot (cm) after 14 days and 30 days and root lengths (cm) after 30 days.

Lengths of root and shoots had been shown in Table 4.13 and Figure 4.41; PGPR contributed to increase lengths for Barley and Malt plants shoots and roots more than controls. The measurement of lengths were more for trials treated with PGPR and irrigated with brackish water compared to trials irrigated with fresh water this lead to PGPR promote vigorous growth for both plants under salt stress.

Beside it was noticed PGPR under high concentration of salt, it enhanced plant growth promotion for roots and shoots to overcome stress, even between individual trials treated with different concentration of water concentration it was noticed that lengths for root and shoot were significant in measurement more than other [Shan, 2009].

For trials treated with UW3 +UW4 compared to trials treated separately with strains, differences in lengths were significant. This indicates performance of trials with both strains show high effective to tolerate to

salinity and same performance of photosynthetic, as trials treated separately.

Table 4.14 and Figure 4.42 include measurements for Malt plant lengths for shoot (cm) and root (cm) after 14 days and after 30 days.

Table 4.14: Measurements of Malt plant lengths for shoot (cm) after 14 days and 30 days and root lengths (cm) after 30 days.

Malt plant Num	Treatment	Length of Shoot after 14 days	Length of Shoot after 30 days	Length of root after 30 days	Significant Value
1	Control Malt irrigated with fresh water	1- 3 cm	1- 2 cm	1- 3 cm	
2	Control Malt irrigated with 6000 mg/L of brackish water	1- 2 cm	2- 4 cm	1- 2 cm	
3	Control Malt irrigated with 10000 mg/L of brackish water	2-3 cm	2-4 cm	4- 5 cm	
4	Treated Malt seeds with UW3 irrigated with fresh water	2-3 cm	3- 5 cm	4- 5 cm	
5	Treated Malt seeds with UW3 irrigated with 6000 mg/L of brackish water	2-4 cm	2- 4 cm	5- 6 cm	Sig
6	Treated Malt seeds with UW3 irrigated with 10000 mg/L of brackish water	3-4 cm	2-3 cm	5-7 cm	Sig
7	Treated Malt seeds with UW4 irrigated with fresh water	1- 2 cm	1- 3 cm	3- 4 cm	
8	Treated Malt seeds with UW4 irrigated with 6000 mg/L of brackish water	2- 4 cm	2- 4 cm	4- 6 cm	Sig
9	Treated Malt seeds with UW4 irrigated with 10000 mg/L of brackish water	3-4 cm	3-5 cm	2-3 cm	
10	Treated Malt seeds with UW3 + UW4 irrigated with fresh water	1- 3cm	2- 3 cm	1- 3 cm	
12	Treated Malt seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	2-3 cm	1- 2 cm	1- 2 cm	
13	Treated Malt seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	2-4 cm	2-3 cm	2-3 cm	Sig
14	Treated Malt seeds with$UW3+H_2O_2$ irrigated with fresh water	1- 2 cm	1- 3 cm	1- 3 cm	
15	Treated Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	2- 3 cm	1- 2 cm	3- 5 cm	
16	Treated Malt seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	2-4 cm	2-3 cm	3-4 cm	
17	Treated Malt seeds with H_2O_2 irrigated with fresh water	1- 2 cm	1- 3 cm	2- 6 cm	
18	Treated Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	1-3 cm	2- 4 cm	3- 5 cm	
19	Treated Malt seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	2-3 cm	1-4 cm	2-3 cm	

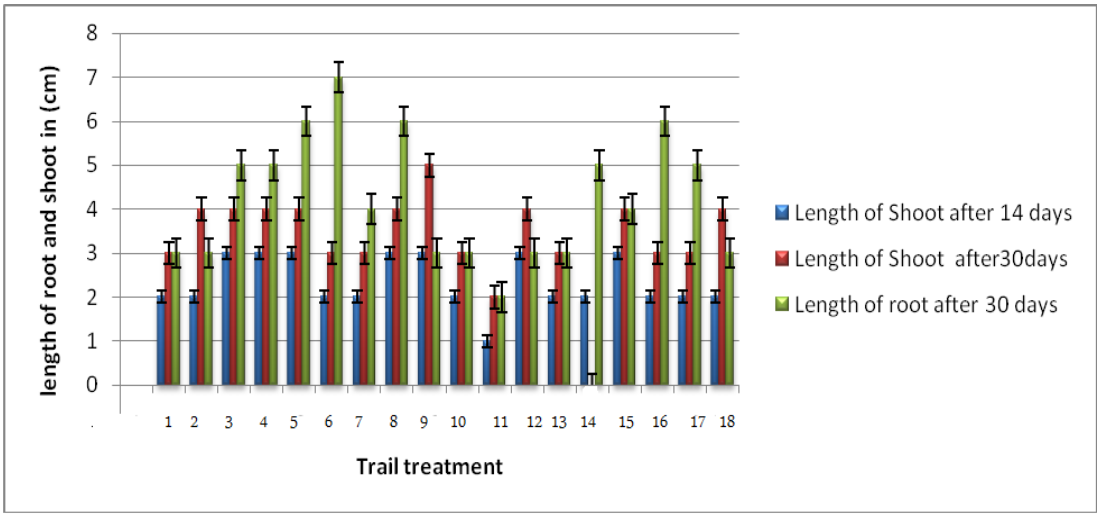


Figure 4.42: Measurements of Malt plant lengths of shoot (cm) after 14 days and 30 days and root lengths (cm) after 30 days.

Lengths of root and shoots had been shown in Table 4.14 and Figure 4.41 and Figure 4.42; where PGPR contributed to increase lengths for Barley and Malt plants shoots and roots more than controls. The measurement of lengths were more for trials treated with PGPR irrigated with brackish water compared to trials irrigated with fresh water this mean PGPR promote vigorous growth for both plants under salt stress .

For trials treated with UW3 +UW4 compared to trials treated with strain separately, difference was not significant higher especially for 10000 mg/L. This indicates performance of trials with both strains show same effective to tolerate to salinity as trials treated separately.

Followed pictures represent photos for some trials for comparing between them in visual differences:

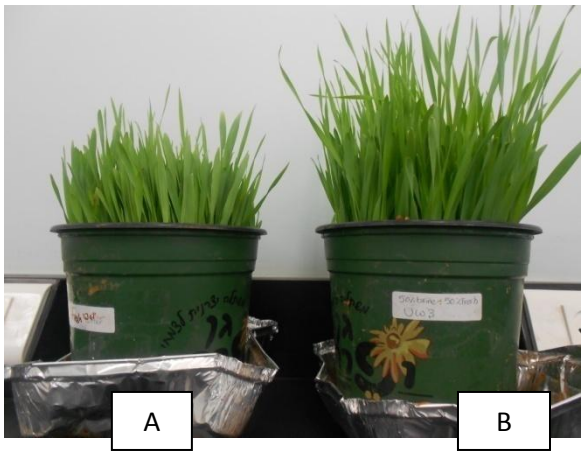


Figure 4.43: (A) represents trial of control Barley plant irrigated with fresh water, (B) represents trials of treatment of Barley plant with UW3+UW4 irrigated with 6000 mg/L.

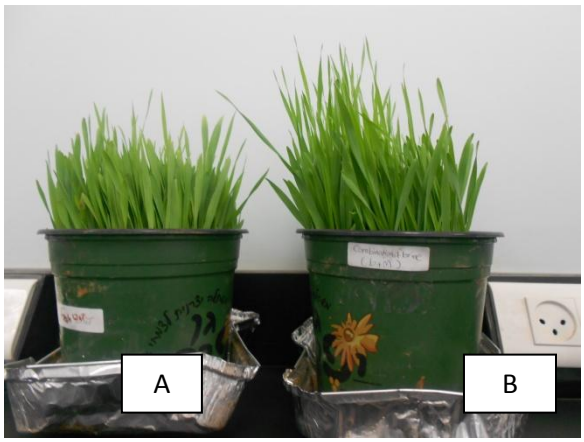


Figure 4.44: (A) represents trial of control Barley plant irrigated with fresh water, (B) represents trials of treatment of Barley plant with UW3 irrigated with 6000 mg/L.

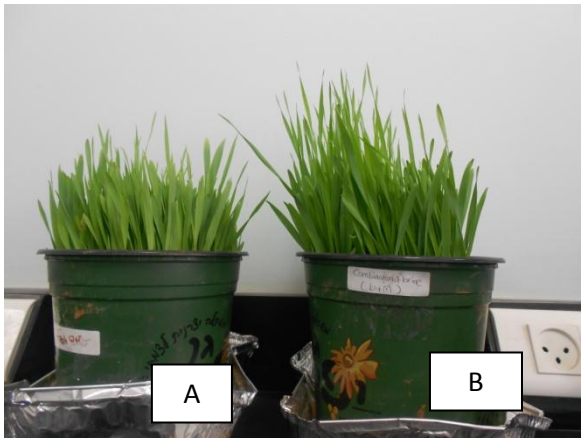


Figure 4.45: (A) represents trial of control Barley plant irrigated with fresh water, (B) represents trials of treatment of Barley plant with UW4 irrigated with 6000 mg/L.

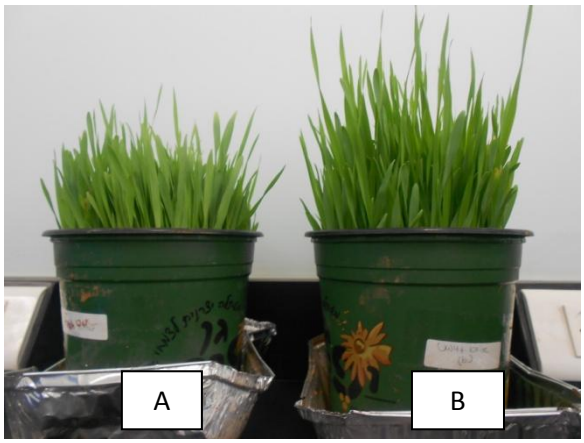


Figure 4.46: (A) represents trial of control Barley plant irrigated with fresh water, (B) represents trials of treatment of Barley plant with UW3+UW4 irrigated with 10000 mg/L.

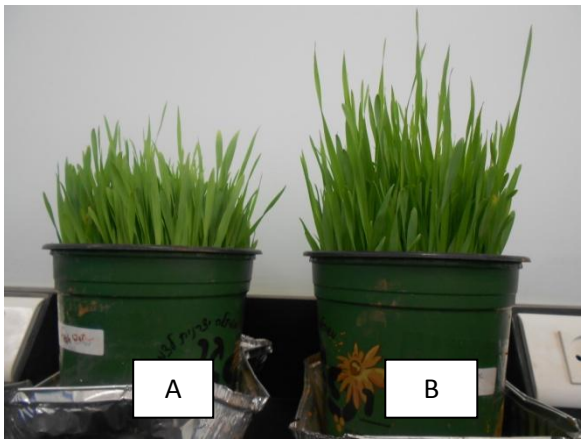


Figure 4.47: (A) represents trial of control Barley plant irrigated with fresh water, (B) represents trials of treatment of Barley plant with UW3 irrigated with 10000 mg/L.

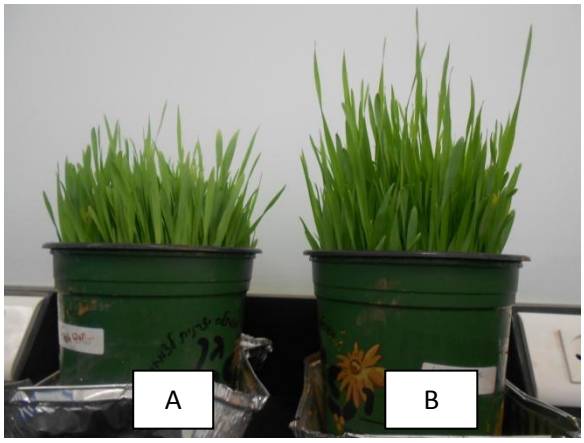


Figure 4.48: (A) represents trial of control Barley plant irrigated with fresh water, (B) represents trials of treatment of Barley plant with UW4 irrigated with 10000 mg/L.

Figures 4.43-4.48 showed leaves of Barley plants treated with PGPR as taller thicker and green darker color compared to untreated ones, besides their roots were longer compared to untreated plants. Thus, PGPR affected photosynthetic activity even under irrigation with salt solution.

For control trials without PGPR irrigated with two different concentration of brackish water; the colors of their leaves were visibly pale green. Some leaves turned to yellow and shorter -smaller. Some followed by premature necrosis, even they reached their growth cycle end before crop coefficient.

To distinguish between plants species which one responded more to bacteria strain .T-test applied to it, where Barley and Malt plant consider tolerant species to salty conditions, but response of Barley plant to these microbes were more than Malt according to T-test in Table 4.15. This attributed could be due to large surface area for Barley seeds that has compared to Malt seeds, more bacteria strains have been adhesion to surface of Barley seeds. Another reason may be related to some specie – specific differences in physiology and anatomy as well as specific differences in conditions required for optimal growth for Malt plant differ from Barley plant. These indicate Malt plant may need different PGPR strains other than those UW3, UW4 for their optimal growth condition.

Table 4.15: T-test to distinguish between Barley plant and Malt plant responses to bacteria.

Group Statistics

	Plant	N	Mean	Std. Deviation	Std. Error Mean
Mass	Barely	12	126.3	84.00	24.24
	Malt	12	1.6	.800	.23

4.6. Salt accumulation in plant:

Salt accumulation test was used in this study to determine effectiveness of phytoextraction mechanism of tested plants, it was used to determine the amount of salt ions have been eliminated from brackish water. This method was carried out trials by taking roots and shoots of plants for all trials are shown in Table 4.16.

Table4.16: Salt accumulation measurement of Na and Cl ions in (mg/g of dry weight) for Barley plant shoots tissue.

#	Treatment	Na (mg/g dry weight)	Cl (mg/g dry weight)	NaCl (mg/g dry weight)	Total Dry mass(g)	weight total ion in total dry mass (mg)/0.114m^2 of pot	Concentration of mmol / 0.114m^2 of pot	Ratio of Cl/Na	Significant Value
1	Control Barley plant irrigated with fresh water	0.659	0.457	1.116	76.1	84.926	1.826	0.693	
2	Control Barley plant irrigated with 6000 mg/L of brackish water	0.956	0.975	1.931	6.835	13.198	0.283	1.019	Sig
3	Control Barley plant irrigated with 10000 mg/L of brackish water	1.974	1.564	3.538	10.258	36.292	0.780	0.792	
4	Treated Barley plant with UW3 irrigated with fresh water	2.378	1.326	3.704	118.8	440.035	9.4631	0.557	
5	Treated Barley plant with UW3 irrigated with 6000 mg/L of brackish water	7.666	5.524	13.19	180.6	2382.114	51.228	0.720	
6	Treated Barley plant with UW3 irrigated with 10000 mg/L of brackish water	23.65	15.324	38.978	189.8	7398.024	159.097	0.647	Sig
7	Treated Barley plant with UW4 irrigated with fresh water	3.475	2.436	5.911	118.8	702.226	15.101	0.601	
8	Treated Barley plant with UW4 irrigated with 6000 mg/L of brackish water	6.146	4.223	10.369	180.6	1872.641	40.271	0.787	Sig

9	Treated Barley plant with UW4 irrigated with 10000 mg/L of brackish water	26.81	11.014	37.828	219.8	8314.594	178.808	0.910	Sig
10	Treated Barley plant with UW3 + UW4 irrigated with fresh water	3.008	0.786	3.794	97.5	369.915	7.9551	0.261	
11	Treated Barley plant with UW3+ UW4 irrigated with 6000 mg/L of brackish water	9.147	3.020	12.167	203.7	2478.417	53.299	0.330	Sig
12	Treated Barley plant with UW3+UW4 irrigated with 10000 mg/L of brackish water	28.05	10.004	38.058	219.6	8357.536	179.731	0.356	Sig
13	Treated Barley plant with UW3+H_2O_2 irrigated with fresh water	2.078	1.341	3.419	88.8	303.607	6.529	0.645	
14	Treated Barley plant with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	5.457	3.224	8.681	180.6	1567.788	33.715	0.690	Sig
15	Treated Barley plant with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	18.35	7.972	26.327	189.80	4996.864	107.459	0.434	Sig
16	Treated Barley plant with H_2O_2 irrigated with fresh water	2.378	1.326	3.704	69.100	255.946	5.504	0.557	Sig
17	Treated Barley plant with H_2O_2 irrigated with 6000 mg/L of brackish water	2.765	1.524	4.289	7.735	33.175	0.713	0.551	
18	Treated Barley plant with H_2O_2 irrigated with 10000 mg/L of brackish water	4.954	2.324	7.278	63.192	459.911	9.890	0.469	

For weights of salt accumulation of Na/ ICl ions (mg/g dry weight) compared to theoretical weight are shown in Figure 4.49.

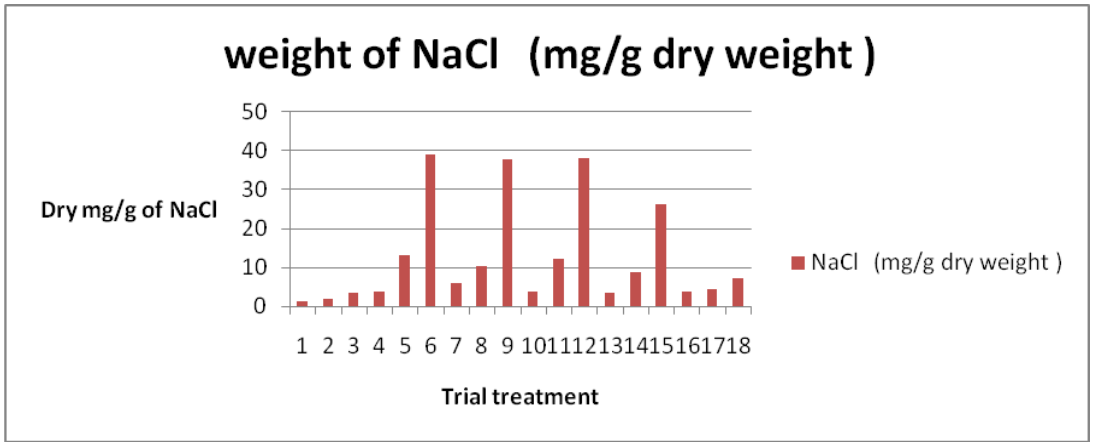


Figure 4.49: Measurements of salt accumulation of Na Cl ions (mg/g dry weight) in Barley plant shoot tissue.

Plant shoot tissue that analyzed for ion accumulation (Table4.16 and Figure4.49) showed total ion weight in total dry mass (g) for Barley seeds treated with UW3, trial of Barley seeds treated with UW4 and UW3 + UW4 and irrigated with 6000 mg/L of brackish water were (2382.1 mg, 1872.6 mg and 2478.4 mg) compared to control (13.2 mg).

Measurements for trial of Barley seeds treated with UW3, trial of Barley seeds with UW4 and UW3+ UW4 irrigated with 10000 mg/L of brackish water (7398.0 mg, 8314.6 mg, and 8357.5 mg) compared to control Barley irrigated with 10000 mg/L of brackish water (36.3 mg).

Measurements for trials of Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water and irrigated with 10000 mg/L of brackish water (1567.8 mg, 4996.9 mg) compared to control Barley irrigated with

6000 mg/L of brackish water (13.2 mg) and control Barley irrigated with 10000 mg/L of brackish water (36.3 mg)

These measurements indicate PGPR enhance for more salt accumulation inside plant where salt didn't stay in soil to cause any salinity for it and result of salt accumulation were more for trail treated with PGPR over untreated trials [mac neill, 2011].

NaCl accumulation in plant tissue for total dry mass ranged from 36.3-8357.5 mg and for ratio of Cl/Na 0.6-1.01 for experimental results compared to theoretical atomic weight equal 1.5. These results indicate that accumulations of Cl^- ions in plant tissue were uneven where Na^+ accumulations were greater than Cl; suggesting that plant utilizes more Cl^- for their biosynthesis [Shan, 2009].

Moreover, these concentrations of salt don't effect to use these plants as forage food for animals, when compared with theoretical ratio.

4.7 Assessment of plant cell membrane stability using the electrolyte leakage methods.

This method describes assessing membrane permeability in relation to salt stress. In this study increase in salt affect plant membrane permeability where measurement of electrolyte leakage methods as TDS in mg/L in Barley plant root tissue trials is shown in Table 4.17 and Figure 4.50.

Table4.17: Measurements of electrolyte leakage methods as TDS mg/L in Barley plant root tissue trials.

Num	Treatment	TDS mg/L	Significant result
1	Control Barley irrigated with fresh water	304	Sig
2	Control Barley irrigated with 6000 mg/L of brackish water	503	--
3	Control Barley irrigated with 10000 mg/L of brackish water	754	--
4	Treated Barley seeds with UW3 irrigated with fresh water	302	Sig
5	Treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water	302	Sig
6	Treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water	513	Sig
7	Treated Barley seeds with UW4 irrigated with fresh water	104	Sig
8	Treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water	303	Sig
9	Treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water	554	Sig
10	Treated Barley seeds with UW3 + UW4 irrigated with fresh water	202	Sig
11	Treated Barley seeds with UW3+ UW4 irrigated with 6000 mg/L of brackish water	302	Sig
12	Treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water	513	Sig
13	Treated Barley seeds with UW3+H_2O_2 irrigated with fresh water	204	Sig
14	Treated Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L of brackish water	323	Sig
15	Treated Barley seeds with UW3+ H_2O_2 irrigated with 10000 mg/L of brackish water	524	Sig
16	Treated Barley seeds with H_2O_2 irrigated with fresh water	202	Sig
17	Treated Barley seeds with H_2O_2 irrigated with 6000 mg/L of brackish water	502	--
18	Treated Barley seeds with H_2O_2 irrigated with 10000 mg/L of brackish water	813	--

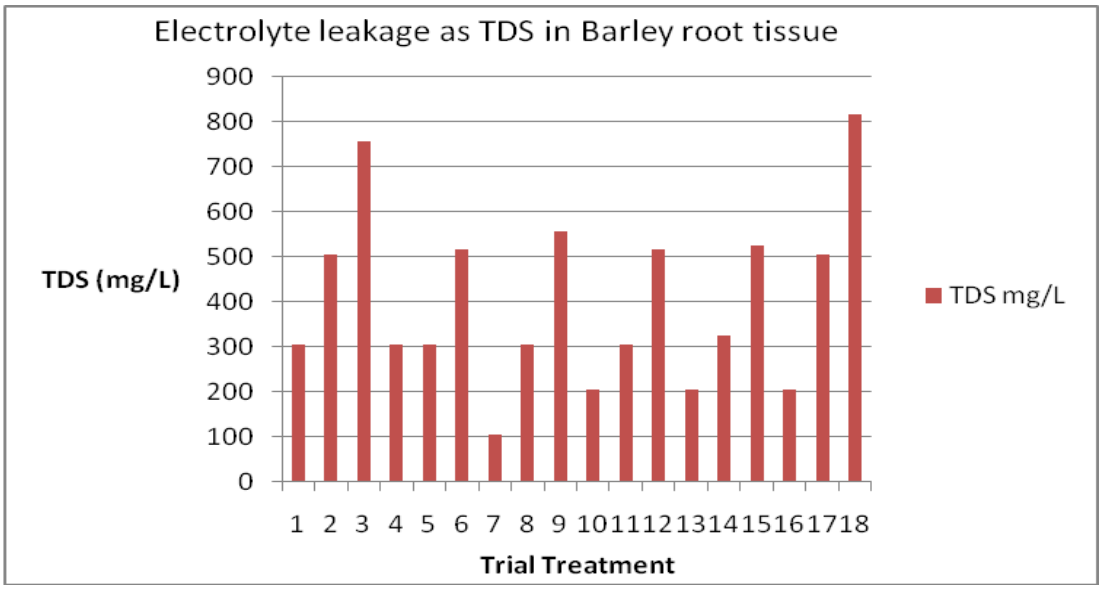


Figure4.50: Measurements of electrolyte leakage methods as TDS in mg/L in Barley plant root tissue trials.

This experiment was performed using Barley plants for all trials and TDS measured as shown in Table4.17 and Figure4.50. The measurements of ion leakage plant tissue are a method for assessing membrane permeability in relation to salt stress. In this study increase in salts affect plant membrane permeability as indicated by higher ion leakage.

Results revealed that salinity had increased the amount of electrolyte leakage from plant cell membrane in general for control trials and one treated only with H_2O_2 and salinity made cell membrane more permeable which observed in results compared to control fresh water, even though plant cell membranes in trials treated with PGPRs, were found having less electron leakage, compared to control one treated irrigated with brackish water. In this tale, implicate PGPR in protection of plant cell membranes were possible by promoting synthesis of lipids that considered as structural constituents of most of cellular membrane [Shan, 2009].

Conclusion:

1- Biomass measurements showed a significant mass increase for those plants treated with PGPRs compared with those control (untreated); which biomass production could enhance phytoremediation efficiency, as well as be used as forage food for animals.

2- Specifically, trials treated with PGPRs had showed significant improvements in salt accumulation for the plants (Barley and Malt) that used in these experiments, indicated that these two plants successfully can be used in phytoremediation process in combination of the PGPRs UW3 and/or UW4,with an advantage of Barley over Malt plant.

3- Results had showed that these PGPRs increase the cell membrane stability as demonstrated by less electrolyte leakage from plant cells relative to plants that were not treated with PGPR.

4- Results from PAM studies indicated plants treated with PGPR had increased photosynthesis rate thus prevented salinity damage to photosystems compared to those untreated ones.

.

Recommended future work:

The results of this research study are highly recommended to be implemented in area space field. In addition to that we highly recommend using other plant species with different strains of PGPRs and compare their responses to brackish water conditions, besides testing other strains combined with other plant species irrigated with different concentration of salts, beside investigating ability for human consumption such these crops .

Beside performance of PGPR can be studied for their high ability of producing more biomass within time.

References

1. Aard. (2007). Government report: **Salt tolerance of plants.** ***Alberta Agriculture and Rural Development*.**

2. Al Agha E.,et al. (2005). **Desalination in the Gaza Strip: drinking water supply and environmental impact**.

3. Alghoul,M.,et al. (2009). **Renewable and sustainable energy: reviews**.13:2661-2667.

4. Al-Karaki G. (2001). **Germination, sodium, and potassium concentrations of Barley seeds as influenced by salinity**. *Journal of Plant Nutrition* 24(3):511-522.

5. Alva, A., et al. (1991). Relationship **between ionic strength and electrical conductivity**. *Soil Science, 152*(4), 239-242.

6. Andersson B., Barber J. (1994). **Composition, organization and dynamics of thylakoid membranes.** *Advances in Molecular and Cell Biology* 10:1-53.

7. Apel K., Hirt H. (2011). **Reactive oxygen species: Metabolism oxidative stress and signal transduction**.53:373-399.

8. Apse M., et al. (1999). **Salt tolerance conferred by over expression of a vacuolarNa^+/H^+antiport in *Arabidopsis*.** *Science* 285:1256-1258.

9. Arnot T., et al. (2011).**A review of reverse osmosis membrane materials for desalination –development to date and suture potential**. *Journal of Membrane Science*.

10. Ashraf, M. (2004). **Some important physiological selection criteria for salt tolerance in plants**. *Flora, 199*(5), 361-376.

11. Assaf. (2004).**Water as human right: the understanding of water in Palestine.** ***Boell. de-k-assaf-Global Issues Papers.***

12. Babu T., et al. (2001). **Synergistic effects of a photo oxidized polycyclic aromatic hydrocarbon and copper on photo synthesis and plant growth: evidence that in vivo formation of reactive oxygen species is a mechanism of copper toxicity.** *Enviornmental Toxicology and Chemistry* 20:1351-1358.

13. Baker N. (2008). **Chlorophyll fluorescence: a probe of photosynthesis in vivo.** Annual Review of Plant Biology 59:89-113.

14. Bates L., et al. (1973). **Rapid determination of free proline for water stress studies.** *Plant and Soil* 39(1):205-207.

15. Beauregard M., et al. (1987). **Sulfate inhibition of photosystem II oxygen evolving complex**. *Applied Biochemistry and Biotechnology* 16:109-117.

16. Beer E. (2008). **Measuring rate of photosynthesis of two tropical sea grasses by pulse amplitude modulated fluorometry.** *Aquatic Botany*.

17. Belkhodja, R., et al. (1994). **Chlorophyll fluorescence as a possible tool for salinity tolerance screening in Barley (*Hordeum vulgareL*.).** *Plant Physiology, 104*(2), 667-673.

18. Bjrkman, O., Demming B. (1987). **Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins**. *Planta, 170*(4), 489-504.

19. Blaha, G., et al. (2000).**Preparation of functional ribosomal complexes and effect of buffer conditions on tRNA positions observed by cryoelectron microscopy**. *Methods in Enzymology, 317*, 292-309.

20. Blumwald E, Aharon G. (2000). **Sodium transport in plant cells.** *Biochimica et Biophysica Acta* 1465:140-151

21. Bohn, et al. (1985). **Soil chemistry (Second ed.).** *New York: John Wiley & Sons Inc*.

22. Brand-Williams, W., et al. (1995). **Use of a free radical method to evaluate antioxidant activity.** *Lebensmittel-Wissenschaft Und Technology/Food Science and Technology, 28*, 25-30.

23. Breckle S. (1995). **How do halophytes overcome salinity?** *Biology of Salt Tolerant Plants: Karachi*. p 199-213.

24. Breckle S. (1990). **Salinity tolerance of different halophyte types. In N. El Bassam, M.Dambroth & B. C. Loughman (Eds.),** *Genetic aspects of plant mineral nutrition* (pp. 167-175).

25. Burnett D.,Siddiqui M. (2006) .**Recovery of fresh water from desalination of brackish produced during oil and gas production operation**

26. Cakmak, I. (2000). **Possible roles of zinc in protecting plant cells from damage by reactive oxygen species.** *New Phytologist, 146*(2), 185-205.

27. Cakmak, I. (2005). **The role of potassium in alleviating detrimental effects of abiotic stresses in plants**. *Journal of Plant Nutrition and Soil Science, 168*, 521-530.

28. Carden D., et al. (2003). **Single-cell measurements of the contributions of cytosolic Na^+ and K^+ to salt tolerance**. *Plant Physiology* 131:676683

29. Chang P. (2007). **The use of Plant Growth Promoting Rhizobacteria (PGPR) and Anarbuscular Mycorrhizal Fungus (AMF) to improve plant growth in saline soils for phytoremediation**. *Waterloo: University of Waterloo*.

30. Chang, P. (2008). **Use of Plant Growth Promoting Rhizobacteria (PGPR) and an Arbuscular Mycorrhizal Fungus (AMF) to improve plant growth in saline soils for phytoremediation** .*Waterloo, Ont.: University of Waterloo*.

31. Chang W., Rui Y. (2005). **Review on brackish disposal from desalination plants.**

32. Cheng Z., Glick B. (2007). 1 **-aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt.** *Canadian Journal of Microbiology* 53(7):912-918.

33. Cheng, Z., et al. (2007). **1-aminocyclopropane-1-carboxylate deaminase from *pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt**. *Canadian Journal of Microbiology*, *53*, 912-918.

34. Cramer G. (2002). **Sodium-calcium interactions under salinity stress.** *Environment-Plants-Molecules. Netherlands: Kluwer Academic Publishers*. p 205-228.

35. Das A., Parida K. (2005). **Salt tolerance and salinity affect on plants a review.** *Ecotoxicology and Environmental Safety*. 60(3): 324–349.

36. Davenport R., Tester M. (2003).**Na^+ tolerance and Na^+ transport in higher plants.** *Annuals of Botany*. 91(5):503-527.

37. Environmental Sciences Division. (2001). **Salt contamination assessment & remediation guidelines**. *Alberta Environment.*

38. Executive summery for the strategic water sector plant in Palestine 2011-2013.

39. Flexas, J., et al. (2004). **Diffusive and metabolic limitations to photosynthesis under drought and salinity in C-3 plants.** *Plant Biology, 6*(3), 269-279.

40. Flowers, T. J., Yeo, A. R. (1988). **Ion relations of salt tolerance.** *New York: Longman Scientific & Technical*.

41. Frahy G., Schopfer P. (2001).**Release of reactive oxygen intermediates super oxide radical hydrogen peroxide and hydroxyl radicals and peroxidase in germinating radish seeds controlled by lights, gibbevellin and abscisic acid.** 25(4).

42. Gerhardt, K. E., et al. (2009). **Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges**. *Plant Science, 176*, 20-30.

43. Glenn, E.., Brown, J., Blumwald, E. (1999). **Salt tolerance and crop potential of halophytes.** *Critical Reviews in plant sciences, 18*(2), 227-255.

44. Glick B. (2004). **Bacterial ACC deaminase and the alleviation of plant stress.** *Advances in Applied Microbiology* 56:291-312.

45. Glick B. (1995). **The enhancement of plant-growth by free-living bacteria.** *Canadian Journal of Microbiology* 41(2):109-117.

46. Glick B., Bashan Y. (1997). **Genetic manipulation of plant growth promoting bacteria to enhance biocontrol of fungal phyto phathogens.** . *Biotechnology Advances* 15:353378.

47. Glick B., Penrose D. (1998). **A model for the lowering of plant ethylene concentrations by Plant Growth Promoting bacteria.** *Journal of Theoretical Biology* 190(1):63-68.

48. Handaly J., et al. (2005). **Impact of land of reject brackish water from desalination plants on soil and ground water.** *European Desalination Society.*

49. Hopkins, W. D. (1995). **Introduction to plant physiology**. New York: J. Wiley.

50. Huang X., et al. (2004). **A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils.** *Environmental Pollution* 130(3):465-476.

51. James R., et al. (2006). **Photosynthetic capacity is related to the cellular and sub cellular partitioning of Na^+, K^+ and Cl^- in salt-affected Barley and durum wheat.** *Plant Cell and Environment* 29(12):2185-2197.

52. Janzen H., Chang C. (1988). **Cation concentrations in the saturation** extract and soil solution extract of soil salinized with various sulfate salts. *Communications in Soil Science and Plant Analysis* 19(4):405-430.

53. Juneau P, Popovic R. (1999). **Evidence for the rapid phyto toxicity and environmental stress evaluation using the PAM fluorometric method: importance and future application.** *Ecotoxicology* 8(6):449-455.

54. Kamilova F., Lugtenberg B. (2009). **Plant Growth Promoting Rhizobacteria**. 63:541-556.

55. Karley AJ., et al. (2000). **Where do all the ions go? The cellular basis of differential ion accumulation in leaf cells.** *Trends in Plant Science* 5(11):465-470.

56. Kende H. (1993). **Ethylene biosynthesis**. *Annual Review of Plant Physiology and Plant Molecular Biology* 44:283-307.

57. Kirkhan M., Wahla I. (2008). **Heavy metal displacement in salt water irrigated during phytoremediation.** *Environmental Pollution Journal*. 155.

58. Krause G., Weis E. (1991). **Chlorophyll fluorescence and photosynthesis: the basics.** *Annual Review of Plant Physiology and Plant Molecular Biology* 42:313-349.

59. Lees H. (2005). **The effects of cadmium and 1, 2-ATQ on Reactive Oxygen Species (ROS) production, photosynthesis and gene expression in *Lemna gibba* (Duckweed).***Waterloo: University of Waterloo*.

60. Loah, L. (2007).**Plant responses to plant growth promoting rhizobacteria.**

61. Long S, Baker N. (1986). **Saline terrestrial environments. Photosynthesis in Contrasting Environments**. *New York: Elsevier*. p 63-102.

62. Mac neill, G. (2011). **Plant Growth Promoting Rhizobacteria enhanced phytoremediation of saline soils and salt uptake into plant biomass**. Waterloo University, Canada.

63. Malaeh, L. (2011). **Reverse Osmosis technology for water treatment. State of the art review**. *Desalination.*

64. Marie A.,Vengosh A. (2001). **Source of salinity in ground water from Jericho area, Jordan Valley**. *Wily Online Library-Ground water*.

65. Mayak S, et al. (2004). **Plant growth-promoting bacteria confer resistance in tomato plants to salt stress**. *Plant Physiology and Biochemistry* 42(6):565-572.

66. Mayak S, et al. (2004). **Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper.** *Plant Science* 166:525-530.

67. Meloni D., Oliva C. (2003). **Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress**. *Environmental and Experimental Botany* 49:69-76.

68. Miller, G., et al. (2010). **Reactive oxygen species homeostasis and signaling during drought and salinity stresses**. *Plant Cell and Environment*, *33*(4), 453-467.

69. Mittler R. (2002). **Oxidative stress, antioxidants and stress tolerance.** *Trends Plant Science* 7:405-410.

70. Munees A., Mulugeta K. (2014). **Mechanisms of application of plant growth promoting rhizobacteria: current perspective**. 26 (1): 1-20.

71. Munns R. (1993). **Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses.** *Plant, Cell and Environment* 16:15-24.

72. Munns R, Tester M. (2008). **Mechanisms of Salinity Tolerance**. *Annual Review of Plant Biology* 59:651-681.

73. Naidoo G, Somaru R. (2008). **Morphological and physiological responses of the halophyte**, *Odyssea paucinervis* (Staph) (Poaceae), to salinity. *Flora* 203:437-447.

74. Netondo G., et al. (2004). **Sorghum and salinity: I. response of growth, water relations, and ion accumulation to NaCl salinity**. *Crop Science* 44:797-805.

75. Niazi M., et al. (1991). **Salinity tolerance in different cultivars of Barley (*Hordeum vulgare* L.)**. *Biologia Plantarum* 34(5-6):465-469.

76. Northcote K. (1972). **Australian soils with saline and sodic properties**

77. Olesen K, Andreasson L-E. (2003). **The function of the chloride ion in photosynthetic oxygen evolution.** *Biochemistry* 42(7):2025-2035.

78. Palestinian Water Authority,2014.

79. Papageorgiou G. (2004). **Chlorophyll *a* fluorescence: a signature of photosynthesis**. *Advances in Photosynthesis and Respiration*: 43-63.

80. Parida A., Das A. (2005). **Salt tolerance and salinity effects on plants: a review**. *Ecotoxicology and Environmental Safety* 60:324 -349.

81. Penrose D., Glick B. (2003). **Methods for isolating and characterizing ACC deaminase containing plant growth-promoting rhizobacteria. Physiologia Plant arum** 118(1):1015.

82. Qadir M, et al. (2007). **Phytoremediation of sodicand saline-sodic soils**. *Advances in Agronomy*, Vol 96. P197-247.

83. Qadir M, et al. (1996). **Reclamation of a saline-sodic soil by gypsum and *Leptochloa fusca*.** *Geoderma* 74:207-217.

84. Ravishenkar G., Suresh B. (2004).**Phytoremediation a novel and promising approach for environmental cleanup.** *Central Food Technological Research institute, Indiana*. 24(2, 3):97-124.

85. Shan, S. (2009). **Enhanced phytoremediation of salt impacted soils using plant growth promoting rhizobacteria (PGPR).** Waterloo University, Canada.

86. Smits E. (2005).**Phytoremediation**. *Annual Review of Plant Biology* 56:15-39.

87. Su Y, et al. (2008). **Phytoextraction and accumulation of mercury in three plant species**: Indian mustard (*Brassica juncea*), beard grass (*Polypogon monospeliensis*), and Chinese brake fern (*Pteris vittata*). *International Journal of Phytoremediation* 10(6):547-560.

88. Tchobanoglous, et al. (2003). **Wastewater Engineering (Treatment Disposal Reuse)** / Metcalf & Eddy, Inc. (4th ed.). *McGraw-Hill Book Company*.

89. Tester, M., Davenport, R. (2003). **Na^+ tolerance and Na^+ transport in higher plants**. *Annals of Botany*, *91*(5), 503-527

90. USEPA. (2000). **Government report: Introduction to phytoremediation. The U.S. Environmental Protection Agency**.

91. Veselov D, et al. (2008). **The effects of NaCl treatment of water relations, growth, and ABA content in Barley cultivars differing in drought tolerance.** *Journal of Plant Growth Regulation* 27:380-386.

92. Wahid, A., et al. S. (2007). **Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins.** *Journal of Plant Physiology*, 164, 283-294.

93. Walton, N. (1989). **Electrical conductivity and total dissolved solids - what is their precise relationship*?*** *Desalination, 7*2, 275-292.

94. Wool house H. (1983). **Toxicity and tolerance in the responses of plants to metals.** *Encyclopedia of Plant Physiology*. 245-300.

95. Wu, S. (2009**). Enhanced phytoremediation of salt-impacted soils using plant growth-promoting rhizobacteria (PGPR).** *Waterloo, Ont.: University of Waterloo*.

96. Yasser H. (2006).**Virtual water as a policy instrument for achieving water security in Palestine**. *Jabalia Camp. Water Resources in the Middle East.*

97. Zhang H, et al. (2005). **Soil salinity using saturated paste and 1: 1 soil to water extracts**. *Soil Society of America Journal* 69(4):1146-1151.

98. Zhao G., et al. (2007). **Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity**. *Crop Science* 47(123-131).

99. Zhong, H. (2011). **Salt mass balance study and plant physiological responses for an enhanced salt phytoremediation system**. Waterloo University, Canada.

100. Zhu JK. (2004). **Plant salt tolerance**. 6(1.2).

100. Zhu J-K. (2001). **Plant salt tolerance**. *Trends in Plant Science* 6(2):66-71.

101. **http//www.who.int/en**.

Annexes

Annex.1 Measurements of soil salinity as TDS after 30 days of cultivation period.

num	Name of parameter	Trial num 1 TDS (mg/l)	Trial num 2 TDS (mg/l)	Trial num 3 TDS (mg/l)	Average	Standard deviation
1	ECe control soil irrigated with fresh water	65.0	65.6	64.2	64.7	0.2
2	EC 1:2 control soil irrigated with fresh water	45.8	49.0	45.3	46.7	2.007
3	ECe control soil irrigated with 6000 mg/L brackish water	90.5	89.6	90.4	90.1	0.4
4	EC 1:2 control soil irrigated with 6000 mg/L brackish water	50.6	50.7	50.9	50.7	0.1
5	ECe control soil irrigated with 10000 mg/L brackish water	95.8	95.7	94.6	95.3	0.6
6	EC 1:2 control soil irrigated with 10000 mg/L brackish water	60.4	61.2	59.8	60.4	0.7
7	ECe soil contain Barley seeds with UW3 irrigated with fresh water	65.4	65.7	64.5	65.2	0.6
8	EC 1:2 soil contain Barley seeds with UW3 irrigated with fresh water	44.8	44.5	44.6	44.6	0.1
9	ECe soil contain Barley seeds with UW3irrigated with 6000 mg/L brackish water	69.0	69.2	69.4	69.2	0.2
10	EC 1:2 soil contain Barley seeds with UW3irrigated with 6000 mg/L brackish water	50.7	52.7	51.3	51.5	1.0
11	ECe soil contain Barley seeds with UW3with 10000 mg/L brackish water	70.6	72.5	69.5	70.8	1.5
12	EC 1:2 soil contain Barley seeds with UW3irrigated with 10000 mg/L brackish water	50.3	52.5	48.7	50.5	1.9
14	ECe soil contain Malt seeds with UW3 irrigated with fresh water	66.4	65.9	66.5	66.2	0.3
15	EC 1:2 soil contain Malt seeds with UW3 irrigated with fresh water	43.8	43.5	43.6	44.6	0.1
16	ECe soil contain Malt seeds with UW3irrigated with 6000 mg/L brackish water	89.0	89.2	89.4	89.2	0.2
17	EC 1:2 soil contain Malt seeds with UW3irrigated with 6000 mg/L brackish water	60.7	60.7	60.3	60.5	0.2
18	ECe soil contain Malt seeds with UW3with 10000 mg/L Brackish water	96.6	95.8	96.5	96.3	0.4
19	EC 1:2 soil contain Malt seeds with UW3irrigated with 10000 mg/L brackish water	60.3	60.5	60.7	60.5	0.2
20	ECe soil contain Barley seeds with UW4 irrigated with fresh water	65.3	65.4	65.0	60.5	0.2
21	EC 1:2 soil contain Barley seeds with UW4 irrigated with fresh water	46.3	46.5	46.4	46.4	0.1
22	ECe soil contain Barley seeds with UW4irrigated with 6000 mg/L brackish water	69.0	69.2	69.4	69.2	0.2
23	EC 1:2 soil contain Barley seeds with UW4irrigated with 6000 mg/L brackish water	50.7	52.7	51.3	51.5	1.0
24	ECe soil contain Barley seeds with UW4with 10000 mg/L brackish water	73.5	72.9	73.4	73.2	0.3
25	EC 1:2 soil contain Barley seeds with UW4irrigated with 10000 mg/L brackish water	51.2	51.7	51.4	51.4	0.2

26	ECe soil contain Malt seeds with UW4 irrigated with fresh water	59.6	59.7	59.5	59.6	0.1
27	EC 1:2 soil contain Malt seeds with UW4 irrigated with fresh water	45.8	46.7	45.8	46.1	0.5
28	ECe soil contain Malt seeds with UW4irrigated with 6000 mg/L brackish water	89.0	89.2	89.4	89.2	0.2
29	EC 1:2 soil contain Malt seeds with UW4irrigated with 6000 mg/L brackish water	61.5	61.5	61.3	61.4	0.1
30	ECe soil contain Malt seeds with UW4with 10000 mg/L Brackish water	87.1	88.2	87.4	87.5	0.5
31	EC 1:2 soil contain Malt seeds with UW4irrigated with 10000 mg/L brackish water	59.9	60.0	60.1	60	0.1
32	ECe soil contain Barley seeds with UW3+UW4 irrigated with fresh water	63.0	63.5	63.8	63.4	0.4
33	EC 1:2 soil contain Barley seeds with UW3+UW4 irrigated with fresh water	42.1	43.0	42.6	42.5	0.4
34	ECe soil contain Barley seeds with UW3+UW4 irrigated with 6000 mg/L brackish water	64.0	64.2	64.3	64.1	0.1
35	EC 1:2 soil contain Barley seeds with UW3 +UW4 irrigated with 6000 mg/L brackish water	45.3	45.7	50.3	47.1	2.7
36	ECe soil contain Barley seeds with UW3+UW4with 10000 mg/L brackish water	68.4	68.2	68.1	68.2	0.1
37	EC 1:2 soil contain Barley seeds with UW3+UW4irrigated with 10000 mg/L Brackish water	43.3	43.5	44.7	43.8	0.7
38	ECe soil contain Malt seeds with UW3+UW4 irrigated with fresh water	57.4	57.4	57.5	57.4	0.1
39	ECe soil contain Malt seeds with UW3+UW4 irrigated with fresh water	46.8	46.7	46.8	46.7	0.1
40	ECe soil contain Malt seeds with UW3+UW4 irrigated with 6000 mg/L brackish water	84.0	84.2	84.7	84.3	0.3
41	EC 1:2 soil contain Malt seeds with UW3+UW4irrigated with 6000 mg/L brackish water	62.5	62.4	63.	62.6	0.3
42	ECe soil contain Malt seeds with UW3+UW4with 10000 mg/L brackish water	87.1	88.2	87.4	87.5	0.5
43	EC 1:2 soil contain Malt seeds with UW3+UW4 irrigated with 10000 mg/L brackish water	56.9	56.0	56.5	56.4	0.4
44	ECe soil contain Barley seeds with UW3+ H_2O_2irrigated with fresh water	64.0	64.3	64.2	64.1	0.1
45	EC 1:2 soil contain Barley seeds with UW3+ H_2O_2irrigated with fresh water	43.8	43.5	43.6	43.6	0.1
46	ECe soil contain Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L brackish water	87.5	87.6	90.4	88.5	1.6
47	EC 1:2 soil contain Barley seeds with UW3+ H_2O_2irrigated with 6000 mg/L brackish water	46.6	46.8	48.9	47.7	1.2
48	ECe soil contain Barley seeds with UW3+ $H_2O_2$with 10000 mg/L brackish water	93.0	92.5	91.9	92.5	0.5
49	EC 1:2 soil contain Barley seeds with UW3+ H_2O_2irrigated with 10000 mg/L brackish water	58.4	58.2	59.0	58.5	1.2
50	ECe soil contain Malt seeds with UW3+ H_2O_2irrigated with fresh water	63.4	63.0	63.2	63.2	0.5
51	EC 1:2 soil contain Malt seeds with UW3+ H_2O_2irrigated with fresh water	43.8	45.0	45.3	44.7	0.4
52	ECe soil contain Malt seeds with UW3+ H_2O_2 irrigated with 6000 mg/L brackish water	82.5	83.6	82.4	82.8	0.2

53	EC 1:2 soil contain Malt seeds with UW3+ H_2O_2irrigated with 6000 mg/L brackish water	48.6	49.7	50.6	49.6	1.0
54	ECe soil contain Malt seeds with UW3+ H $_2O_2$with 10000 mg/L brackish water	93.8	92.7	92.6	93.0	0.6
55	EC 1:2 soil contain Malt seeds with UW3+ H 2O2irrigated with 10000 mg/L brackish water	57.4	58.2	58.8	58.1	0.7
56	ECe soil contain Barley seeds with H $_2O_2$irrigated with fresh water	63.0	63.0	63.2	63.1	0.1
57	EC 1:2 soil contain Barley seeds with H $_2O_2$irrigated with fresh water	42.8	42.0	42.3	42.4	0.4
58	ECe soil contain Barley seeds with H $_2O_2$ irrigated with 6000 mg/L brackish water	88.05	89.0	88.4	88.5	0.4
59	EC 1:2 soil contain Barley seeds with H_2O_2irrigated with 6000 mg/L brackish water	47.6	47.7	47.9	47.7	0.1
60	ECe soil contain Barley seeds with H $_2O_2$with 10000 mg/L brackish water	91.0	91.7	91.6	91.4	0.3
61	EC 1:2 soil contain Barley seeds with H $_2O_2$irrigated with 10000 mg/L	58.4	58.2	58.8	58.4	0.3
62	ECe soil contain Malt seeds with H $_2O_2$irrigated with fresh water	64.0	64.3	64.4	64.2	0.2
63	EC 1:2 soil contain Malt seeds with H $_2O_2$irrigated with fresh water	43.8	43.0	43.3	43.3	0.4
64	ECe soil contain Malt seeds with H_2O_2 irrigated with 6000 mg/L brackish water	83.05	83.0	83.4	83.15	0.2
65	EC 1:2 soil contain Malt seeds with H_2O_2irrigated with 6000 mg/L brackish water	50.6	50.7	50.9	50.73	0.1
66	ECe soil contain Malt seeds with H $_2O_2$with 10000 mg/L brackish water	94.0	94.3	94.6	94.3	0.3
67	EC 1:2 soil contain Malt seeds with H_2O_2irrigated with 10000 mg/L brackish water	59.4	59.2	59.5	59.3	1.5

Annex.2: Calculated experimental measurement of Electrical conductivity (EC) in unit ds/l each trials after 20 days, each parameter was performed in triplicate at Temp 17 ^{o}C

num	Name of parameter	Trial num 1 ds/l	Trial num 2 ds/l	Trial num 3 ds/l	Average	SD
1	ECe control soil irrigated with fresh water	0.101	0.102	0.100	0.101	0.001
2	EC 1:2 control soil irrigated with fresh water	0.071	0.076	0.070	0.072	0.003
3	ECe control soil irrigated with 6000 mg/L brackish water	0.142	0.14	0.141	0.140	0.007
4	EC 1:2 control soil irrigated with 6000 mg/L brackish water	0.079	0.079	0.079	0.079	0.002
5	ECe control soil irrigated with 10000 mg/L brackish water	0.149	0.149	0.147	0.149	0.001
6	EC 1:2 control soil irrigated with 10000 mg/L brackish water	0.094	0.095	0.093	0.094	0.001
7	ECe soil contain Barley seeds with UW3 irrigated with fresh water	0.102	0.102	0.100	0.101	0.009
8	EC 1:2 soil contain Barley seeds with UW3 irrigated with fresh water	0.101	0.102	0.100	0.101	0.001
9	ECe soil contain Barley seeds with UW3irrigated with 6000 mg/L brackish water	0.07	0.069	0.069	0.069	0.002
10	EC 1:2 soil contain Barley seeds with UW3irrigated with 6000 mg/L brackish water	0.107	0.108	0.108	0.108	0.003
11	ECe soil contain Barley seeds with UW3with 10000 mg/L brackish water	0.079	0.082	0.080	0.080	0.001
12	EC 1:2 soil contain Barley seeds with UW3irrigated with 10000 mg/L brackish water	0.110	0.113	0.108	0.110	0.002
14	ECe soil contain Malt seeds with UW3 irrigated with fresh water	0.078	0.082	0.076	0.078	0.003
15	EC 1:2 soil contain Malt seeds with UW3 irrigated with fresh water	0.103	0.102	0.103	0.103	0.006
16	ECe soil contain Malt seeds with UW3irrigated with 6000 mg/L brackish water	0.139	0.139	0.139	0.139	0.003
17	EC 1:2 soil contain Malt seeds with UW3irrigated with 6000 mg/L brackish water	0.094	0.094	0.094	0.101	0.003

18	ECe soil contain Malt seeds with UW3with 10000 mg/L brackish water	0.150	0.149	0.150	0.150	0.001
19	EC 1:2 soil contain Malt seeds with UW3irrigated with 10000 mg/L brackish water	0.094	0.094	0.094	0.094	0.003
20	ECe soil contain Barley seeds with UW4 irrigated with fresh water	0.102	0.102	0.101	0.101	0.001
21	EC 1:2 soil contain Barley seeds with UW4 irrigated with fresh water	0.072	0.073	0.072	0.072	0.005
22	ECe soil contain Barley seeds with UW4irrigated with 6000 mg/L brackish water	0.107	0.108	0.108	0.108	0.003
23	EC 1:2 soil contain Barley seeds with UW4irrigated with 6000 mg/L brackish water	0.079	0.082	0.080	0.081	0.001
24	ECe soil contain Barley seeds with UW4with 10000 mg/L brackish water	0.114	0.113	0.114	0.114	0.008
25	EC 1:2 soil contain Barley seeds with UW4irrigated with 10000 mg/L brackish water	0.08	0.080	0.080	0.080	0.003
26	ECe soil contain Malt seeds with UW4 irrigated with fresh water	0.093	0.093	0.092	0.093	0.001
27	EC 1:2 soil contain Malt seeds with UW4 irrigated with fresh water	0.0715625	0.0729687	0.071562	0.07203125	0.008
28	ECe soil contain Malt seeds with UW4irrigated with 6000 mg/L brackish water	0.139	0.139	0.139	0.1393	0.001
29	EC 1:2 soil contain Malt seeds with UW4irrigated with 6000 mg/L brackish water	0.096	0.096	0.095	0.095	0.006
30	ECe soil contain Malt seeds with UW4with 10000 mg/L Brackish water	0.136	0.137	0.136	0.137	0.007
31	EC 1:2 soil contain Malt seeds with UW4irrigated with 10000 mg/L brackish water	0.093	0.093	0.093	0.093	0.002
32	ECe soil contain Barley seeds with UW3+UW4 irrigated with fresh water	0.098	0.099	0.099	0.099	0.002
33	EC 1:2 soil contain Barley seeds with UW3+UW4 irrigated with fresh water	0.065	0.067	0.066	0.066	0.004
34	ECe soil contain Barley seeds with UW3+UW4 irrigated with 6000 mg/L brackish water	0.1	0.100	0.100	0.100	0.002
35	EC 1:2 soil contain Barley	0.070	0.071	0.078	0.073	0.001

	seeds with UW3 +UW4 irrigated with 6000 mg/L brackish water					
36	ECe soil contain Barley seeds with UW3+UW4with 10000 mg/L brackish water	0.107	0.106	0.106	0.106	0.009
37	EC 1:2 soil contain Barley seeds with UW3+UW4irrigated with 10000 mg/L brackish water	0.067	0.067	0.069	0.068	0.002
38	ECe soil contain Malt seeds with UW3+UW4 irrigated with fresh water	0.089	0.089	0.089	0.089	0.009
39	EC 1:2 soil contain Malt seeds with UW3+UW4 irrigated with fresh water	0.073	0.072	0.073	0.073	0.005
40	ECe soil contain Malt seeds with UW3+UW4 irrigated with 6000 mg/L brackish water	0.131	0.132	0.132	0.132	0.005
41	EC 1:2 soil contain Malt seeds with UW3+UW4irrigated with 6000 mg/L brackish water	0.097	0.097	0.098	0.097	0.001
42	ECe soil contain Malt seeds with UW3+UW4with 10000 mg/L brackish water	0.136	0.137	0.136	0.136	0.001
43	EC 1:2 soil contain Malt seeds with UW3+UW4 irrigated with 10000 mg/L brackish water	0.088	0.087	0.088	0.0.088	0.002
44	ECe soil contain Barley seeds with UW3+ H_2O_2irrigated with fresh water	0.100	0.100	0.100	0.100	0.002
45	EC 1:2 soil contain Barley seeds with UW3+ H_2O_2irrigated with fresh water	0.068	0.067	0.068	0.670	0.001
46	ECe soil contain Barley seeds with UW3+ H_2O_2 irrigated with 6000 mg/L brackish water	0.136	0.136	0.141	0.138	0.008
47	EC 1:2 soil contain Barley seeds with UW3+H_2O_2irrigated with 6000 mg/L brackish water	0.072	0.073	0.076	0.074	0.006
48	ECe soil contain Barley seeds with UW3+ $H_2O_2$with 10000 mg/L brackish water	0.145	0.144	0.143	0.143	0.003
49	EC 1:2 soil contain Barley seeds with UW3+ H2O2irrigated with 10000 mg/L Brackish water	0.091	0.090	0.092	0.091	0.001
50	ECe soil contain Malt seeds with UW3+ H 2O2irrigated with fresh	0.099	0.098	0.098	0.099	0.001

	water					
51	EC 1:2 soil contain Malt seeds with UW3+ H 2O2irrigated with fresh water	0.068	0.070	0.070	0.069	0.001
52	ECe soil contain Malt seeds with UW3+ H 2O2 irrigated with 6000 mg/L Brackish water	0.128	0.130	0.128	0.129	0.001
53	EC 1:2 soil contain Malt seeds with UW3+ H 2O2irrigated with 6000 mg/L Brackish water	0.075	0.077	0.079	0.077	0.001
54	ECe soil contain Malt seeds with UW3+ H 2O2with 10000 mg/L Brackish water	0.146	0.144	0.144	0.145	0.017
55	EC 1:2 soil contain Malt seeds with UW3+ H 2O2irrigated with 10000 mg/L Brackish water	0.089	0.090	0.091	0.086	0.006
56	ECe soil contain Barley seeds with H 2O2irrigated with fresh water	0.098	0.098	0.098	0.098	0.007
57	EC 1:2 soil contain Barley seeds with H 2O2irrigated with fresh water	0.066	0.065	0.066	0.066	0.002
58	ECe soil contain Barley seeds with H 2O2 irrigated with 6000 mg/L Brackish water	0.137	0.139	0.138	0.138	0.005
59	EC 1:2 soil contain Barley seeds with H 2O2irrigated with 6000 mg/L Brackish water	0.074	0.074	0.074	0.74	0.004
60	ECe soil contain Barley seeds with H $_2O_2$with 10000 mg/L Brackish water	0.142	0.143	0.143	0.111	0.003
61	EC 1:2 soil contain Barley seeds with H 2O2irrigated with 10000 mg/L	0.091	0.090	0.091	0.091	0.006
62	ECe soil contain Malt seeds with H_2O_2irrigated with fresh water	0.100	0.100	0.101	0.100	0.003
63	EC 1:2 soil contain Malt seeds with H 2O2irrigated with fresh water	0.068	0.067	0.067	0.067	0.004
64	ECe soil contain Malt seeds with H 2O2 irrigated with 6000 mg/L Brackish water	0.129	0.129	0.130	0.129	0.003
65	EC 1:2 soil contain Malt seeds with	0.079	0.079	0.079	0.079	0.002

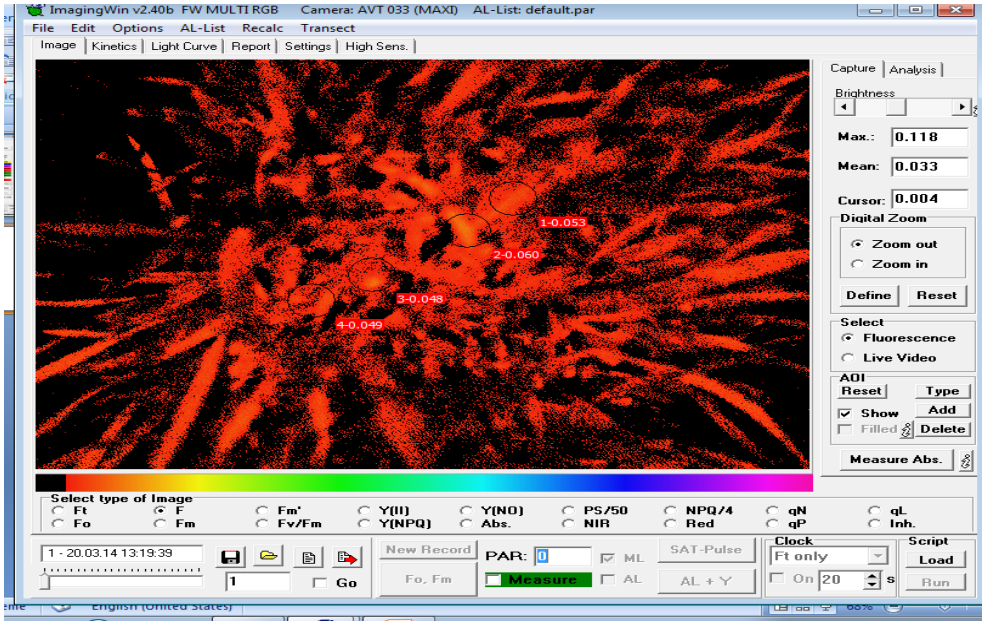
	H 2O2irrigated with 6000 mg/L Brackish water					
66	ECe soil contain Malt seeds with H 2O2with 10000 mg/L brackish water	0.146	0.147	0.147	0.147	0.004
67	EC 1:2 soil contain Malt seeds with H2O2irrigated with 10000 mg/L brackish water	0.092	0.092	0.092	0.097	0.002

Annex.3: Measurement of electrical conductivity of brackish water for two syntheses samples before and for decants water for each trial. Each trial was performed in triplicate

Name of parameter	Trial num 1 TDS (g/l)	Trial num 2 TDS (g/l)	Trial num 3 TDS (g/l)	Average	Standard deviation
EC for 6000mg/L of brackish water before irrigation	5.98	5.89	5.95	5.94	0.04
EC for 10000 mg/L of brackish water before irrigation	9.87	9.94	9.96	9.92	0.04
EC for decent brackish water of Barley seeds with UW3irrigated with 6000 mg/L brackish water	3.65	3.67	3.64	3.65	0.01
EC for decent water of Barley seeds with UW3irrigated with 10000 mg/L brackish water	6.94	6.90	6.87	6.90	0.03
EC for decent brackish water of Malt seeds with UW3irrigated with 6000 mg/L brackish water	4.89	4.90	4.92	4.90	0.01
EC for decent water of Malt seeds with UW3irrigated with 10000 mg/L brackish water	8.70	8.75	8.74	8.73	0.02
EC for decent brackish water of Barley seeds with UW4irrigated with 6000 mg/L brackish water	3.25	3.28	3.26	3.26	0.01
EC for decent water of Barley seeds with UW4 irrigated with 10000 mg/L brackish water	6.17	6.13	6.15	6.15	0.02
EC for decent Brackish water of Malt seeds with UW4irrigated with 6000 mg/L Brackish water	4.75	4.76	4.79	4.76	0.02
EC for decent water of Malt seeds with UW4irrigated with 10000 mg/L brackish water	7.77	7.79	7.77	7.77	0.01
EC for decent brackish	2.85	2.87	2.84	2.85	0.01

water of Barley seeds with UW3 +UW4irrigated with 6000 mg/L brackish water					
EC for decent water of Barley seeds with UW3+UW4 irrigated with 10000 mg/L brackish water	6.47	6.43	6.45	6.45	0.02
EC for decent brackish water of Malt seeds with UW3+UW4irrigated with 6000 mg/L brackish water	3.86	3.85	3.82	3.84	0.02
EC for decent water of Malt seeds with UW3+UW4 irrigated with 10000 mg/L Brackish water	7.70	7.65	7.68	7.67	0.02
EC for decent brackish water of Barley seeds with UW3+H2O2irrigated with 6000 mg/L brackish water	3.16	3.15	3.13	3.14	0.01
EC for decent water of Barley seeds with UW3+ H2O2 irrigated with 10000 mg/L brackish water	6.70	6.65	6.68	6.67	0.02
EC for decent brackish water of Barley seeds with H_2O_2irrigated with 6000 mg/L brackish water	4. 88	4.89	4.90	4.89	0.01
EC for decent water of Barley seeds with $H2O_2$irrigated with 10000 mg/L brackish water	8.89	8.87	8.85	8.87	0.02
EC for decent Brackish water of Malt seeds with irrigated with 6000 mg/L brackish water	5.10	5.09	5.02	5.07	0.04
EC for decent brackish water of Malt seeds with irrigated with 10000 mg/L brackish water	9.87	9.94	9.96	9.92	0.04

Annex.4 picture for trials selected random trials



Annex.5: PAM fluometry measurement for Barley plant irrigated with 6000 of brackish water.

Time Min: Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.779	0.019	0.006	0.001
0:00:42	0.159	0.027	0.001	0.001
0:01:02	0.197	0.041	0.251	0.035
0:01:23	0.257	0.046	0.383	0.046
0:01:43	0.308	0.049	0.395	0.053
0:02:03	0.329	0.047	0.392	0.055
0:02:23	0.361	0.044	0.371	0.056
0:02:44	0.372	0.040	0.362	0.055
0:03:04	0.385	0.037	0.350	0.053
0:03:24	0.388	0.038	0.346	0.053
0:03:45	0.388	0.038	0.345	0.052
0:04:05	0.398	0.039	0.337	0.052
0:04:25	0.405	0.035	0.329	0.049
0:04:45	0.416	0.036	0.322	0.048
0:05:06	0.419	0.034	0.319	0.046
0:05:20	0.602	0.036	0.214	0.037
0:05:32	0.613	0.033	0.196	0.030
0:05:46	0.632	0.028	0.175	0.023
0:06:04	0.651	0.023	0.156	0.014
0:06:24	0.666	0.021	0.144	0.011
0:06:48	0.673	0.017	0.131	0.006
0:07:18	0.686	0.016	0.119	0.004
0:07:53	0.694	0.014	0.111	0.005

Annex.6: PAM fluometry measurement for Barley plant irrigated with 10000 of brackish water.

Time Min:Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.779	0.012	0.005	0
0:00:42	0.238	0.041	0	0
0:01:02	0.267	0.055	0.197	0.036
0:01:23	0.301	0.058	0.312	0.055
0:01:43	0.321	0.057	0.335	0.062
0:02:03	0.378	0.052	0.306	0.065
0:02:23	0.396	0.043	0.296	0.060
0:02:44	0.415	0.043	0.281	0.059
0:03:04	0.416	0.036	0.281	0.053
0:03:24	0.404	0.029	0.289	0.047
0:03:45	0.408	0.026	0.289	0.041
0:04:05	0.406	0.027	0.295	0.040
0:04:25	0.408	0.020	0.296	0.032
0:04:45	0.416	0.019	0.295	0.030
0:05:06	0.410	0.022	0.301	0.030
0:05:20	0.603	0.018	0.195	0.019
0:05:32	0.617	0.019	0.179	0.015
0:05:46	0.627	0.015	0.168	0.008
0:06:04	0.635	0.018	0.158	0.009
0:06:24	0.644	0.011	0.148	0.007
0:06:48	0.657	0.017	0.131	0.006
0:07:18	0.665	0.016	0.119	0.004

Annex.7: PAM fluometry measurement for treated Barley seeds with UW3 irrigated with fresh water.

Time Min:Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.786	0.008	0.005	0.001
0:00:42	0.217	0.015	0.005	0.001
0:01:02	0.351	0.008	0.231	0.027
0:01:23	0.429	0.008	0.234	0.026
0:01:43	0.451	0.013	0.210	0.023
0:02:03	0.479	0.013	0.183	0.018
0:02:23	0.484	0.015	0.182	0.014
0:02:44	0.482	0.012	0.193	0.012
0:03:04	0.486	0.014	0.205	0.012
0:03:24	0.489	0.017	0.214	0.013
0:03:45	0.492	0.019	0.225	0.014
0:04:05	0.487	0.021	0.237	0.016
0:04:25	0.488	0.022	0.242	0.017
0:04:45	0.480	0.023	0.250	0.016
0:05:06	0.488	0.023	0.250	0.017
0:05:20	0.631	0.016	0.178	0.010
0:05:32	0.644	0.013	0.164	0.007
0:05:46	0.653	0.009	0.156	0.004
0:06:04	0.657	0.011	0.153	0.005
0:06:24	0.667	0.009	0.143	0.002
0:06:48	0.679	0.008	0.133	0.002
0:07:18	0.688	0.008	0.120	0.002
0:07:53	0.700	0.012	0.115	0.006

Annex.8: PAM fluometry measurement for treated Barley seeds with UW3 irrigated with 6000 mg/L of brackish water.

Time Min: sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.005
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.02
0:02:03	0.318	0.044	0.423	0.0383
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.9: PAM fluometry measurement for treated Barley seeds with UW3 irrigated with 10000 mg/L of brackish water.

Time Min:Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.005
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.048
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.5937	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.10: PAM fluometry measurement for treated Barley seeds with UW4 irrigated with fresh water

Time Min: Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.786	0.008	0.005	0.005
0:00:42	0.217	0.015	0.005	0.001
0:01:02	0.351	0.008	0.231	0.027
0:01:23	0.429	0.008	0.234	0.026
0:01:43	0.451	0.013	0.210	0.023
0:02:03	0.479	0.013	0.183	0.018
0:02:23	0.484	0.015	0.182	0.014
0:02:44	0.482	0.012	0.193	0.012
0:03:04	0.486	0.014	0.205	0.012
0:03:24	0.489	0.017	0.216	0.013
0:03:45	0.490	0.019	0.225	0.014
0:04:05	0.487	0.021	0.237	0.016
0:04:25	0.488	0.022	0.242	0.017
0:04:45	0.480	0.023	0.250	0.0166
0:05:06	0.488	0.023	0.250	0.017
0:05:20	0.631	0.016	0.178	0.010
0:05:32	0.644	0.013	0.164	0.007
0:05:46	0.653	0.009	0.156	0.004
0:06:04	0.657	0.011	0.153	0.005
0:06:24	0.667	0.009	0.143	0.002
0:06:48	0.679	0.008	0.133	0.002
0:07:18	0.688	0.008	0.126	0.002
0:07:53	0.7	0.012	0.115	0.006

Annex.11: PAM fluometry measurement for treated Barley seeds with UW4 irrigated with 6000 mg/L of brackish water.

Time Min:Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.005
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.12: PAM fluometry measurement for treated Barley seeds with UW4 irrigated with 10000 mg/L of brackish water.

Time Min:Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.005
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.019
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.13: PAM fluometry measurement for treated Barley seeds with UW3+UW4 irrigated fresh water

Time Min : sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.786	0.008	0.005	0.001
0:00:42	0.217	0.015	0.005	0.001
0:01:02	0.351	0.008	0.231	0.027
0:01:23	0.429	0.008	0.234	0.026
0:01:43	0.451	0.013	0.210	0.023
0:02:03	0.479	0.013	0.183	0.018
0:02:23	0.484	0.015	0.182	0.014
0:02:44	0.482	0.012	0.193	0.012
0:03:04	0.486	0.014	0.205	0.012
0:03:24	0.489	0.017	0.216	0.013
0:03:45	0.490	0.019	0.225	0.014
0:04:05	0.487	0.021	0.237	0.016
0:04:25	0.488	0.022	0.242	0.017
0:04:45	0.480	0.023	0.250	0.016
0:05:06	0.488	0.023	0.250	0.017
0:05:20	0.631	0.016	0.178	0.010
0:05:32	0.644	0.013	0.164	0.007
0:05:46	0.653	0.009	0.156	0.004
0:06:04	0.657	0.011	0.153	0.005
0:06:24	0.667	0.009	0.143	0.002
0:06:48	0.679	0.008	0.133	0.002
0:07:18	0.688	0.008	0.126	0.002
0:07:53	0.700	0.012	0.115	0.006

Annex.14: PAM fluometry measurement for treated Barley seeds with UW3+UW4 irrigated 6000 mg/L of brackish water.

Time Min : sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.005
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.0053	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.15: PAM fluometry measurement for treated Barley seeds with UW3+UW4 irrigated with 10000 mg/L of brackish water

Time Min: sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0.00	0.005
0:01:02	0.197	0.043	0.2993	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.16: PAM fluometry measurement for treated Barley seeds with UW3+H_2O_2 irrigated with fresh water

Time Min: Sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.779	0.019	0.006	0.001
0:00:42	0.159	0.027	0.001	0.001
0:01:02	0.197	0.041	0.251	0.035
0:01:23	0.257	0.046	0.383	0.046
0:01:43	0.308	0.049	0.395	0.053
0:02:03	0.329	0.047	0.392	0.055
0:02:23	0.361	0.044	0.371	0.056
0:02:44	0.372	0.040	0.362	0.055
0:03:04	0.385	0.037	0.350	0.053
0:03:24	0.388	0.038	0.346	0.053
0:03:45	0.388	0.038	0.345	0.052
0:04:05	0.398	0.039	0.337	0.052
0:04:25	0.405	0.035	0.329	0.049
0:04:45	0.416	0.036	0.322	0.048
0:05:06	0.419	0.034	0.319	0.046
0:05:20	0.602	0.036	0.214	0.037
0:05:32	0.613	0.033	0.196	0.030
0:05:46	0.632	0.028	0.175	0.023
0:06:04	0.651	0.023	0.156	0.014
0:06:24	0.66	0.021	0.144	0.011
0:06:48	0.673	0.017	0.131	0.006
0:07:18	0.686	0.016	0.119	0.004
0:07:53	0.694	0.014	0.11	0.005
0:08:35	0.700	0.012	0.106	0.004

Annex.17: PAM fluometry measurement for treated Barley seeds with UW3+H_2O_2 irrigated with 6000 mg/L of brackish water

Time Min:sec	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.005
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.615	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

Annex.18: PAM fluometry measurement for treated Barley seeds with UW3+H_2O_2 irrigated with 10000 mg/L of brackish water

Time	Average Y(II)	Standard deviation	Average NPQ	Standard deviation
0:00:00	0.769	0.009	0.006	0.001
0:00:42	0.155	0.030	0	0.001
0:01:02	0.197	0.043	0.299	0.028
0:01:23	0.258	0.047	0.422	0.018
0:01:43	0.283	0.043	0.442	0.024
0:02:03	0.318	0.044	0.423	0.038
0:02:23	0.346	0.040	0.401	0.041
0:02:44	0.353	0.034	0.389	0.041
0:03:04	0.363	0.033	0.379	0.044
0:03:24	0.373	0.028	0.366	0.043
0:03:45	0.385	0.023	0.351	0.039
0:04:05	0.388	0.025	0.346	0.042
0:04:25	0.397	0.018	0.332	0.036
0:04:45	0.397	0.015	0.328	0.033
0:05:06	0.412	0.013	0.313	0.028
0:05:20	0.593	0.009	0.207	0.016
0:05:32	0.600	0.004	0.195	0.012
0:05:46	0.612	0.005	0.185	0.010
0:06:04	0.624	0.003	0.172	0.007
0:06:24	0.641	0.002	0.159	0.004
0:06:48	0.646	0.007	0.151	0.004
0:07:18	0.658	0.005	0.141	0.006

جامعة النجاح الوطنية

كلية الدراسات العليا

إستخدام تقنية علاج النبات في علاج المياه المالحة الناتجة من محطات التناضح العكسي

إعداد

رناد جلال يحيى حامد

إشراف

د. شحده جودة

د. رائد الكوني

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين.

2014

إستخدام تقنية علاج النبات في علاج المياه المالحة الناتجة من محطات التناضح العكسي

اعداد

رناد جلال يحيى حامد

اشراف

د. شحده جودة

د. رائد الكوني

الملخص

تعتبر المياه عالية الملوحة ناتجا ثانويا يصدر عن محطات التناضح العكسي بعد عملية تحلية المياه. بحيث أن نسبة محتواها من أيونات الأملاح الذائبة مابين mg/L(10000-5000). وهذه الأيونات هي أيون الصوديوم وأيون الكلور وأيونات الكالسيوم والمغنيسيوم والبوتاسيوم والكربونات والكبريتات.

تعد هذه المياه ملوث للبيئة لاحتوائها على تلك التراكيز العالية من الأملاح، بحيث أنها تسبب مشاكل للتربة والنباتات وتحد من تطور المجال الزراعي في فلسطين .

في الآونة الأخيرة تم تطبيق الأبحاث في علاج تلك المياه، من خلال تخفيف تراكيز الاملاح بإضافة المياه الحلوة، وتقنية أخرى تسمى بتقنية علاج النبات، بحيث تعتمد هذه التقنية بشكل رئيسي على النبات المتحمل للملوحة في عملية إستخراج الأملاح وتجميعها في الكتلة الحيوية.

لكن بشكل عام، عند مرحلة معينة تعمل التراكيز العالية من الأملاح على تثبيط نمو النبتة حتى وإن كانت من النوع المتحمل للملوحة، لذلك درست بعض الابحاث تأثير نوع من أنواع البكتيريا المسماه بPGPR في تعزيز نمو النباتات وتحسينه من خلال خفض إنتاج مركب الإثيلين المنتج في فترة إجهاد النبتة وتثبيط نموها، وتقوم هذه البكتيريا على تحسين عملية البناء الضوئي تحت الجهد المتعرضة له، مما يزيد من قابليتها لإمتصاص أكبر قدر من الأملاح وتخزينه في كتلتها الحيوية.

في هذه الورقة البحثية تم اعتماد تقنية علاج النبات وتهجين بذور النبات بالسلالتينUW3 و UW4 المعزولة من مختبر البرفسور غليك واترلو في جامعة كندا لزيادة قابلية النبتة في التخلص من الأملاح أثناء عملية الري، وتجميعها في الكتلة الحيوية ومن ثم حصادها، وهكذا يتم التخلص من المياه عالية الملوحة بطرق ودية للبيئة.

شملت المعاملات في التجربة معاملات لبذور الشعير والملت المهجنة بسلالةUW3 ، معاملات مهجنة بسلالة ال UW4 ، معاملات مهجنة بتلك السلالتين معا، ومعاملات دون أي تهجين أو معالجة لتعتبر المرجعية، كما تم إضافة معاملات أخرى احتوت تهجين البذور بسلالة الUW3 مع الهيدروجين بيروكسيد، ومعاملات تمت معاملتها بالهيدرجين بيروكسيد لوحده. وتم تقسيم كل معاملة وري مجموعة بالماء العذب، وأخرى بمحلول المياه عالي الملوحة تركيز 6000 mg/L، وأخرى بمحلول 10000 mg/L. وتم تنفيذها في دفيئة زراعية صممت في حديقة المنزل.

أظهرت النتائج أن تأثير PGPR أدى إلى زيادة في نمو النبات و النشاط الضوئي والاستقرار الغشائي، وكما لوحظ زيادة أطوال الجذور للمعاملات المعالجة بتلك السلالات حتى وإن كانت تحت جهد الأملاح مقارنة مع المعاملات المرجعية المروية سواء بالمياه العذبة أو التراكيز الأخرى من محاليل المياه المالحة. وكما لوحظ زيادة الكتلة الحيوية لمعاملات ال PGPR بحيث كانت للمعاملات المهجنة بسلالةUW3 (237.31% ,249.40% ,156.11%)، والمهجنة بسلالة ال UW4 (156.11% ,237.31% ,288.83%)، والمهجنة بتلك السلالتين مع بعضهما(128.12% ,267.67% ,288.56%) بينما المعاملات المرجعية كانت (100% ,150.08% ,8.98 %).

وكما أظهرت الدراسة أن فحص تسرب الالكترونات للمعاملات مع PGPR المروية بمحاليل المياه المالحة وتساوي 304 mg/L وهي نفس القيم للمعاملات المروية بالمياه العذبة، مما يعني أن تسرب الالكترونات داخل الغشاء أقل من المعاملات دون ال PGPR والمروية بمحاليل المياه المالحة بحيث أن تسرب الالكترونات داخل الغشاء كان أعلى مما أدى إلى دمار الخلية.

وكما تم استخدام جهاز PAM لقياس سير عملية البناء الضوئي داخل النبتة إن كانت ضمن معدلاتها الطبيبعة ، فأظهرت النتائج للمعاملات التي خضعت للمعالجة بسلالات ال PGPR والمروية بمحاليل المياه المالحة لم تتأثر لديها سير عملية البناء الضوئي داخلها ولم تتعرض لأي إجهاد ملحي على عكس المعاملات المرجعية والمروية بمحاليل المياه المالحة بحيث أظهرت القياسات أن هناك تراجع ملحوظ لعملية البناء الضوئي مما يدل على أن النتبة تعاني من إجهاد وزيادة في تركيز مركب الاثيليلن المثبط لنموها .