Enhanced Phytoremediation of Salt-Impacted Soils Using Plant Growth-Promoting Rhizobacteria (PGPR)

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Soil salinity is a widespread problem that limits crop yield throughout the world. The accumulation of soluble salts in the soil can inhibit plant growth by increasing the osmotic potential of interstitial water, inducing ion toxicity and nutrient imbalances in plants. Over the last decade, considerable effort has been put into developing economical and effective methods to reclaim these damaged soils.

Phytoremediation is a technique that uses plants to extract, contain, immobilize and degrade contaminants in soil. The most common process for salt bioremediation is phytoextraction which uses plants to accumulate salt in the shoots, which is then removed by harvesting the foliage. As developing significant plant biomass in saline soils is an issue, a group of free-living rhizobacteria, called plant growth promoting rhizobacteria (PGPR), can be applied to plant seeds to aid plant growth by alleviating salt stress.

The principle objective of this research was to test the efficacy of PGPR in improving the growth of plants on salt-impacted soils through greenhouse and field studies. In this research, previously isolated PGPR strains of *Pseudomonas putida*. UW3, *Pseudomonas putida* UW4, and *Pseudomonas corrugata* CMH3 were applied to barley (*Hordeum valgare* C.V. AC ranger), oats (*Avena sativa* C.V. CDC baler), tall wheatgrass (*Agropyron elongatum*), and tall fescue (*festuca arundinacea* C.V. Inferno). PGPR effects on plant growth, membrane stability, and photosynthetic activity under salt stress were examined.

Greenhouse studies showed that plants treated with PGPR resulted in an increase in plant biomass by up to 500% in salt-impacted soils. Electrolyte leakage assay showed that plants treated with PGPR resulted in 50% less electrolyte leakage from membranes.

Several chlorophyll a fluorescence parameters, F_v/F_m, effective quantum yield, F_s, qP, and qN obtained from pulse amplitude modulation (PAM) fluorometry showed that PGPR-treated plants resulted in improvement in photosynthesis under salt stress.

Field studies showed that PGPR promoted shoot dry biomass production by 27% to 230%. The NaCl accumulation in plant shoots increased by 7% to 98% with PGPR treatment. The averaged soil salinity level at the CMS and CMN site decreased by 20% and 60%, respectively, during the 2008 field season. However, there was no evidence of a decrease in soil salinity at the AL site. Based on the improvements of plant biomass production and NaCl uptake by PGPR observed in the 2008 field studies, the phytoremediation efficiency on salt-impacted sites is expected to increase by 30-60% with PGPR treatments. Based on the average data of 2007 and 2008 field season, the time required to remove 25% of NaCl of the top 50 cm soil at the CMS, CMN and AL site is estimated to be six, twelve, and sixteen years, respectively, with PGPR treatments. The remediation efficiency is expected to accelerate during the remediation process as the soil properties and soil salinity levels improve over time.

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List of Abbreviations

ACC	1-aminocyclopropane-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)
EC_e	Electrical-conductivity of a saturated soil paste extract
Fd	Ferredoxin
FNR	Ferredoxin $NADP^+$-reductase
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
PAH	Polycyclic aromatic hydrocarbons
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 plastoquinol pool
PQH_2	Reduced plastoquinol pool
ROS	Reactive oxygen species
SAM	S-adenosyl-methionine
SAR	Sodium adsorption ratio
SP	Saturating pulse used in PAM fluorometry
TSB	Tryptic soy broth

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 $qN = 0$); no 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′

Chapter 1 - Introduction

Salinity is a major environmental factor that causes degradation of the physical-chemical properties of soil resulting in major impacts on crop productivity. Globally, approximately 1 billion ha of land (7% of all land area) are affected by soil salinity (Flowers et al. 1997). This is estimated to have a negative impact on one-third of the world's food production (Munns 2002). This is clearly an enormous problem, and of great concern. The problem is escalating due to increasing salt release and accumulation from various natural and anthropogenic sources. Salt in the environment can result from natural weathering of geological formations or from anthropogenic activities such as brine contamination from petroleum production activities and long-term irrigation (AE 2001).

To minimize the effects of soil salinization, much research has been put into finding economical and effective methods to re-establish vegetation in salt-impacted soils (AE 2001; USEPA 2000). Some methods that have been used for removal of salt in soil include disposal of surface layers, use of electro-kinetic extraction, soil washing with clean water, or soil mixing with organic materials to improve soil structure (AE 2001; USEPA 2000). Unfortunately, these techniques are often impractical and costly as well as having other environmental drawbacks such as appropriate disposal of the contaminants.

Phytoremediation is a technique that uses plants to extract, contain, immobilize, degrade, or combinations of these to diminish contaminants in soil (Kömives and Gullner 2000; USEPA 2000). Phytoremediation has shown to be advantageous in several aspects: 1) it is economical, 2) it preserves the physical-chemical properties of soil, and 3) has the potential to achieve rapid remediation (Huang et al. 2004).

The most common form of phytoremediation for saline soil is phytoextraction, which uses plants to accumulate salt in shoots and the salt can then be removed from the

soil via harvesting the foliage (USEPA 2000). However, efficient removal of salt requires sufficient above-ground plant biomass, which is an issue as salt stress inhibits plant germination and growth. To overcome the salt stress on plants, it has been found that a group of free-living rhizobacteria called plant growth promoting rhizobacteria (PGPR) that contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase can be applied to plant seeds to lower the plant stress hormone, ethylene, and promote plant growth under salt stress.

In this study, a combination of several salt tolerant plants and PGPR are applied together to remediate three salt-impacted sites located in Saskatchewan, Canada. Both lab and field work were conducted to examine the effects of PGPR on plants under salt stress as well as to evaluate the feasibility and efficiency of using plants with the aid of PGPR to remediate salt-impacted sites.

1.1 Definition of soil salinity and salinity parameters

Soil salinity is a term used to describe the amount of mineral salts present in soil (Richard 1954). The mineral salts constitute a mixture of electrolytes. The major cations in saline soils include Na^+, Ca^{2+}, Mg^{2+}, and K^+; the major anions include Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^-. These constituents are usually reported in units of mg/L (ppm), mmol/L or mmol charge/L (meq/L) in solution extracted from a soil saturated with water (Tanji 2002).

Salinity is often measured as electrical conductivity (EC), a measure of the ability of a substance to conduct electricity. EC is used to express the magnitude of the total dissolved salts (electrolytes) in soils (AE 2001). Units for EC are generally reported as

milliSimens/cm (mS/cm, equivalent to μmhos/cm) for lower salt-impacted soils or deciSiemens/m (dS/m, equivalent to mmhos/cm) for higher salt-impacted soils (Tanji 2002), where 1 dS/m is approximately equal to 10 mM of total salts (Cramer 2002). The salt content in the soil is commonly estimated from electrical conductivity of saturated soil-paste (EC_e). However, due to the ease of measurement, EC values are often expressed in a fixed ratio (w/v) of soil to water extract (i.e. $EC_{1:1}$ or $EC_{1:2}$). The EC value obtained from fixed ratio extraction can be correlated back to the EC_e (Zhang et al. 2005) by determining a conversion factor, K (Equation 1). Depending on the soil properties, the K values range from two to four.

$$EC_e = K \times EC_{1:2} \qquad \text{(Equation 1)}$$

According to the Canadian Environmental Quality Guidelines (CCME 1991), the acceptable level of salt in soil expressed as EC_e is < 2 dS/m for agriculture land and < 4 dS/m for industrial land (AARD 2007).

Soil salinity can be divided into several ranges from non-saline to very strongly saline according to their electrical conductivity values (Table 1.1). Generally, salinity levels vary widely by season and soil depth, i.e. salinity levels vary widely from spring to fall (AARD 2007). Usually, salinity increases on soil surface just after spring thaw due to runoff and spring rains and will generally drop to lower levels after the arrival of autumn (AARD 2007). For surface soil, an EC_e higher than 2 dS/m is considered saline.

Table 1.1. Range of salinity based on electrical conductivity and soil depth (AARD 2007)

Soil depth (cm)	Non- saline	Weakly saline	Moderately saline	Strongly saline	Very strongly saline
0-60	< 2 dS/m	2-4 dS/m	4-8 dS/m	8-16 dS/m	> 16 dS/m
60-120	< 4 dS/m	4-8 dS/m	8-16 dS/m	16-24 dS/m	> 24 dS/m

In addition to salinity, high sodium concentrations can also impair soil quality and structure by causing surface cracking and clay dispersion (AE 2001). Poor soil structure can adversely affect plant germination, root elongation and penetration. Sodium concentration in soil (sodicity) is measured as the sodium adsorption ratio (SAR). This is a calculation of the amount of sodium (Na^+) relative to calcium (Ca^{2+}) and magnesium (Mg^{2+}) in soil solution (Equation 2), and indicates the contribution of Na^+ to total salinity. The concentration of Na^+, Ca^{2+}, Mg^{2+} are usually expressed in mM. The structure of the soil degrades as SAR increases, with the optimal values for SAR being less than 4.0. SAR values higher than 15 indicate that the soil is severely degraded (AE 2001).

$$SAR = \frac{[Na^+]}{\sqrt{([Ca^{2+}] + [Mg^{2+}]) / 2)}} \qquad \text{(Equation 2)}$$

To lower the SAR value and improve soil structure, gypsum ($CaSO_4 \cdot 2H_2O$), which is moderately water soluble and the most commonly applied calcium amendment, can be added to provide Ca^{2+} to replace excess Na^{2+} in the soil cation exchange complex (Qadir et al. 1996; Qadir et al. 2003). Another method is to add organic matter to counteract the adverse effects of exchangeable sodium. Over time, organic matter improves soil structure, permeability, aeration, and nutrients (AE 2001).

1.2 Effects of salinity on plants

Salinity is one of the most severe environmental stresses on plants (Munns and Tester 2008; Tester and Davenport 2003; White and Broadley 2001). The most common salt ions that inhibit plant growth are sodium and chloride (Tester and Davenport 2003). Na^+ is more toxic to plants than Cl^-, because Na^+ is the primary cause of ion-specific

damage, resulting in a range of disorders from enzyme activation to protein synthesis (Tester and Davenport 2003). Conversely, an excess of Cl^- that accumulates in shoots may inhibit photosynthesis (Flowers 1988). In general, the growth of shoots is affected by salinity to a greater extent than growth of roots. Salt primarily limits plant growth in three ways: 1) osmotic effects that lower the ability of plants to take up water from the soil, 2) ion-specific damage of excess Na^+ and Cl^-, and 3) nutrient deficiencies because elevated levels of Na^+ compete with the uptake of other nutrients by interfering with ion transporters (Tester and Davenport 2003). Symptoms of damage to plants include: growth inhibition, leaf discoloration, anatomical and morphological changes such as changes in cell wall structure (Tester and Davenport 2003).

1.2.1 Impaired growth

Highly saline soil (EC_e > 16 dS/m) can severely interfere with germination and growth of plants. As water and nutrients move from areas of low salt concentration to areas of high salt concentration, soil salinity prevents plant roots from taking up water and other nutrients into the plant, resulting in osmotic and nutrient imbalances that impair proper plant growth. Munns (2002) has summarized the sequential physiological responses of plants under salinity stress. The root tip acts as a finely tuned sensor for various kinds of stress (Colmer et al. 1994). A sudden increase in soil salinity will cause plant cells to shrink due water loss and immediate changes in expansion rates resulted from the osmotic effects of salt around the roots (Cramer and Bowman 1991; Munns 2002; Neumann 1993). After several hours, plant cells can restore their original shape; however, a decrease in cell elongation rates is observed in both leaves and roots (Hsiao and Xu

2000; Munns 2002). Continued exposure for a few days results in a decrease in plant growth (i.e., slower cell division and impaired cell elongation). In this case, leaves are often more sensitive to salinity than roots (Hsiao and Xu 2000; Munns 2002). Changes in plant cell dimension are observed more for area than depth, therefore, leaves appear to be smaller and thicker (Munns and Tester 2008). The effects of salinity become more apparent after a few weeks of exposure (Munns and Tester 2008). Yellowing or death of older leaves may be visible in salt-sensitive plants, where salt levels are high, due to increase uptake or inability to store salt in vacuoles (Karley et al. 2000; Munns and Tester 2008; Tester and Davenport 2003). Only the salt-tolerant plants are able to grow for several months under moderate salinity; however, early flowering or decreased production of florets may result (Munns 2002).

1.2.2 Toxicity of excess Na^+

Salinity imposes both ionic and osmotic stresses on plants. Influx of salt ions may cause excessive accumulation of Na^+ and Cl^- in the cytosol and lower the apoplastic water potential (Binzel et al. 1988). As salinity of the soil increases, osmotic damage can occur in plants as a result of the build up of excess salt. For most plant species, Na^+ is considered to be more toxic than Cl^- (Munns and Tester 2008). Once Na^+ is taken up by the roots, it can be rapidly translocated to shoots via the xylem where it accumulates as water evaporates. Further, recirculation of Na^+ back to roots is limited, suggesting that Na^+ transport is somewhat unidirectional resulting in accumulation in shoots and foliage (Tester and Davenport 2003). Na^+-specific damage is associated with the accumulation of Na^+ in leaves, resulting in necrosis and shortening the lifetime of individual leaves,

ultimately reducing net crop yield (Munns 1993). Plants need to maintain turgor pressure and water uptake. Without proper intracellular compartmentalization (i.e., vacuole storage), excess of Na^+ is toxic in plants due osmotic imbalances. Moreover, high soil Na^+ can also cause deficiencies of other nutrients by interfering with ion transporters. K^+ homeostasis is essential for cellular functions and this can be disrupted due to competition of Na^+ for K^+ binding sites. More than 50 enzymes are activated by K^+ in plant cells; however, Na^+ cannot substitute in this role, hence enzyme activity can be inhibited in the presence of excess Na^+ (Bhandal and Malik 1988). Also, protein synthesis requires high concentrations of K^+ for the binding of tRNA to ribosomes; therefore, competition of K^+ binding sites by Na^+ can cause severe damage in plant cells (Wyn Jones et al. 1979).

1.2.3 Toxicity of excess Cl^-

Chloride ion (Cl^-) is an essential micronutrient for higher plants. It is involved in oxygen evolution reactions in photosynthesis (Olesen and Andreasson 2003), maintaining electrical charge across membranes (Läuchli and Lüttge 2002), and adjusting osmotic potential in vacuoles and the cytosol (Flowers 1988). Toxicity and inhibition of photosynthesis may be occur if Cl^- is present in excess. In some plant species, such as soybean, citrus and grapevine, only small amounts of Na^+ reach the leaves whereas Cl^- can continue to accumulate in the leaves; therefore, Cl^- is considered a more toxic component in the aforementioned plants (Läuchli 1984; Munns and Tester 2008; Storey and Walker 1999).

1.3 Salt tolerance of plants

Salt resistance in plants is a complex phenomenon (Breckle 1990; Breckle 1995; Munns 1993). Adaptive physiological and biochemical responses of salt tolerant plants under salinity stress include: 1) osmotic adjustment and compartmentalization of salt into safe storage places such as vacuoles (James et al. 2006); 2) adjustments in ion transport from roots to leaves, such as controlled uptake, extrusion and sequestration of ions (Xiong and Zhu 2002); 3) production of phytohormones such as abscisic acid (ABA) and ethylene (Xiong and Zhu 2002); and 4) production of osmolytes, such as proline to maintain conformation of macromolecules (Ueda et al. 2007). Some examples of tolerance mechanisms under salinity stress can be found in Table 1.2.

To cope with salt stress, the most efficient way is to selectively take up ions into plants and exclude those that are toxic. When Na^+ gets into plants, it is stored in vacuoles within plant cells to maintain osmotic potential in the vacuole and cytoplasm. Translocation of Na^+ is achieved via Na^+ diffusion channels, Na^+ pumps, and Na^+/H^+ antiporters (Apse et al. 1999; Blumwald et al. 2000). As Na^+ accumulates in the vacuole, osmotic potential in the cytoplasm must be balanced with that in the vacuole. This is achieved by synthesis and accumulation of organic solutes that do not inhibit biochemical reactions in plants, such as proline and sucrose (Hu et al. 2000; Ueda et al. 2007; Xiong and Zhu 2002). In addition to osmotic and ionic stress, salinity also causes oxidative stress by producing excess reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and singlet oxygen ($^1O_2$) that can disrupt cellular structures and molecules (Mittler 2002). Antioxidant compounds, such as

Table 1.2. Tolerance mechanisms of halophytes on saline soil (adapted from Breckle 1990)

Avoidance	
1	Growth only during favourable seasons
2	Grow only in favourable areas
3	Limitation of root growth to selected soil horizons
Adaptation processes	
1	Selectivity against Na^+ and Cl^-
2	Exclusion of salt from shoots
3	Diversion of salt out of assimilating tissues
4	Compartmentalization of salt within plant, tissue, and cells
5	Synthesis of organic solutes and osmolytes
7	Disposal of older plant parts ("salt-filled organs")
Tolerance	
1	Increase salt tolerance of tissues, cells, and organelles
2	Increase in halo-succulence
	a) Increase in leaf-succulence
	b) Increase in stem-succulence, reduction of leaves

ascorbic acid, glutathione, thioredoxin and carotenoids, can scavenge these ROS and enhance salt tolerance of plants (Xiong and Zhu 2002).

Plant species vary in how well they tolerate salt-affected soils. Some plants will tolerate high levels of salinity while others can tolerate little or no salinity. The relative growth of plants in the presence of salinity is termed their salt tolerance. Summary of the tolerance levels of various crops and forage grasses can be found in Table 1.3. Examples of salt tolerant crops include: oats, barley, wheat, and sugarbeet. Salt tolerant grasses include: tall wheatgrass and alkaligrass.

1.4 Phytoremediation as a potential technology for remediation of salt-impacted soil

Soil salinity severely diminishes available vegetative lands; as a result, much effort has been put into research on economical and effective methods to restore vegetation on these salt-impacted soils. Conventional methods for removal of salt from soil include disposal of surface layers and soil washing. However, these methods are impractical, labour intensive, expensive, and destructive to soil structure. For the aforementioned reasons, phytoremediation has been studied extensively for the past 30 years as a potential solution to restoring salt-impacted lands (USEPA 2000).

Phytoremediation is a technique that uses plants to mitigate organic or inorganic contaminants in soils. This technique has been widely studied to remediate metals, petroleum waste, pesticides, and salt-impacted soils (Bose et al. 2008; Huang et al. 2005; Huang et al. 2004; Lunney et al. 2004; Olson et al. 2008; Qadir et al. 2007; Su et al. 2008; Zeeb et al. 2006). Phytoremediation can be classified based on the contaminant

Table 1.3. Salt tolerance of various types of plants (adapted from AE 2001)

Salt Tolerance EC (dS/m)	Field Crops	Angiosperm	Forages	Angiosperm
Very High			beardless wildrye	M
>20			fulks altai grass	M
			levonns alkaligrass	M
High	kochia	D	altai wildrye	M
16-20	sugar beets	D	tall wheatgrass	M
			slender wheat grass	M
8-16	6-row barley	M	birdsfoot trefoil	D
	sunflower	D	sweetclover	D
	2-row barley	M	alfalfa	D
	fall rye	M	bromegrass	M
	winter wheat	M		
	spring wheat	M		
Moderate	oats	M	crested wheatgrass	M
4-8	yellow mustard	D	intermediate	M
	meadow fescue	M	wheatgrass	M
	canola	D	reed canary grass	M
	corn	M		
Low	timothy	M	white dutch clover	D
0-4	peas	D	alsike clover	D
	field beans	D	red clover	D

- D represents dicotyledonous
- M represents monocotyledonous

fate or mechanism of remediation: degradation, extraction, volatilization, transformation, filtration, or a combination of these (Cunningham and Ow 1996; Kömives and Gullner 2000; Salt et al. 1998). The most common plant mechanism for salt remediation is phytoextraction in which plants take up and accumulate salt in the above-ground portions of the plants. This foliage can be harvested from a given site and transported to another location (Kömives and Gullner 2000).

Phytoremediation could become a cost-effective and environmentally sound technology for remediation of salt-impacted sites if it can be properly developed. There are certain limitations that must be overcome for this plant-based remediation system to come into common usage. Phytoremediation can be time-consuming because it requires several growing seasons to lower the level of contaminants in soil. It is also limited to soil depths that are in the rooting zone (USEPA 2000). Furthermore, successful remediation of soil with high levels of salt is hard to achieve by the fact that plant growth and germination is inhibited by salinity. As a result, finding salt tolerant plants that have deep and vigorous root growth, as well as sufficient above-ground biomass production are some of the basic criteria for the selection of plants for remediation of salt-impacted sites.

1.5 Effect of plant growth-promoting rhizobacteria (PGPR) on plant growth under salt stress

Saline soil remediation can be performed via plant growth if sufficient biomass can be generated. The efficiency of this method depends on the production of above-ground plant biomass; greater biomass results in more rapid remediation. However, salinity can severely diminish plant growth and trigger a wide range of negative responses in plants.

Examples of these responses include: diminished water potential, smaller leaf size, alteration of cellular metabolism, and increased ethylene production (ethylene is a stress hormone that inhibits plant growth). Hence, one of the challenges of research is to improve plants growth under conditions of salt stress.

Plant growth-promoting rhizobacteria (PGPR) are found in association with the roots (rhizosphere) of many different plants (Kloepper et al. 1989) and affect plant growth and development either indirectly or directly (Glick 1995). The indirect promotion of plant growth occurs when these bacteria decrease or prevent some of the deleterious effects of a phytopathogenic organism, usually a fungus, by a number of different mechanisms. Alternatively, the direct promotion of plant growth by PGPR is to provide plants with compounds that are synthesized by the bacterium, or to facilitate the uptake of nutrients from the environment (Glick 1995). For example, atmospheric nitrogen can be fixed by PGPR and supplied to plants, as well as synthesizing siderophores, which can solubilize and sequester iron from soil and make it available to the plant cells. Furthermore, PGPR are able to synthesize phytohormones such as auxins, cytokinins or gibberellins that could stimulate cell division and help plants to tolerate a variety of environmental stresses (Glick 2004; Glick and Bashan 1997).

It has been found that a number of PGPR contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme cleaves ACC, the precursor of ethylene in plants, to ammonia and α-ketobutyrate (Glick et al. 1998). Therefore, the presence of PGPR with ACC deaminase may lower the levels of ethylene in developing or stressed plants, enhance the survival of some seedlings, and facilitate the formation of longer roots (Figure 1.1). In addition to ACC deaminase, some groups of

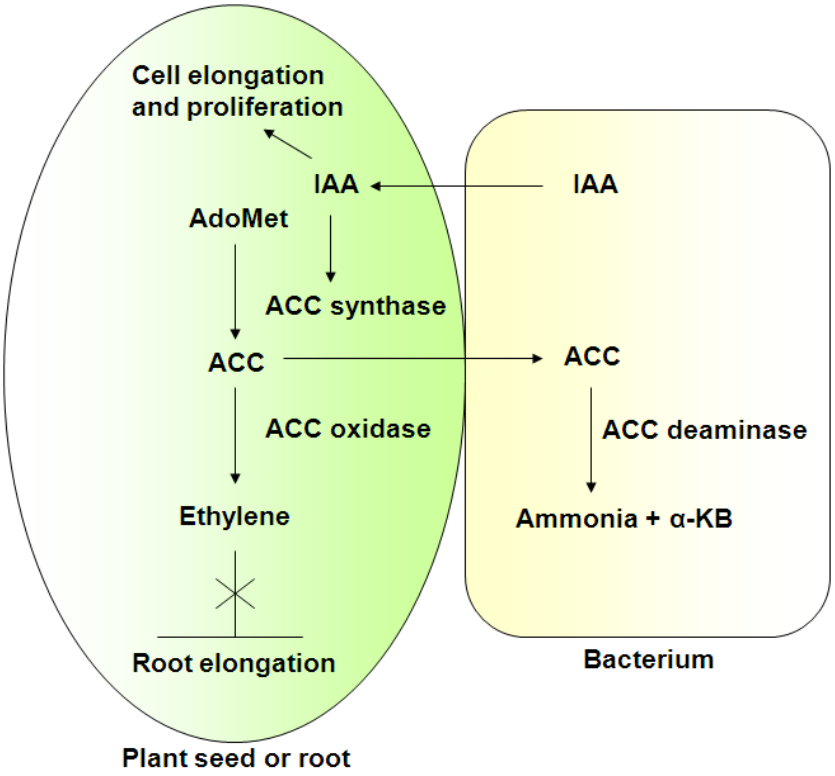


Figure 1.1. Schematic diagram of how PGPR containing ACC deaminase lower the ethylene precursor, ACC (Glick et al. 1998). The ACC deaminase of the bacterium may lower ethylene levels in plants by degrading ACC to ammonia and α-ketobutyrate. Decreasing ethylene in plants may alleviate stress and thereby improve plant growth. Some groups of PGPR are also capable of producing phytohormone, IAA, which further stimulates plant growth by conferring plant cell proliferation as well as root elongation.

PGPR may also synthesize and secrete indole-3-acetic acid (IAA), which can be absorbed by plant seeds or roots (Fallik et al. 1994; Hong et al. 1991). The assimilated IAA can stimulate plant cell proliferation and elongation. Meanwhile, IAA stimulates the activity of ACC synthase to convert S-adenosyl-methionine (SAM) into ACC (Kende 1993). A significant portion of ACC may be exuded from the roots or seeds and taken up by PGPR containing ACC deaminase activity to hydrolyze ACC to yield ammonia and α-ketobutyrate. As the uptake and hydrolysis of ACC by the PGPR decreases the ACC level in plants, the biosynthesis of the stress hormone ethylene is impeded, facilitating plant growth under stress conditions.

It has been shown that PGPR promotes plant growth under saline conditions in several laboratory and field studies (Chang 2007; Cheng et al. 2007; Lifshitz et al. 1987; Mayak et al. 2004a; Mayak et al. 2004b; Nadeem et al. 2007; Saravanakumar and Samiyappan 2007). Mayak et al (2004a) reported that an ACC-deaminase-containing PGPR, *Achromobacter piechaudii* ARV8, can significantly lowered ethylene production and increased biomass production of tomato plants grown in the presence of up to 172 mM NaCl. Recently, Nadeem et al. (2007) reported that several strains of ACC-deaminase-containing PGPR significantly increased plant height, root length, total biomass and grain yield in maize under salt stress. Saravanakumar and Samiyappan (2007) reported that ACC-deaminase containing *Pseudomonas fluorescens* strain TDK1 significantly promoted plant growth in groundnut seedlings under salt stress relative to the strains lacking ACC-deaminase and untreated control treatments. Cheng et al. (2007) also found that inoculation of *Pseudomonas putida* UW4 containing ACC-deaminase significantly improved shoot biomass of canola, whereas inoculation of the mutant strain of UW4

lacking ACC- deaminase activity (UW4/AcdS$^-$) did not promote plant growth. The effect of PGPR on plant growth promotion in phytoremediation studies of salt-impacted soils was confirmed by Greenberg et al. (2008). Results from each of these cases supported the proposed model that PGPR with ACC-deaminase activity can lower ethylene production in plants and hence facilitate plant growth under saline stress.

1.6 Electrolyte leakage method for assessing cell membrane stability in plants

The plant cell membrane plays an important role in the maintenance of the micro-environment and normal metabolism of the plant cell, and is often one of the first targets of many plant stresses such as low and high temperatures (Ismail and Hall 1999; Maheswary et al. 1999; Saelim and Zwiazek 2000), air pollution (Garty et al. 2000), acid conditions (Spencer and Ksander 1999), metals (De and Mukherjee 1996), and salt (Chen et al. 1999). Salt stress will lead to damage in plant cell membrane and increases its permeability. As a result, electrolytes that are contained within the membrane will leak into surrounding tissues (Campos et al. 2003). Therefore, the maintenance of cell membrane stability and integrity is important to salt tolerance in plants. The degree of injury in cell membranes can be estimated through measurements of electrolyte leakage from cells by comparing the conductivity of the leaked contents from plant tissues in water (McNabb and Takahashi 2000). This electrolyte leakage technique is an appealing method for estimation of plant cell damage and hardiness because it is simple, rapid, uses readily available and inexpensive equipment, and is suited to analyzing large numbers of samples. In the present study, an electrolyte leakage method was employed to assess cell membrane

damage in plants under salinity stress, and to determine whether PGPR improves plant cell membrane stability.

1.7 Pulse amplitude modulation (PAM) fluorometry for measurement of photosynthetic activity in plants

Photosynthesis is a physiological process in plants that couples energy of light to form: 1) carbohydrates, 2) proton motive force, and 3) adenosine triphosphate (ATP) as energy sources (Papageorgiou 2004). Photosynthesis is initiated by absorption of light and conversion of photon energy to chemical energy. During this procedure, H_2O is split to O_2 (which is released into the atmosphere) and to electrons and protons, which participate in the electrochemical reactions where redox and proton gradients are coupled to phosphorylation of adenosine diphosphate (ADP) and the fixation of CO_2 to sugars (Papageorgiou 2004).

The photosynthetic electron transport chain consists of three protein complexes: PSII, the cytochrome b_6/f complex, and PSI (Figure 1.2) (Andersson and Barber 1994). Photosystem II (PSII) includes the light-harvesting center II (LHCII), the oxygen-evolving complex (OEC) on the lumen side of the membrane, the reaction center P_{680}, the primary electron acceptor pheophytin (Pheo) and the secondary acceptors Q_A and Q_B. When Q_B is reduced twice, it migrates to the lipid bilayer as part of the reduced plastoquinol pool (PQH_2). At the cytochrome b_6f complex (cyt b_6f), PQH_2 is oxidized to PQ, which migrates back to the Q_B binding site of PSII. The cyt b_6f complex transfers the electrons to the next mobile component plastocyanin (PC), which in turn migrates into the lumen. Photosystem I (PSI) contains the light-harvesting center I (LHCI), the reaction center P_{700} and a number

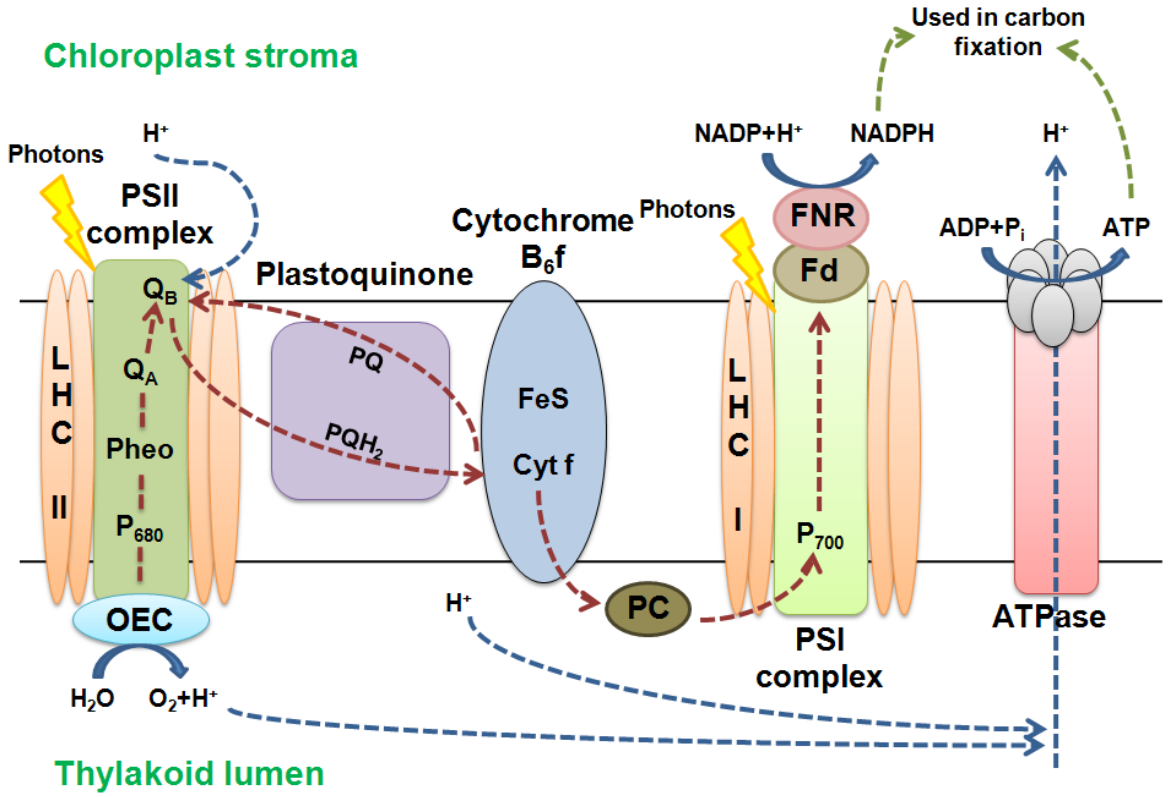


Figure 1.2. Schematic picture (without stoichiometry) of the thylakoid membrane showing the components of photosynthetic electron transport chain (Andersson and Barber 1994).

of electron acceptors. The electrons are transferred from PSI to NADPH via ferredoxin (Fd) and ferredoxin $NADP^+$-reductase (FNR). The formation of NADPH initiates the transport of protons into the thylakoid space. The additional proton light-driven transport of electrons from H_2O to $NADP^+$ forming NADPH initiates the transport of protons into the thylakoid space. Additional protons are split off from water by the OEC, yielding O_2. The resulting pH gradient across the membrane powers the synthesis of ATP via the ATP synthase/hydrolase complex (ATPase).

Environmental stresses such as air pollutants, herbicides, heavy metals and salinity often result in deleterious effects on photosynthesis in plants (Beauregard et al. 1987; Bowyer et al. 1991; Fuerst et al. 1985; Jiang et al. 2006; Krupa et al. 1993; Pell et al. 1997; Woolhouse 1983). Diminishments in plant growth due to salt stress are often associated with a decrease in photosynthesis. It has been found that salt stress has negative impacts on photosynthetic efficiency in *Brassica* putida (Nazir et al. 2001) and wheat (Raza et al. 2006). During salt stress, a net decrease in photosynthetic rate, possibly due to decrease in stomatal conductance, was found in cotton (Meloni and Oliva 2003), leading to a decrease in the water evaporation rate or CO_2 uptake through the stomata in plant leaves (Brugnoli and Björkman 1992). Furthermore, osmotic stress and ionic toxicity are often associated with salt stress. Studies show that osmotic stress results in a decrease of chloroplast volume and an increase in Na^+ ion concentration in the cytosol which ultimately can inactivate photosynthetic electron transport (Allakhverdiev et al. 1996; Price and Hendry 1991). The inhibition of photosynthesis is a good measure of the physiological state of the plant. Changes in overall rate of photosynthesis activity, photosynthetic electron transport efficiency, intactness of PSII and possible photoinhibition may result when plants are

under stress (Juneau and Popovic 1999; Krause and Weis 1991; Maxwell and Johnson 2000; Rosenqvist and Kooten 2003). Therefore, measurement of photosynthesis can be used as an indication of the extent to which plants are salt stressed.

Chlorophyll fluorescence has become a key technique in plant biology to assess photosynthetic activity (Baker 2008). It acts an indicator of plant adaptation to the environment or stress. The advantage of chlorophyll fluorescence over many other techniques is that it can provide rapid and non-destructive measurements (DeEll and Toivonen 2003; Schreiber et al. 1994). This technique has been used to measure cellular responses to salinity and degrees of salt stress in plant leaves (Jiang et al. 2006; Smillie and Nott 1982). The principal of chlorophyll fluorescence analysis is that light absorbed by chlorophyll can be used in 3 ways: 1) energy to drive photosynthesis; 2) dissipation as heat; or 3) remission as light (chlorophyll a fluorescence). These three processes are in competition such that an increase in efficiency of one form will result in a decrease in the other two (Baker 2008; Butler 1978; Maxwell and Johnson 2000).

For this study, chlorophyll *a* fluorescence was measured with pulse amplitude modulation (PAM) fluorometry. PAM fluorometry gives information on the functionality of PSII such as flow of electrons and rate of photosynthesis. With this technique, a small amount of light is modulated (emitted in pulses) and used as the measuring light. While heat dissipation is relatively constant, comparison of several chlorophyll *a* fluorescence parameters (e.g. F_v/F_m, yield, qP, and qN) can be use to assess the efficiency of photochemistry in plants and to study the effect of salinity on photosynthetic electron transport (Maxwell and Johnson 2000). Nomenclature of chlorophyll fluorescence derived from PAM induction curve can be found in Figure 1.3.

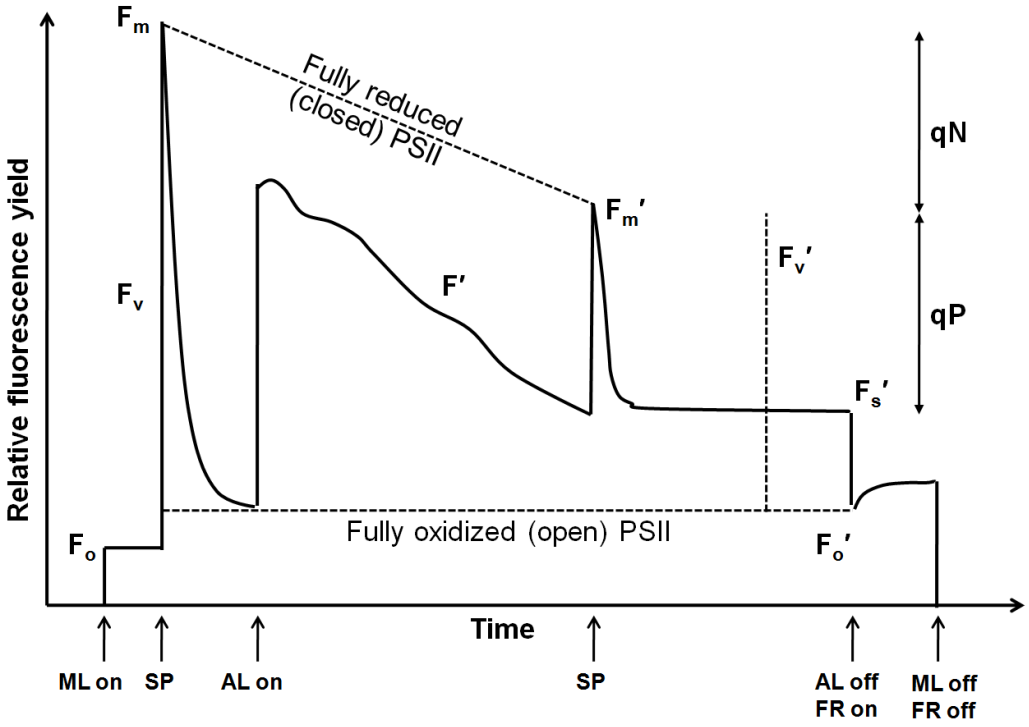


Figure 1.3. Nomenclature of PAM fluorescence parameters derived from recordings of a dark-adpated leaf. ML= modulated measuring light, SP= saturating pulse, AL = actinic light, FR= far-red light (Van Kooten et al. 1987).

F_m is the maximal fluorescence of dark-adapted tissue and F_0 is the minimal fluorescence (background fluorescence). From F_m and F_0, the F_v/F_m ratio was calculated. The parameter F_v/F_m $[(F_m-F_0)/F_m]$ is a measure of maximum quantum yield of PSII, or the potential quantum efficiency if all PSII centers were open (Maxwell and Johnson 2000). The measurement of F_v/F_m provides a measure of the intactness of the PSII/LHC complex. It gives information on the probability that a trapped photon will end up in the reaction center and cause a photochemical event. Any change in the state of photosystem II will cause a decrease in the value of F_v/F_m (Maxwell and Johnson 2000).

The optimal value of F_v/F_m varies between 0.79 to 0.83 for most plant species (Björkman and Demmig 1987; Johnson et al. 1993), and lower values indicate that the plant is stressed or not at optimal health. Yield of steady-state photosynthesis $[(F_m' - F_s)/F_m']$ can be calculated from the maximal fluorescence in light-adapted tissue (F_m') and stead state fluorescence (F_s). The yield of photosynthesis is a measurement of photosynthetic efficiency (Genty et al. 1989), which is proportional to the light absorbed by chlorophyll associated with PSII that is used in photochemistry. It can provide an indication of overall photosynthesis (Maxwell and Johnson 2000). The parameter qP $[(F_m' - F_s)/(F_m' - F_o')]$ is a measure of photochemical quenching, which is an indication of the proportion of PSII reaction centers that are open and equals the approximate oxidation of PSII (Dietz et al. 1985; Schreiber et al. 1994; Schreiber et al. 1986; Weis and Berry 1987). The term qN $[1- (F_m' - F_o)/(F_m-F_0)]$ measures the non-photochemical quenching of fluorescence, which is related to the dissipation of energy as heat and indicates the extent of photoinhibition (Maxwell and Johnson 2000; Rosenqvist and Kooten 2003; Schreiber et al. 1986).

1.8 Research objectives

Upstream petroleum production causes salinity problems in Western Canada. Phytoremediation offers a potentially efficient, cost-effective, and non-destructive technology for removal of salt from salt-impacted soils. Successful phytoremediation relies on the ability of plants to grow and accumulate salts in the shoots, removing the salt to the above-ground plant biomass. Thus, it is crucial to ensure that enough plant biomass is produced for efficient remediation. However, salinity is highly phyto-toxic and can inhibit plant germination and growth. By lowering the salt stress on plants with the use of PGPR, more biomass of plants could be produced, and ultimately increase phytoremediation efficiency on salt-impacted soil. Therefore, the goal of this research was to improve plant growth on highly saline soils using PGPR treatment of seed, to examine the potential for PGPR to alleviate salt stress, as well as to evaluate the feasibility of using plants with the aid of PGPR for phytoremediation on salt-impacted sites.

Three naturally occuring, non-pathogenic PGPR were used for greenhouse and field trials in this research. They are *Pseudomonas putida* UW4 (Glick et al. 1995), *Pseudomonas putida* UW3 (Glick et al. 1995) and *Pseudomonas corrugata* CMH3 (Chang 2007). All of these bacterial strains have high 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and are naturally occurring in rhizosphere soil (Penrose and Glick 2003). However, plants have been shown to respond differently dependent on the PGPR used (Chang 2007). Therefore, effects of different PGPR to different plants were examined in this research based on plant biomass production, photosynthetic activity, cellular membrane stability, and salt uptake ability. The specific goals of this research are:

1. Selection of salt tolerant plant species that are able to grow on moderately to highly saline soil for feasible phytoremediation. In this research, several salt tolerant cereal and grass plant species were tested in greenhouse and field trials; barley (*Hordeum vulgare*), oats (*Avena sativa*), tall fescue (*Festuca arundinacea*), and tall wheatgrass (*Agropyron elongatum*). It is expected that these salt tolerant plant species will respond to PGPR and will be able to grow on highly salt-impacted soils.
2. Selection of PGPR/plant combinations for optimization of plant growth improvement. In this study, three PGPR strains, UW3 (*Pseudomonas putida*), UW4 *(Pseudomonas putida*) and CMH3 *(Pseudomonas corrugate*) were coated on plant seeds either separately or in combination to examine their effects on various plant species. Because plants might respond to PGPR differently, it is important to select the best combinations of PGPR and plants for optimal phytoremediation results.
3. Study the effect of PGPR on plants in terms of biomass production. As phytoremediation of salt relies on the amount of above-ground plant biomass that can be produced, it is important to quantify PGPR effects in terms of plant growth promotion. This goal is achieved by measurement of the fresh and dry weights of plant shoots and roots. It is expected that plants will produce greater above-ground biomass due to treatment of PGPR.
4. Study the effect of PGPR on plants in terms of photosynthetic activity. Decreases in plant growth due to salt stress are often associated with an impairment of photosynthesis. In this research, salinity stress on photosynthetic activity in plants was measured by chlorophyll *a* fluorescence using pulse amplitude modulated (PAM) fluorometry. The question of whether PGPR have an effect on relieving photosynthetic

stresses due to salt was also examined. It is expected that PGPR will relieve photosynthetic stresses in plants, while promoting better plant growth.

5. Study the effect of PGPR on plant cell integrity. Salinity stress can lead to damage in plant cell membrane, and thus increase in its permeability. In this research, the effect of soil salinity on plant cell membrane was examined by measurements of electrolyte leakage from cells. Effects of PGPR on maintaining membrane stability was examined by comparing the conductivity of leaked ions from plants treated with and without PGPR. It is expected that PGPR will alleviate the extent of membrane damage in plants due to salinity stress, and helps plants to maintain their membrane integrity.
6. Measure Na, Cl, Ca, Mg, and K accumulation in plants. Phytoremediation of salt-impacted soil relies on plants to accumulate ions in above-ground biomass that can be removed from soil via harvesting the foliage. It is expected that PGPR may increase salt uptake in certain plant species.
7. Assessment of PGPR enhanced salt phytoremediation in the field. The ultimate goal of this research is to employ PGPR to enhance phytoremediation of salt-impacted soils in field applications. In this research, phytoremediation efficiency of salt-impacted soils with PGPR was evaluated on three oil fields in Saskatchewan, Canada. Soil salinity levels of field sites were monitored through a two-year study. It is expected that PGPR will improve phytoremediation efficiency on salt-impacted soil by producing greater plant matter. The increased plant biomass production would lead to greater salt accumulation in plant tissues. Hence, an ultimate increase in salt removal from soils and remediation efficiency is expected.

Chapter 2 - Material and Methods

2.1 Research field sites

In this research, three salt-impacted sites was used. Soil salinity of the field sites was monitored through a two-year long study (May 2007-Nov 2008). Field data for 2007 was documented previously (Chang 2007). The present work focused on 2008 results. This research was divided into greenhouse and field studies. All greenhouse experiments used soils from the field sites to study the application of PGPR to enhance phytoremediation of salt-impacted soils.

2.1.1 Cannington Manor South (CMS) and North (CMN) sites.

Both Cannington Manor South (CMS) and North (CMN) sites are located in Cannington Manor, Carlyle, Saskatchewan, Canada. The suspected cause of salt contamination for these two sites was leakage of a brine water storage tank in the winter years ago, and the brine water spread over a wide area on the frozen ground. The land has been treated with of gypsum ($CaSO_4$) and planted with foxtail barley (*Hordeum jubatum*) in attempts to re-establish vegetation over the past four decades. A 4-inch layer of compost was mixed into the top soil of both sites before the planting in May 2007.

The CMS and CMN sites are 400 m apart from each other and the CMS site is at a lower elevation leading to frequent flooding in Spring. The planting designs for 2008 of CMS and CMN sites are presented in Figure 2.1 and Figure 2.2, respectively. The CMS site is rectangular-shaped, 0.14 hectare (0.33 acre) in size with dimensions of 90 m $\times$ 15 m.

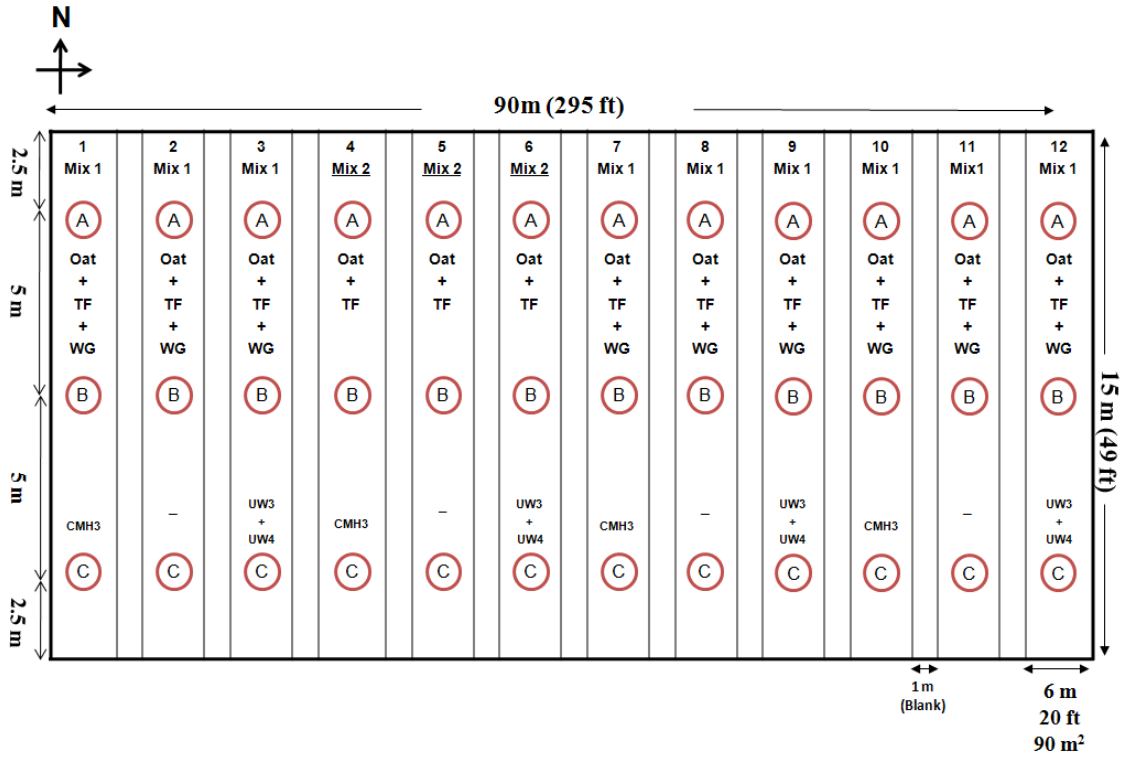


Figure 2.1. Planting and soil sampling design for Cannington Manor South (CMS) site in 2008. For each strip, plants were sown as a mix. Mix 1: oats + inferno tall fescue (TF) + tall wheatgrass (TW). Mix 2: oats + inferno tall fescue (TF). ◯ Indicates the area on the site from where soil samples were taken.

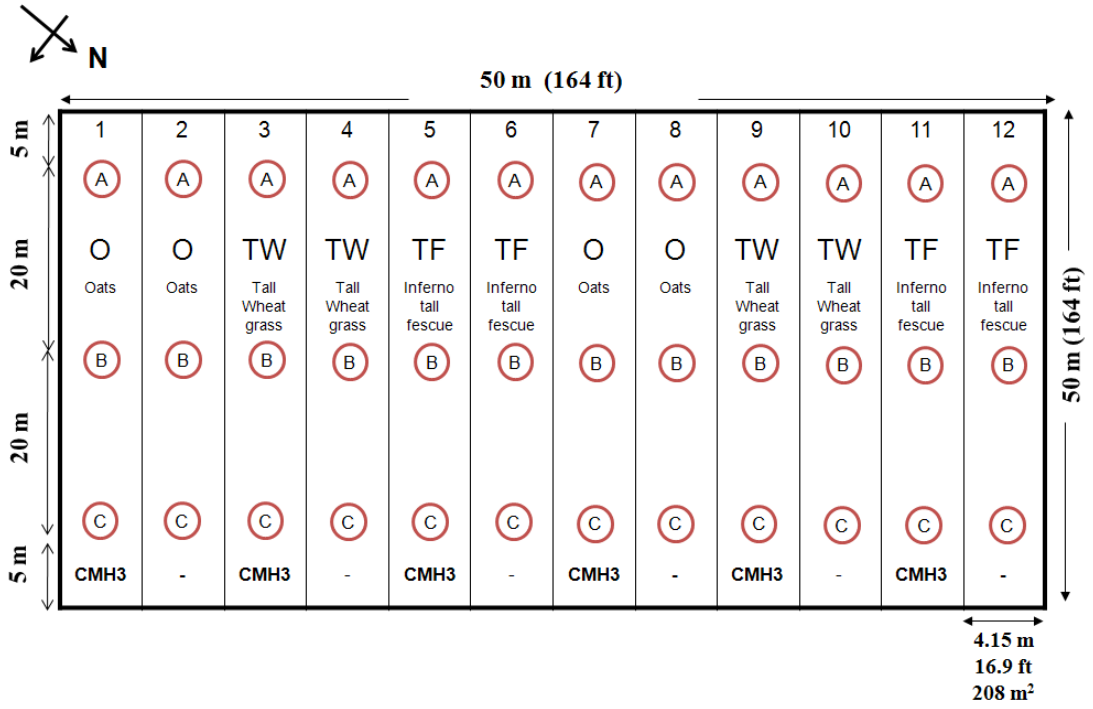


Figure 2.2. Planting and soil sampling design for Cannington Manor North (CMN) site in 2008. Three plant species were sown separately on each strip: oats (O), inferno tall fescue (TF), and tall wheatgrass (TW). ◯ Indicates the area on the site from where soil samples were taken.

The salt level of the CMS site is considered high with an average EC_e of 17.6 dS/m (ranging from 5 to 36 dS/m) at the end of season in 2007, and an average ECe of 14.5 dS/m (ranging from 2 to 32 dS/m) before planting in May 2008. The CMN site is square-shaped and 0.25 hectare in size (0.62 acre) with dimensions of 50 m × 50 m. The salt level of the CMN site is considered low in contrast to CMS. The average EC_e of entire CMN was 6.5 dS/m (section EC_e ranging from 2 to 25 dS/m) at end of season in 2007, and an average EC_e of 7.1 dS/m (section EC_e ranging from 2 to 16 dS/m) before planting in May 2008.

2.1.2 Alameda site (AL)

The Alameda site (AL) is located in Alameda, near Estevan, Saskatchewan, Canada. The history and source of salt contamination is unknown, however, it is suspected that the saline soils came from a flare pit a few years ago. A 4-inch layer of compost was mixed into the top soil of the site before planting in May 2007.

Planting design for 2008 of AL site is presented in Figure 2.3. The AL site is a 0.16 hectare (0.4 acre) irregularly shaped plot with overall dimensions of approximately 85 m × 25 m. The salt level of AL site is extremely high. The average EC_e of the entire AL site is 23.5 dS/m (section EC_e ranging from 11 to 37 dS/m) at end of season in 2007, and an average EC_e of 27.0 dS/m (section EC_e ranging from 11 to 45 dS/m) before planting in May 2008.

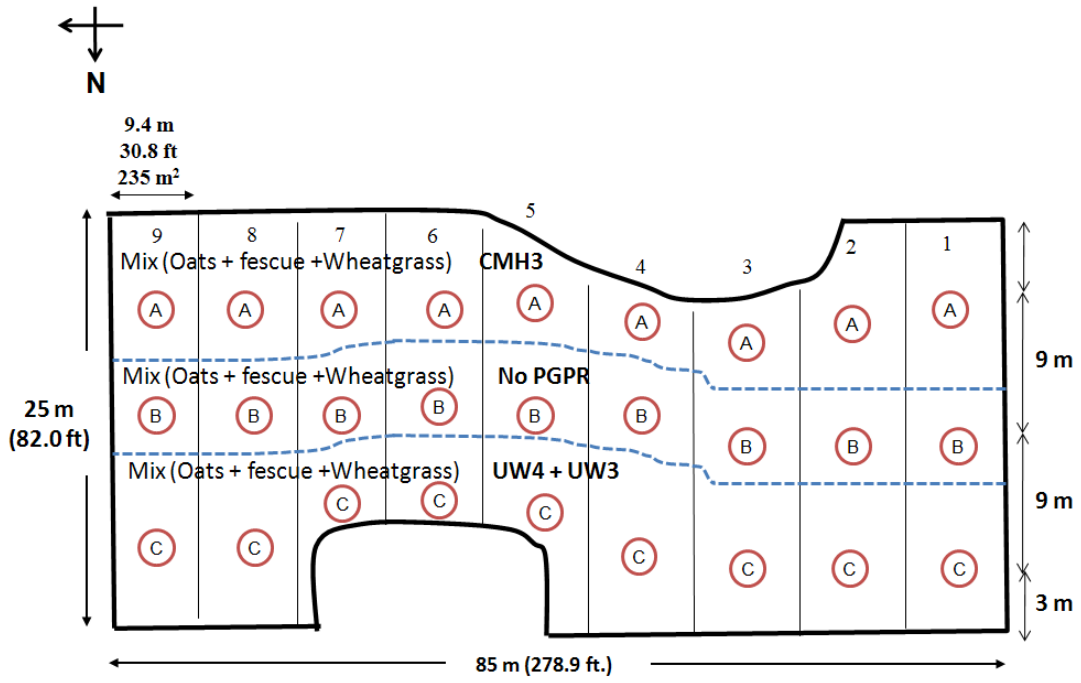


Figure 2.3. Planting and soil sampling design for Alameda (AL) site in 2008. Plants were sown as a mix with oats, inferno tall fescue and tall wheatgrass. ◯ Indicates the area on the site from where soil samples were taken.

2.2 Measurement of soil salinity

Soil salinity was measured as electrical-conductivity based on $EC_{1:2}$ (1:2 ratio of soil to water extract) and EC_e (soil saturated with water). Measurements were carried out according to published methods (Chang 2007; Janzen and Chang 1988; Rhodes et al. 2002) with slight modifications. Soil samples were air dried to remove moisture, pulverized, and then sieved using a 4-mm particle size sieve. All EC_e measurement was performed in triplicate. An aliquot of 50 g soil was mixed with sufficient ddH_2O (de-ionized and distilled water) in a 100-mL beaker to reach saturation. The characteristics that are required for saturation include: 1) a shiny appearance of the soil paste; 2) the paste flows slightly when dispersion is made in the surface; and 3) soil paste slides cleanly from an aluminum spatula. The sample was allowed to settle for at least 4 hours and was checked to ensure the saturation criteria were met. If free water had accumulated on the surface, small amounts of soil were added and the paste remixed. If the soil had stiffened or dried, ddH_2O was added and the paste remixed. This was repeated until all saturation criteria were met. The mixture was filtered through a Buchner funnel or transferred to a 50 mL Falcon sterile culture tube and centrifuged at 2000 r.p.m. for 10 minutes. The electrical-conductivity of the filtrate or supernatant was then measured with an electrical-conductivity meter (Oakton Instruments, Vernon Hills, IL, US).

For $EC_{1:2}$ measurements, samples were performed in triplicate. An aliquot of 15 g of soil was mixed with 30 mL ddH_2O in a 50 mL plastic Falcon culture tube. The mixture was shaken on a shaker table at 80 r.p.m. for 30 minutes before centrifugation at 2000 r.p.m. for 10 minutes. The electrical-conductivity of the supernatant was measured with an electrical-conductivity meter (Oakton Instruments, Vernon Hills, IL, US). The K values

were determined by the ratio between the $EC_{1:2}$ and EC_e according to equation 1. The calculated K value for CMS, CMN and AL site was 2.0, 2.5, and 2.6, respectively.

2.3 Proliferation of PGPR from stock cultures to prepare for seed treatments

Experimental procedures were carried out according to published methods with modifications (Lifshitz et al. 1987; Penrose and Glick 2003). An aliquot of 100 µL of bacterial glycerol stock was added to 50 mL of sterile tryptic soy broth (TSB) medium contained in a 250 mL Erlenmeyer flask and grown at 23 ± 1 ℃ on a rotary shaker at 80 r.p.m. for 24 hours.

2.4 Seed treatment with PGPR

Seed treatment with PGPR was followed by published methods (Greenberg et al. 2008; Greenberg et al. 2007). PGPR strains of *Pseudomonas putida* UW3 (Glick et al. 1995) , *Pseudomonas putida* UW4 (Glick et al. 1995), and *Pseudomonas corrugata* CMH3 (Chang 2007) were prepared by the method stated in Section 2.3. The bacterial culture grown in TSB was transferred aseptically into a sterile 50 mL Falcon tube, centrifuged at 2000 r.p.m. for 20 minutes. The cell pellets were washed and resuspended with 50 mL of 0.1% (w/v) sodium pyrophosphate to remove secondary metabolites, and centrifuged again at 2000 r.p.m. for 20 minutes. The final bacterial pellet was resuspended in sterile ddH_2O (de-ionized and distilled water) to an absorbance of 2.0 at 600nm.

A polymer (Sigma, Oakville, Canada) was also added to facilitate adhesion of the bacterial cells to the seed surface. To prepare the polymer, 15.0 g of methylcellulose powder (Sigma, Oakville, Canada) was dissolved in 1.0 L of ddH_2O and stirred for one

hour. The solution was then autoclaved for at 131 ºC and 30 psi for 30 minutes, after which a gelatinous solid formed. Upon cooling, the gel liquefied (reverse-gelatinisation) into a slurry form. This polymer was added to the bacterial suspension at a rate of 200 mL per liter of bacterial suspension. A commercial non-toxic blue colorant (Color Coat Blue, Becker Underwood, Saskatchewan) was added into the bacterial-polymer slurry at a ratio of 17.5 mL to 1 L of slurry. The presence of colorant was necessary to meet safety regulations requiring all treated seeds to be visibly colored to avoid use for animal consumption.

Regardless of the seed type, an aliquot of 5 mL of the blue bacterial-polymer slurry were applied to 500 mL equivalent of seeds using a seed treater (HEGE 11, Wintersteiger Inc., Austria) and the machine ran for one minute. The dried seeds were immediately transferred into sealed plastic bags and stored at 4 ºC for a maximum of two weeks prior to usage.

2.5 Growth of cereals and grasses on salt-impacted soil

Several salt tolerant plant species were tested for their response to PGPR: barley (*Hordeum vulgare* C.V. AC ranger), oats (*Avena sativa* C.V. CDC baler), tall fescue (*Festuca arundinacea* C.V. Inferno), and tall wheatgrass (*Agropyron elongatum*). Barley was purchased from Cribit Seeds (ON); oats and tall fescue were purchased from Ontario Seed Company (ON); tall wheatgrass was purchased from Wagon Wheel Seed Corp (SK). Seeds were inoculated with PGPR according to the method stated in Section 2.4. Control seeds that were coated with the methylcellulose polymer and colorant, but de-ionized and distilled water (ddH_2O) was substituted for the bacterial culture in the slurry.

Salt-impacted soil from the field sites was sieved through 50-mm mesh and mixed to be as homogeneous as possible before use. Properties of the salt-impacted soils can be found in Appendix (Table 1). Plant seeds treated with and without PGPR were planted in plastic 6 × 6 × 5 cm (length × width × height) pots with 2 small holes at the bottom for drainage. Each pot was filled with 80 – 100 cm^3 of sieved soil, either 15 of cereal seeds (i.e. barley or oats) or 0.30 g of grass seeds (approximately 150 seeds for tall fescue, and 90 seeds for tall wheatgrass) were sown. After evenly spreading out the seeds on soil, a thin layer (0.5 - 0.8 cm) of sieved soil was applied to cover the seeds. All pots were contained in a tray (without holes) to prevent salt leaching from soil and were placed in the greenhouse. The day time temperature ranged from 25 – 35 ℃ and the night time temperature ranged from 18 – 27 ℃. Plants were irrigated once or twice before germination and irrigated daily after germination. Lighting source was natural sun light with no supplemental lighting. After plants were established, fresh and dry weight of shoots and roots were measured at various growth stages. For some experiments, plant membrane stability and photosynthetic activity were also examined.

2.6 Assessment of plant cell membrane stability using the electrolyte leakage method

The ion leakage measurement was modified from published procedures (Bajji et al. 2002; Campos et al. 2003). Fresh plant samples for this assay were taken from greenhouse after 12 days of growth on salt-impacted soil (under conditions stated above). Fresh shoot samples (1 g fresh weight) of similar size or phase of growth were cut into approximately 3 cm long segments, washed with ddH_2O, and blotted dry with a Kimwipe. Segments were

submerged in 10 mL of ddH_2O in a 20 mL test tube and the test tube was placed into a vacuum dessiccator. Using a vacuum pump (Savant, VP 100, New York, USA), the samples were subject to a vacuum at a rate of 100L/min for 2 hours. EC value of the solution was then measured at room temperature of 23 ± 1 ℃ using an electrical-conductivity meter (Oakton Instruments, IL).

2.7 Measurement of photosynthesis via chlorophyll *a* fluorescence with a pulse amplitude modulated (PAM) fluorometer

Chlorophyll *a* fluorescence is measured with pulse amplitude modulated (PAM) fluorometer (PAM-2100, Heinz Walz GmbH, Eichenring, Germany). For this assay, four types of plants were grown on soils from either Alameda (EC_e = 30 dS/m) or Cannington Manor South (EC_e = 40 dS/m) salt-impacted sites for 20 to 40 days (sowing method and greenhouse conditions were as stated in Section 2.5). Plant species that are relatively salt tolerant were tested: barley (AC ranger) (*Hordeum vulgare*), oats (CDC baler) (*Avena sativa*), tall fescue (Inferno) (*Festuca arundinacea*), and tall wheatgrass (*Agropyron elongatum*).

Plants with and without PGPR treatments were grown in the greenhouse for 20 to 40 days (under conditions stated previously). Whole plants were dark adapted for 30 min prior to PAM analyses to ensure all PSII reaction centers were open. PAM measurements were made on attached leaves with the aid of a 0.8 cm diameter fiber optic cable. The minimal fluorescence in dark-adapted tissue, $F_{o,}$ was adjusted to 0.400 ± 0.040 by changing the fluence rate of the measuring light (gain) (Babu et al. 2001; Lees 2005; Ueckermann 2008). The maximal fluorescence in dark-adapted tissue, F_m, was measured by a single

non-modulated saturating 0.6 s light pulse (2000 µmol m^{-2} s^{-1} of PAR). After 30 seconds, fluorescence in steady state, F_s, was measured using the non-modulated 640-700nm actinic radiation (70 µmol m^{-2} s^{-1}) for 14 minutes after the fluorescence reached steady state (Babu et al. 2001; Lees 2005; Ueckermann 2008). A single non-modulated saturating 0.6 s light pulse were triggered every minute to measure the maximal fluorescence during steady state photosynthesis, F_m', in the presence of actinic light (Babu et al. 2001; Lees 2005; Ueckermann 2008). The PAM parameters derived first were F_v/F_m (maximal photosystem II [PSII] activity) followed by photochemical quenching (qP; net energy storage), non-photochemical quenching (qN; energy loss), and Yield (PSII activity at steady state). These parameters were all calculated using PamWin software (PC software PamWin V 2.00, Heinz Walz GmbH, Germany).

2.8 Measurement of PGPR growth curve at saline condition to test tolerance of PGPR to salt

Bacteria were cultured as stated previously except sodium chloride (NaCl) was added to Tryptic Soy Broth (TSB) to make the growth media saline. Salt (NaCl) concentrations ranging from 0 - 2 % (w/v) were used. PGPR strains of *Psudomonas putida* UW3 and *Pseudomonas putida* UW4 (Glick et al. 1995) were inoculated in saline TSB growth media and grown at root temperature (23±1 ℃) on a rotary shaker at 80 r.p.m for 26 hours. Optical density (OD) readings at 600 nm were taken at different time intervals to assess the growth and tolerance of bacteria under salt stress. According to the Beer's law, the relationship between concentration and absorbance was not linear at high

concentration. Therefore, dilutions were made to lower the absorbance lies between 0 - 1 measured by the spectrophotometer.

2.9 Plant growth pouch assays to examine salt tolerant range of barley and canola

The growth pouch assays were modified from published procedures (Penrose and Glick 2003). PGPR were grown in regular Tryptic Soy Broth (TSB) for 24 hours as stated previously. The bacteria cells were then centrifuged at 2000 r.p.m. for 20 minutes, and the cell pellets were resuspended in sterile 0.03 *M* $MgSO_4$ and the concentration adjusted to an absorbance of 0.15 measured at 600nm.

Triplicate seed-pack growth pouches (Mega International, Minneapolis, Minn.) received 17 mL of ddH_2O or NaCl solution ranging from 0.1 – 2.0 % (w/v). The EC value for each salt solution was previously measured using an electrical-conductivity meter (Oakton Instruments, IL) at room temperature (23±1 ℃) (Table 2.1). Pouches were placed upright in a rack with two empty pouches placed at each end of each rack. The racks were placed in an autoclavable plastic bin containing ddH_2O at a depth of 3 cm and covered with aluminum foil, then autoclaved at 131˚C and 30 psi for 20 minutes.

Barley (*Hordeum vulgare*) (common) and canola (*Brassica napus*) seeds were immersed in 70 % ethanol for 1 min in glass Petri dishes followed by 1 % sodium hypochlorite (bleach) for 10 minutes under aseptic conditions. The bleach solution was suctioned off and the seeds were thoroughly rinsed with sterile ddH_2O five times. Each dish was incubated at room temperature for 1 hour with bacterial suspensions in sterile 0.03 *M* $MgSO_4$ or sterile 0.03 *M* $MgSO_4$ for with and without PGPR treatment, respectively. Ten canola or eight barley seeds sterilized and treated with sterile

Table 2.1. Electrical conductivity (EC) values of various salt concentrations (w/v) at 23 ℃

Concentration of NaCl solution (w/v)	Molarity (mmol/L)	EC value (dS/m) ± S.E.
0.0 % (dd H_2O)	0.00	< 0.002 ± 0.00
0.1 %	17.11	2.08 ± 0.00
0.2 %	34.22	3.99 ± 0.00
0.4 %	68.44	7.58 ± 0.04
0.6 %	102.66	11.02 ± 0.01
0.8 %	136.89	14.32 ± 0.01
1.0 %	171.11	17.53 ± 0.02
1.2 %	205.33	22.27 ± 0.07
1.4 %	239.55	25.80 ± 0.10
2.0 %	342.22	34.87 ± 0.03
3.0 %	513.33	52.06 ± 0.11

Measurements of electrical conductivity were performed in three independent replicates (N=3). Errors values are standard errors (S.E.).

0.03 M $MgSO_4$ or PGPR suspensions in sterile 0.03M $MgSO_4$ were placed aseptically into each growth pouch with sterilized forceps, then incubated at growth chamber at 23±1˚C in the dark for 2 days followed with 12 hours of light (18 μmol $m^{-2}s^{-1}$)/dark cycle for 4 days. Percent germination, shoot length and root length of plants were measured on the sixth day of growth.

2.10 Field studies to assess the effects of PGPR on plant growth

To complement greenhouse experiments, field experiments were also performed to test the feasibility of PGPR aided phytoremediation on salt-impacted sites. All three field sites, Cannington Manor North (CMN), Cannington Manor South (CMS), and Alameda (AL), were tilled before planting. PGPR strains of *Pseudomonas putida* UW3, *Pseudomonas putida* UW4 (Glick et al. 1995), and *Pseudomonas corrugata* CMH3 were used to treat plant seeds prior to planting at field sites. Treatment was as above using the HEGE seed treater (HEGE 11, Wintersteiger Inc., Austria).

On the field, seeds were sown by a Brilliant^{TM} drop-spreader at a density of 65 g per m^2 for grasses and 40 g of seeds per m^2 for cereal plants. Each of the salt-impacted sites was divided into strips with different combinations of plant species and PGPR treatments to suit the purpose of finding the best methodology for field sites (Figure 2.1, Figure 2.2, and Figure 2.3). For soil salinity measurements, triplicate soil samples were taken from each strip subdivided into three sections: A, B, and C (Figure 2.1, Figure 2.2, and Figure 2.3). Three top 20 cm soil samples were taken randomly within each subsection by an auger and mixed thoroughly as a composite sample for salinity measurement. For each site, ten soil samples were selected for $EC_{1:2}$ and EC_e

measurement to determine the average K factor that used to convert $EC_{1:2}$ to EC_e for rest of the soil samples.

After a growth period of two and four months, soil samples for each site were taken back to the lab for salinity measurements. Plant biomass from an area of 0.25 m^2 at each strip was recorded for each site. The plant samples were washed, air dried and removed all grains before sent to ALS Environmental Inc. (Waterloo, ON) and Maxxam analytics Inc. (Missisauga, ON) for analysis of Na and Cl ion concentrations in tissues. Percent vegetation coverage for each site was also recorded to observe the tolerance of plants to saline soil and the effect of PGPR on plant growth on saline soil.

2.11 Salt accumulation in plants

Plant samples from salt-impacted sites were washed and air dried for 5 days prior to analysis by ALS Environmental Inc. (Waterloo, ON) or Maxxam analytics Inc. (Missisauga, ON). For ALS Environmental Inc., plant shoot tissue is analyzed for Na^+, Cl^-, K^+, and Ca^{2+} ion concentrations by method USEPA 6020, where plant tissue was completely decomposed in nitric acid and analyzed by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy). ALS Environmental Inc. measurement of chloride included analysis by IC (Ion Chromatography) according to APHA method 4110B. For sodium analysis by Maxxam Analytics Inc., plant shoot tissue was digested with Aqua Regia, then analyzed with ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) by AOAC 985.01 method. For chloride analysis performed by Maxxam Analytics Inc., plant samples were dispersed in water and acidified by method AOAC 983.14. Soluble chloride

was titrated with silver nitrate to a fixed potentiometric endpoint before multiply by appropriate conversion factors to obtain equivalent concentrations.

2.12 Statistical analysis

All statistical analyse were performed using the software GraphPad Prism 5 (GraphPad Software, Inc). All analysis were analyzed by one-way ANOVA followed by the post-hoc Tukey test (a test that compares all possible pairs of means) at $P < 0.05$. Assumptions were made that 1) the samples being tested are independent; 2) the samples has a normal distribution with unknown mean μ_i; 3) all of the samples have the same standard deviation σ.

Chapter 3 - Results

3.1 Preliminary experiments

Three preliminary experiments were conducted. An experiment was performed to assess the range of salt tolerance for two PGPRs, UW3 and UW4 that were isolated from non-saline impacted soil to ensure their optimal performance on high salt-impacted soil that would be encountered at Cannington Manor South (CMS) and Alameda (AL) sites. Another experiment was performed to calibrate the $EC_{1:2}$ values between in-house and two accredited analytical laboratories. The third preliminary experiment was performed to determine the salt tolerant range for canola and barley as well as the PGPR effects on plant root elongation.

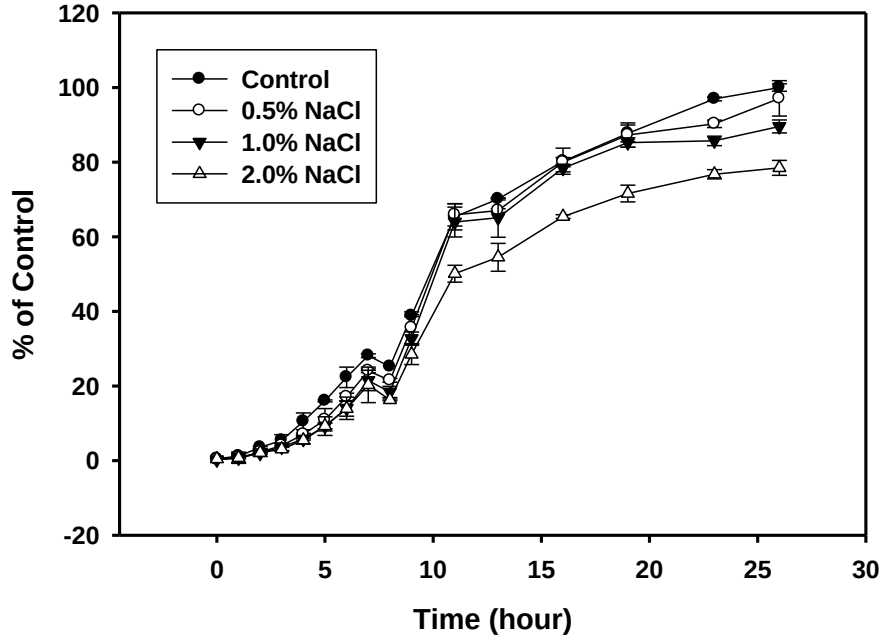
3.1.1 Measurement of PGPR growth under saline conditions

The average salt levels of the salt-impacted soil from the field sites ranged from an EC_e of 20 – 40 dS/m, this is equivalent to an aqueous solution of 1 to 2% of NaCl. It is important to ensure that the growth and performance of the non-indigenous PGPR (isolated from non-salt-impacted soil) will not be adversely affected by high salt concentrations. In this preliminary assay, the PGPR isolated from non-salt-impacted soil, UW3 (*Pseudomonas putida*) and UW4 (*Pseudomonas putida*) were tested for their tolerance to saline conditions ranging from 0 - 2% NaCl (w/v). The optical density (O.D.) value measured at 600 nm of the bacteria grown in saline (0.5%, 1.0% and 2.0% NaCl) tryptic soy broth (TSB) medium was divided by the O.D. value of the bacteria grown in control (0% NaCl) TSB medium. The PGPR isolated from salt-impacted soil, CMH3 (*Pseudomonas corrugata*), was not assessed. This is because CMH3 was isolated from soil of the Cannington Manor South (CMS) site (EC_e of 35 dS/m ≈ 2% salt in soil), and

therefore was speculated that the performance and growth of CMH3 will not be significantly affected at 2% salt. The objective of this assay was to assess the ability of UW3 and UW4 to tolerate the salt levels of the salt-impacted soils from Saskatchewan.

At 0.5% NaCl, the growth of UW3 and UW4 did not decreased (Figure 3.1). At 1.0% NaCl, the growth of UW3 and UW4 decreased by 10% and 20%, respectively. At 2.0 %, the growth of UW3 and UW4 decreased by 22% and 25%, respectively. Since both UW3 and UW4 show tolerance up to 2% salt (no more than 30% inhibition of growth), it was expected that their application to the salt-impacted field would be feasible and would not significantly affect their performance due to salinity.

(A)



(B)

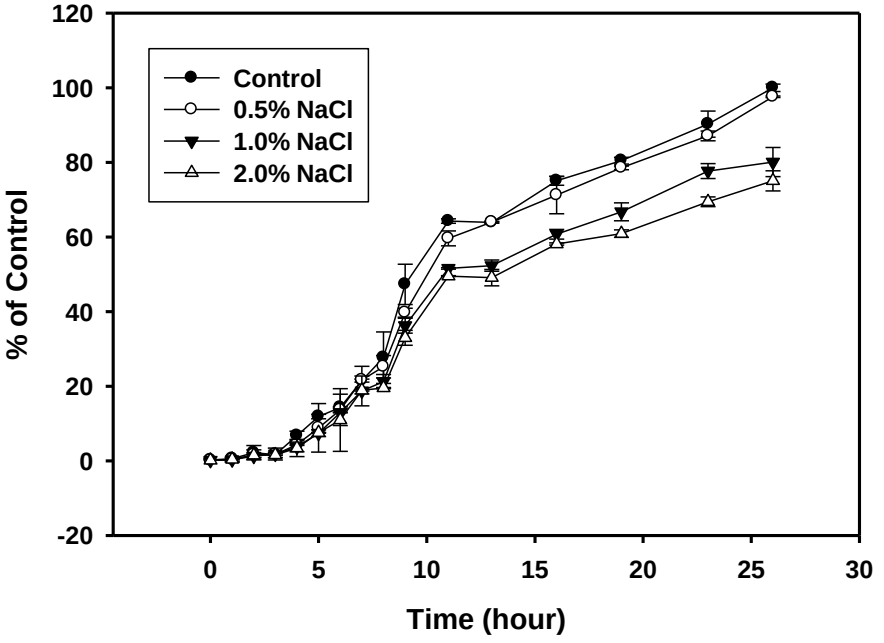


Figure 3.1. Growth curve of UW3 (A) and UW4 (B) in TSB with various salt concentrations over 26 hours at 23±2 ℃. Samples were performed in triplicate (n = 3). Error bars were standard errors (S.E.).

3.1.2 Calibration of in-house and analytical laboratory $EC_{1:2}$ values

Soil salinity measurements for each site were mainly conducted in-house. Triplicate measurements of $EC_{1:2}$ (electrical conductivity at 1 part soil to 2 parts water (w/v)) values were performed for each soil sample and multiplied by a conversion factor, K, to obtain the corresponding EC_e (soil salinity of a saturated paste) values (refer to Equation 1). To confirm in-house $EC_{1:2}$ accuracy, quality assurance was performed by having selected samples analyzed by accredited analytical laboratories. Three to five soil samples at various salinity ranges ($EC_{1:2}$ < 5 dS/m, between 5 to 10 dS/m, between 10 to 20 dS/m, and >20 dS/m) were sent to either ALS Environmental Inc. (Waterloo, ON) or Maxxam Analytics Inc. Laboratory (Missisauga, ON) for $EC_{1:2}$ measurements. EC_e values were not used as a parameter for calibration, as $EC_{1:2}$ is the primary measurement used in accredited analytical laboratories. The $EC_{1:2}$ value measured by the accredited analytical laboratories is multiplied by 2 (to account for dilution), and does not use a K value to convert $EC_{1:2}$ to EC_e (this is a different procedure performed in house). The extraction efficiency of salt ions from a complex soil matrix varies depending on soil porosity and texture, and therefore, by multiplying an $EC_{1:2}$ with an assumed dilution factor may not be representative of the true EC_e value. The standard curve for calibration between in-house and analytical laboratory $EC_{1:2}$ values is presented (Figure 3.2). The in-house $EC_{1:2}$ measurements correlated very well with both of the accredited analytical laboratories, indicated by R^2 value close to 1.0 (R^2_{ALS} 0.994, and R^2_{Maxxam} 0.962). Also, the slope of the curves was approximately equal to 1 and the y intercept was approximately equals to 0. This demonstrated that the in-house salinity measurements were accurate and can be used to assess soil salinity during phytoremediation.

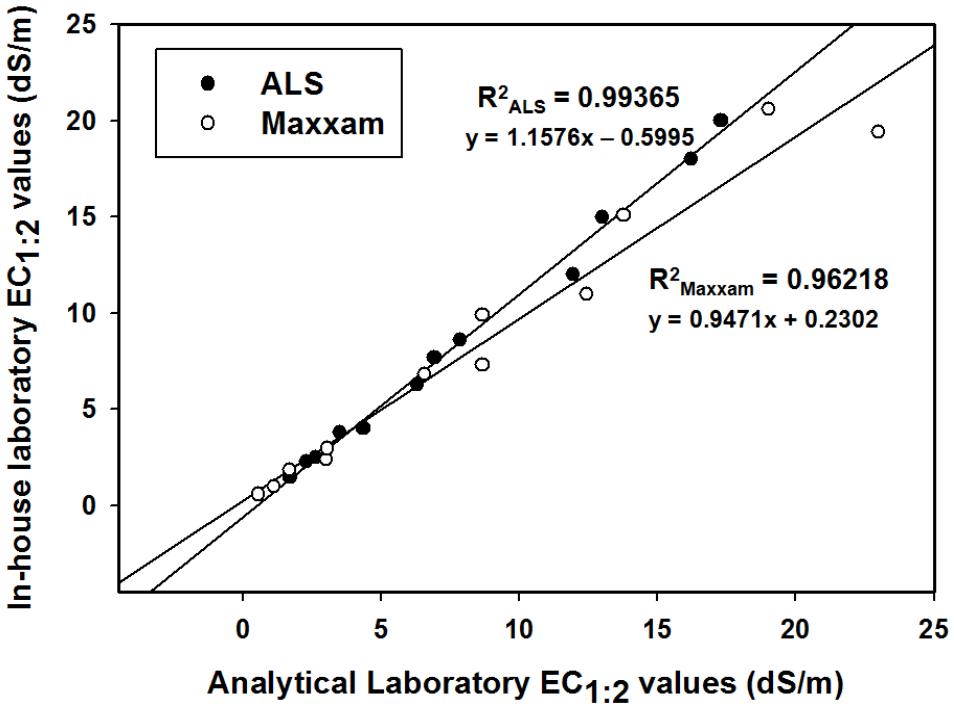


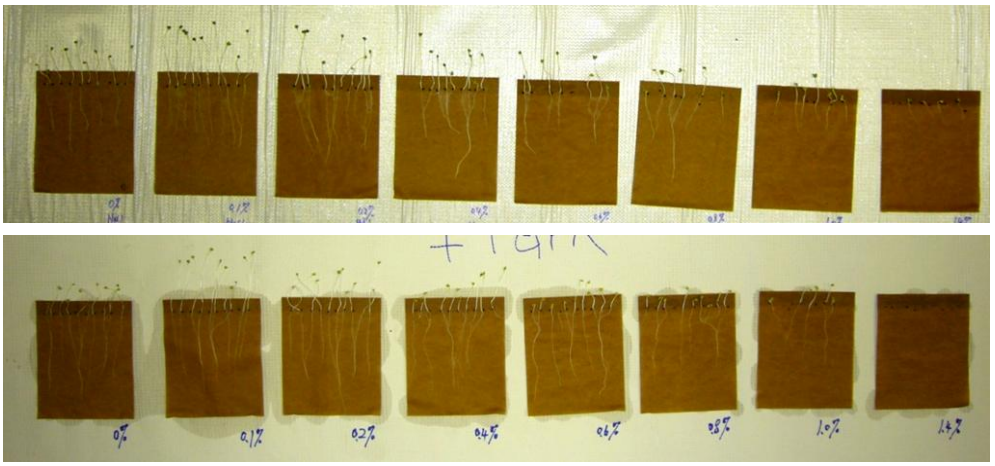
Figure 3.2. Soil $EC_{1:2}$ calibration between the in-house method and accredited analytical laboratory (ALS Laboratory Group and Maxxam Analytics Inc.) results.

3.1.3 Plant growth pouch assays to examine the tolerable salt range of plants

In this assay, the growth of common barley (*Hordeum vulgare*) (Cribit Seeds, ON) and canola (*Brassica napus*) (Ontario Seed Company, ON) in salt concentrations that ranged from 0-1.4% was assessed after 6 days (Figure 3.3). The corresponding electrical conductivity of the salt solutions (w/v) used in this assay can be found in Table 2.1.

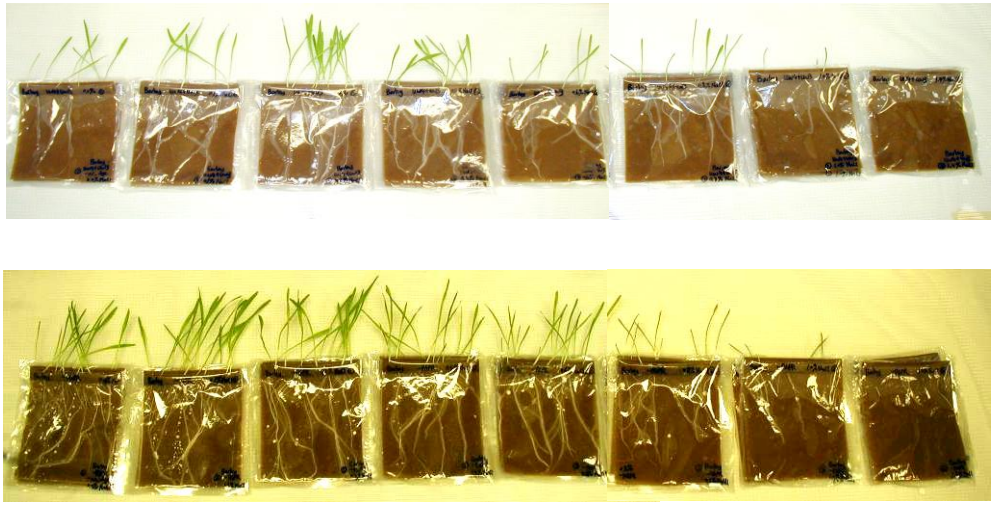
In general, canola was tolerant to increasing salt concentrations up to a NaCl level of 1% (Figure 3.4). However, this trend was not observed with barley. Percent germination of barley varied from approximately 30% at low salt concentrations and increased to 50-70% at 0.2-0.4% NaCl before decreasing to below 20% at 1.0-1.4% NaCl (Figure 3.4). Moreover, a low percent germination was observed in barley even at minimal salt stress (0%-0.1% NaCl), suggesting poor seed quality (Figure 3.4). Upon treatment with UW3 + UW4, percent germination of barley increased by over 200% compared to the untreated (No PGPR) sample (Figure 3.4). The effect of PGPR on canola germination was not pronounced, since the percent germination of the No PGPR samples was already close to 100%. Results from these experiments showed that barley was more responsive to PGPR treatment but less salt tolerant than canola based on germination.

(A)



[NaCl] %	0	0.1	0.2	0.4	0.6	0.8	1.0	1.4
EC (dS/m)	0	2.0	4.0	7.6	11.0	14.3	17.5	25.9

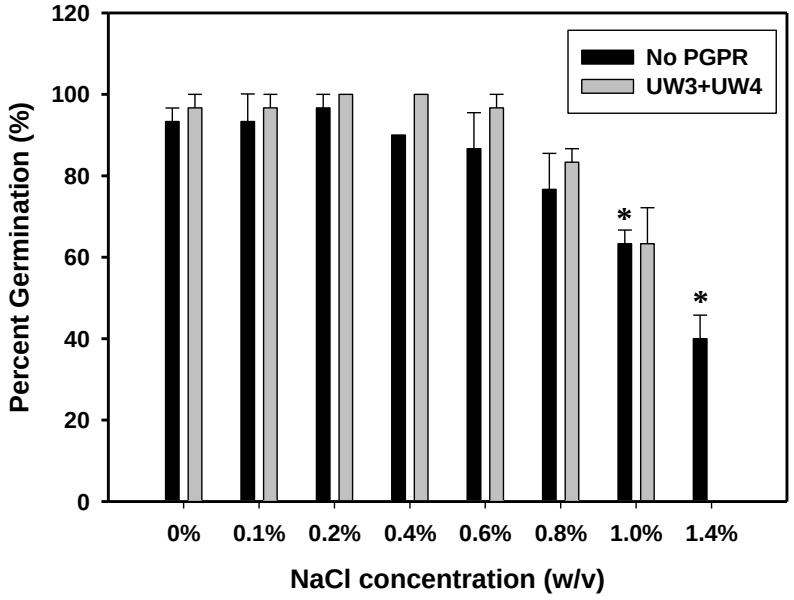
(B)



[NaCl] %	0	0.1	0.2	0.4	0.6	0.8	1.0	1.4
EC (dS/m)	0	2.0	4.0	7.6	11.0	14.3	17.5	25.8

Figure 3.3. Growth of canola (A) and barley (B) in growth pouches after 6 days at increasing salt concentrations. The electrical conductivity (EC) value at each salt concentration is measured in dS/m.

(A)



(B)

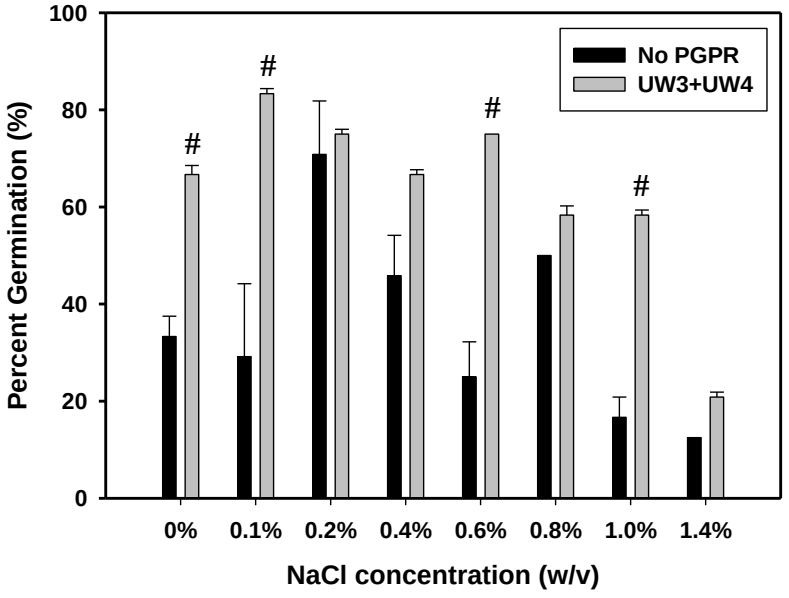
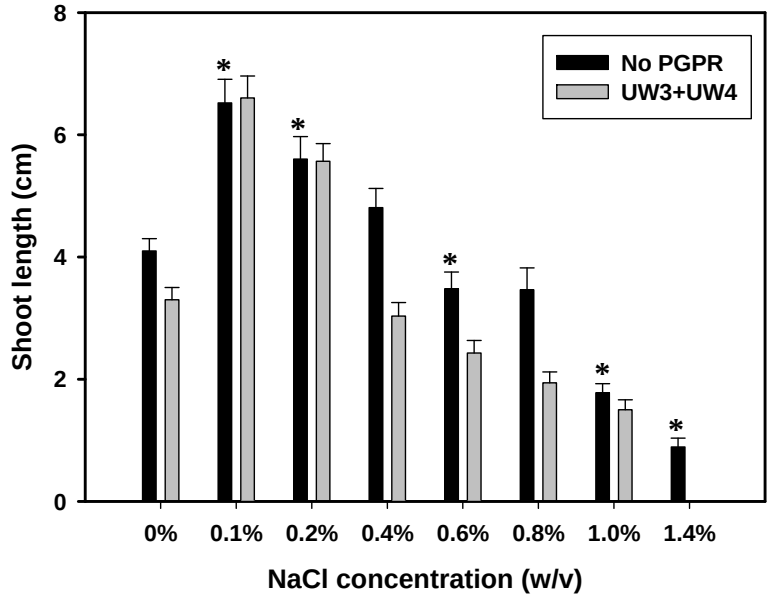


Figure 3.4. Percent germination of canola (A) and barley (B) in growth pouches after 6 days of growth at increasing salt concentrations ranging from 0% -1.4% (w/v). No germination was observed for canola treated with UW3+UW4 at 1.4% NaCl. All measurements were performed in three independent replicates (N = 3). Error bars standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences observed when comparing No PGPR sample at 0% NaCl to all other No PGPR samples at various NaCl concentrations ($P < 0.05$). # indicates significant differences observed comparing No PGPR versus UW3+UW4 treated samples at corresponding NaCl concentrations ($P < 0.05$).

In addition to percent germination, shoot and root length of both plants species after 6 days of incubation in growth pouches was measured (Figure 3.5 and Figure 3.6). It was noted that at 0% NaCl, where better plant growth was expected, shoot and root growth of both plant species actually decreased compared to those in the low salt concentrations (0.1-0.2% NaCl). At high salt concentrations (above 0.8% NaCl), shoot and root growth of both plant species were significantly inhibited by salt (Figure 3.6 and Figure 3.6). Treatment with UW3+UW4 significantly promoted root growth in canola by 65% (Figure 3.5). Specifically, by comparing the root growth of canola seeds at 0.6% NaCl and 1.0% NaCl, the effect of PGPR on root elongation was less evident at 1.0% NaCl (Figure 3.7). These results demonstrated that UW3+UW4 can significantly promote plant growth in canola, exposed to a moderate concentration (0.6%) of NaCl compared to a high concentration (1.0%) of NaCl. In contrast to canola, the effect of PGPR on barley growth was not significant (Figure 3.6), possibly because the roots reached the bottom of the growth pouches before 6 days. Compared to barley, canola exhibited greater salinity tolerance and responsiveness to PGPR treatment in short-term growth pouch assays. However, cereal plants (i.e., barley and oats) were chosen for field and greenhouse experiments, due to its high planting density and above-ground biomass production making it a more suitable plant species for phytoremediation field trials (Chang 2007).

(A)



(B)

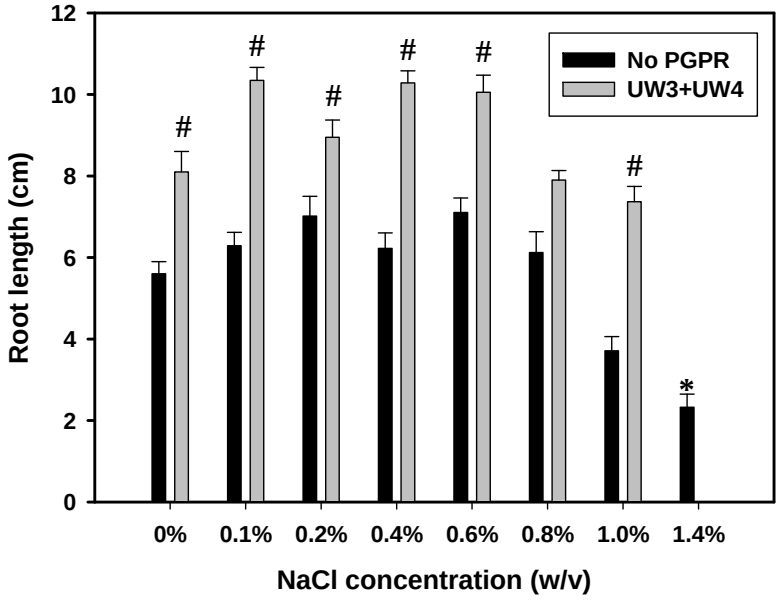
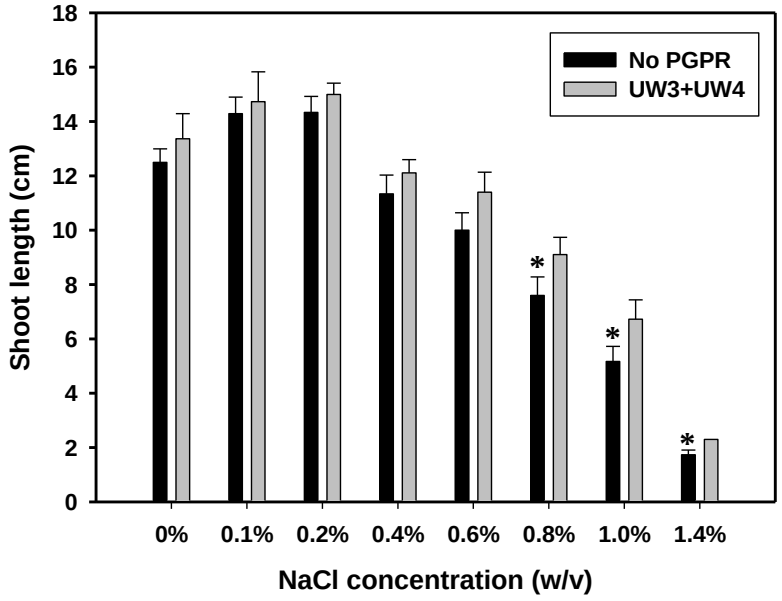


Figure 3.5. Canola shoot length (A) and root length (B) after 6 days of growth at increasing salt concentrations ranging from 0%-1.4% (w/v). No growth was observed for canola treated with UW3+UW4 at 1.4% NaCl. All measurements were performed in three independent replicates (N = 3). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences observed when comparing No PGPR sample at 0% NaCl to all other No PGPR samples at various NaCl concentrations ($P < 0.05$), # indicates significant differences observed comparing No PGPR versus UW3+UW4 treated samples at corresponding NaCl concentrations ($P < 0.05$).

(A)



(B)

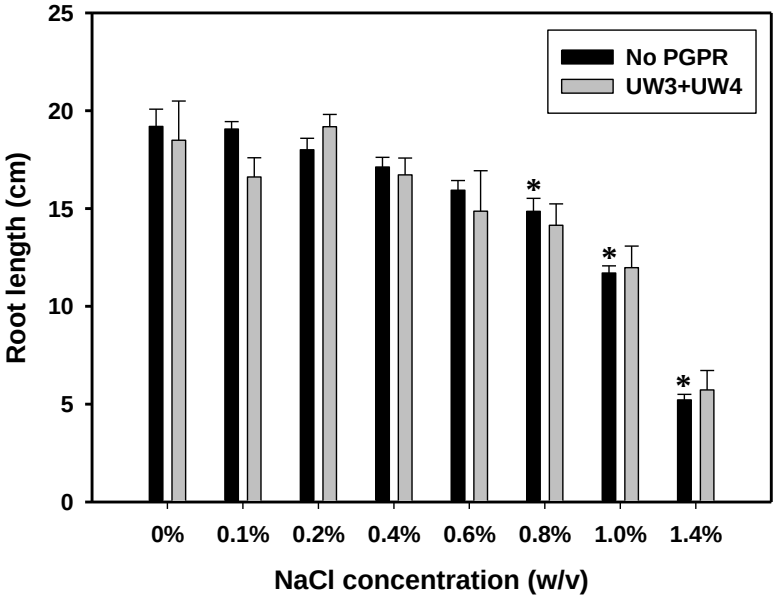


Figure 3.6. Barley shoot length (A) and root length (B) after 6 days of growth at increasing salt concentrations ranging from 0%-1.4% (w/v). All measurements were performed in three independent replicates (N = 3). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences observed when comparing No PGPR sample at 0% NaCl to all other No PGPR samples at various NaCl concentrations ($P < 0.05$). Comparison between No PGPR versus UW3+UW4 treated samples at corresponding NaCl concentrations ($P < 0.05$) were also performed, however, no statistical significant differences were found.

(A)

No PGPR **UW3 + UW4**

(B)

No PGPR **UW3 + UW4**

Figure 3.7. Effect of PGPR on root elongation of canola after 6 days in 0.6 % of NaCl (A) and 1.0% of NaCl (B).

3.2 Greenhouse studies to examine effects of PGPR on plant biomass production

Four greenhouse experiments were carried out to investigate the impact of salinity on shoot and root growth for several plant species grown on salt-impacted soils, as well as the effect of various PGPR combinations on plant biomass production. Experiments were carried out with soil sampled directly from two research sites to mimic its authentic soil conditions and to allow for better prediction of PGPR effects on plant growth promotion for the field trials. The soil salinity for each greenhouse experiment varied due to the availability of soil that was taken from the research sites.

3.2.1 Growth of barley and oats on low salt-impacted soil in green house trials

In this experiment, barley (*Hordeum vulgare* C.V. AC ranger) and oats (*Avena sativa* C.V. CDC baler) were tested on control soil - ProMix^{TM} (EC_e < 2 dS/m), and low salt-impacted soil from a non-research site in Alberta (EC_e = 3.2 dS/m). The properties of the soils can be found in Appendix (Table 1 and Table 2). The impact of salinity and the effect of PGPR on plant growth of these two plants species was examined after 20 days of growth in the greenhouse.

It was found that growth of oats were slightly inhibited by salt, as was shown by visibly decreased shoot length compared to control plants grown on ProMix^{TM} soil (Figure 3.8). Oats grown on salt impacted soil without PGPR treatment exhibited a decrease in dry shoot biomass by 40% and dry root biomass by 50% compared to the control plants grown on ProMix^{TM} soil (Figure 3.9). However, no inhibition on plant biomass production due to salinity was observed in barley.

(A)

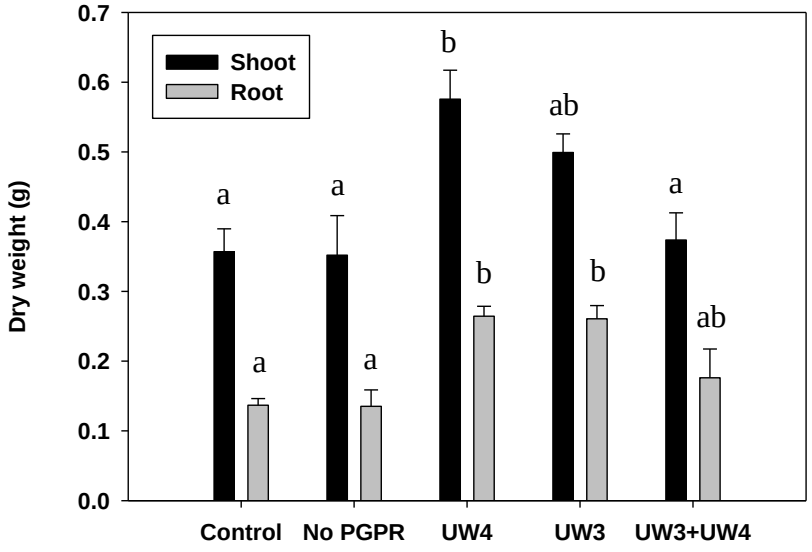
Control (ProMix™) | No PGPR (salt soil) | UW4 (salt soil) | UW3 (salt soil) | UW3 + UW4 (salt soil)

(B)

Control (ProMix™) | No PGPR (salt soil) | UW4 (salt soil) | UW3 (salt soil) | UW3 + UW4 (salt soil)

Figure 3.8. Growth of barley (A) and oats (B) in Alberta low salt-impacted soil (EC_e = 3.2 dS/m) after 20 days of growth.

(A)



(B)

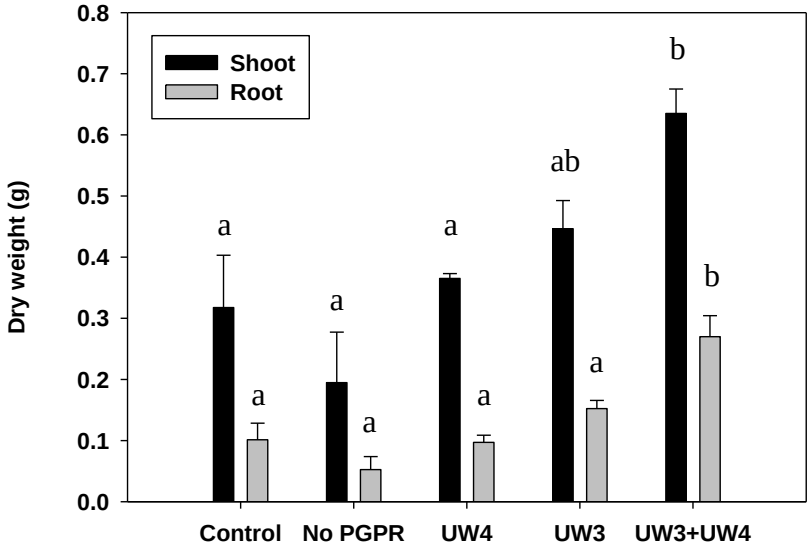


Figure 3.9. Root and shoot dry weight of barley (A) and oats (B) grown in control (ECe < 2 dS/m) and Alberta low salt-impacted (ECe = 3 dS/m) soil with and without PGPR treatment after 20 days. All measurements were performed in four independent replicates (N = 4). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Mean separation was done by shoot and by root. Bars with different letters are significantly different observed at $P < 0.05$.

With PGPR treatment, shoots were visibly taller compared to untreated (No PGPR) plants for both plant species. PGPR-treated plants showed significant improvements on shoot and root growth, as indicated by a significant increase in plant dry weight production (Figure 3.9 A). For barley, there was a 60% increase in dry shoot biomass and a 90% increase in dry root biomass with UW4 treatment compared to the untreated (No PGPR) plants. Barley treated with UW3 also showed improvement on root biomass production, but to a lesser extent than the UW4 treated plants. Shoots biomass of barley were increased by 40% and roots were significantly increased by 90% with UW3 treatment compared untreated plant.

It was observed that when UW3 and UW4 were applied as a mixture (UW3+UW4) on barley, the growth promotion effect was not greater than when UW3 and UW4 were used separately. This is in contrast to the results observed in oats, where applying UW3 and UW4 together yielded a growth improvement exceeding the growth observed when using UW3 and UW4 separately (Figure 3.9 B). Oats treated with UW3+UW4 significantly increased shoot and root dry biomass by 220% and 440%, respectively.

3.2.2 Growth of four plants on Alameda (AL) high salt-impacted soil

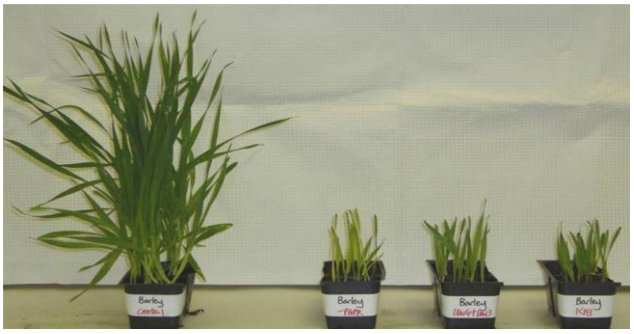
In this set of experiments, two cereal species, barley (*Hordeum vulgare* C.V. AC ranger) and oats (*Avena sativa* C.V. CDC baler), as well as two perennial grass species, tall fescue (*Festuca arundinacea* C.V. Inferno), and tall wheatgrass (*Agropyron elongatum*), were used. The objective of this set of experiment was to examine salinity tolerance of the above four plants species as well as their response to PGPR treatments in high salt-impacted soils. Their growth was assessed on salt-impacted soil from the Alameda (AL)

site (EC_e = 30 dS/m) over 70 days of growth in the greenhouse. The properties of soils used in this experiment can be found in Appendix (Table 1 and Table 2). The effects of the non-indigenous PGPR UW3 and UW4 (applied as a mix), and indigenous bacteria CMH3 on all four plants were examined during their growth cycle.

It was visibly observed that plants grown under saline conditions had smaller, thicker leaves compared to the plants grown on ProMix^{TM} (Figure 3.10). Moreover, the leaves of untreated plants grown in saline soil were pale green in color with some of the older leaves turning yellow, followed by premature necrosis. The PGPR treated plants were a darker green color compared to the untreated plants, suggesting that PGPR may be have an effect on plant photosynthesis. To verify possible PGPR enhancement of photosynthesis in plants, the photosynthetic activity of all four plant species under saline conditions was assessed and will be presented later.

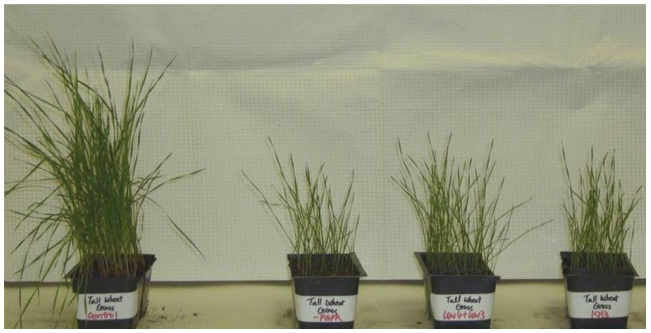
Plant dry biomass data (shoots and roots) for the four plant species after 10, 20, 45 and 70 days of growth were collected (Table 3.1 Table 3.2). It was found that growth of all four plant species was severely impacted in the high salinity soil (EC_e = 30 dS/m). This was indicated by a marked decrease in shoot and root dry biomass compared to plants grown in control (ProMix^{TM}) soil (Table 3.1 and Table 3.2). It was observed that grasses have a longer growing period, lasting up to 70 days in high salt impacted soil, whereas cereal plant species reached the end of their growth cycle after 20 days, suggesting that grasses may be more salt tolerant than cereals. After 20 days of growth, cereal plants that were grown in salt-impacted soil produced only 8-16% of shoot dry biomass compared to the plants that were grown in ProMix^{TM} (Table 3.1). The impact of salinity on grasses was

(A)

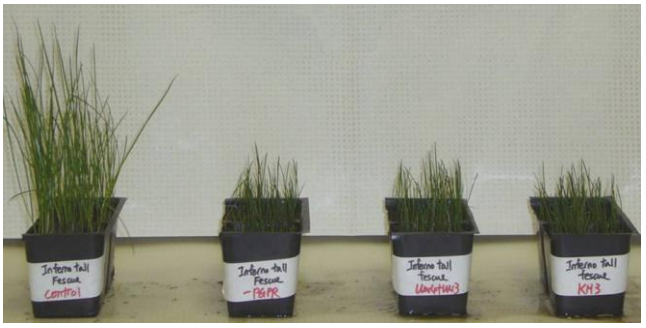


(B)

(C)



(D)



Control (ProMix^{TM}) | No PGPR (Salt soil) | UW3+UW4 (Salt soil) | CMH3 (Salt soil)

Figure 3.10. Growth of barley (A), oats (B), tall wheatgrass (C), and tall fescue (D) after 20 days in salt-impacted soil from Alameda (AL) research site (EC_e = 30 dS/m).

Table 3.1. Dry weight of shoots for four plant species grown on Alameda (AL) salt-impacted soil (EC_e = 30 dS/m) over 70 days in the greenhouse with and without PGPR treatments.

Plants	Treatment	Shoot dry weight (g)			
		10 days	20 days	45 days	70 days
Barley (AC ranger)	Control (ProMix™)	0.3241 ± 0.0179 a	1.3080 ± 0.0085 a	1.9401 ± 0.0206	N/D
	No PGPR	0.1150 ± 0.0004 b	0.0983 ± 0.0007 d	N/D	N/D
	UW3+UW4	0.1318 ± 0.0029 b	0.1891 ± 0.0031 b	N/D	N/D
	CMH3	0.1166 ± 0.0098 b	0.1482 ± 0.0043 c	N/D	N/D
Oats (CDC baler)	Control (ProMix™)	0.2338 ± 0.0086 a	0.9621 ± 0.0031 a	1.5085 ± 0.0722	N/D
	No PGPR	0.0474 ± 0.0021 b	0.1514 ± 0.0100 b	N/D	N/D
	UW3+UW4	0.0509 ± 0.0015 b	0.1794 ± 0.0023 b	N/D	N/D
	CMH3	0.0389 ± 0.0037 b	0.1516 ± 0.0101 b	N/D	N/D
Tall Wheatgrass	Control (ProMix™)	0.0997 ± 0.0045 a	0.5204 ± 0.0050 a	1.1581 ± 0.0179 a	1.1940 ± 0.0013 a
	No PGPR	N/G c	0.0710 ± 0.0117 d	0.2367 ± 0.0098 c	0.2413 ± 0.0131 d
	UW3+UW4	0.0386 ± 0.0040 b	0.1316 ± 0.0045 b	0.2959 ± 0.0135 b	0.3585 ± 0.0209 c
	CMH3	0.0415 ± 0.0090 b	0.1011 ± 0.0095 c	0.2065 ± 0.0071 c	0.4602 ± 0.0082 b
Tall fescue (Inferno)	Control (ProMix™)	0.0080 ± 0.0021 a	0.1336 ± 0.0080 a	0.7506 ± 0.0190 a	N/D
	No PGPR	N/G b	0.0483 ± 0.0048 c	0.0674 ± 0.0055 c	N/D
	UW3+UW4	N/G b	0.0685 ± 0.0046 b	0.0960 ± 0.0048 b	N/D
	CMH3	N/G b	0.0647 ± 0.0034 b	0.0612 ± 0.0043 c	N/D

- N/D indicates plant dry biomass was not determined, because plants were wilted and reached the end of their growth cycle.
- N/G indicates seeds failed to germinate at that time.
- Results were based on non-repeated measures of triplicate samples (N=3) per sampling point. Error values were standard errors (S.E.).
- Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Mean value followed by different letters within a column of the corresponding plant species are significantly different at $P < 0.05$.

Table 3.2. Dry weight of roots for four plant species grown on Alameda (AL) salt-impacted soil (EC_e = 30 dS/m) over 70 days in the greenhouse with and without PGPR treatments.

Plants	Treatment	Root dry weight (g)			
		10 days	20 days	45 days	70 days
Barley (AC ranger)	Control (ProMix™)	0.0825 ± 0.0025 a	0.3770 ± 0.0088 a	0.8477 ± 0.0092	N/D
	No PGPR	0.0348 ± 0.0021 d	0.0343 ± 0.0010 c	N/D	N/D
	UW3+UW4	0.0448 ± 0.0020 c	0.0569 ± 0.0072 b	N/D	N/D
	CMH3	0.0520 ± 0.0011 b	0.0541 ± 0.0012 b	N/D	N/D
Oats (CDC baler)	Control (ProMix™)	0.0587 ± 0.0027 a	0.3228 ± 0.0120 a	0.7358 ± 0.0913	N/D
	No PGPR	0.0265 ± 0.0038 b	0.0699 ± 0.0027 d	N/D	N/D
	UW3+UW4	0.0246 ± 0.0003 b	0.1178 ± 0.0100 c	N/D	N/D
	CMH3	0.0239 ± 0.0008 b	0.1680 ± 0.0015 b	N/D	N/D
Tall Wheatgrass	Control (ProMix™)	0.0894 ± 0.0040 a	0.1582 ± 0.0032 a	0.2367 ± 0.0098 a	0.9163 ± 0.0020 a
	No PGPR	N/G d	0.0586 ± 0.0073 c	0.0952 ± 0.0038 c	0.1283 ± 0.0116 c
	UW3+UW4	0.0241 ± 0.0001 b	0.0904 ± 0.0006 b	0.1769 ± 0.0098 b	0.1798 ± 0.0112 c
	CMH3	0.0172 ± 0.0024 c	0.0677 ± 0.0004 c	0.1562 ± 0.0081 b	0.3227 ± 0.0444 b
Tall fescue (Inferno)	Control (ProMix™)	0.0125 ± 0.0011 a	0.0418 ± 0.0028 a	0.2367 ± 0.0098 a	N/D
	No PGPR	N/G b	0.0291 ± 0.0020 a	0.0500 ± 0.0022 b	N/D
	UW3+UW4	N/G b	0.0459 ± 0.0018 ab	0.0414 ± 0.0014 b	N/D
	CMH3	N/G b	0.0446 ± 0.0094 ab	0.0497 ± 0.0027 b	N/D

- N/D indicates plant dry biomass was not determined, because plants were wilted and reached the end of their growth cycle.
- N/G indicates seeds failed to germinate at that time.
- Results were based on non-repeated measures of triplicate samples (N=3) per sampling point. Error values were standard errors (S.E.).
- Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Mean value followed by different letters within a column of the corresponding plant species are significantly different at $P < 0.05$.

less severe. Grasses that were grown on salt-impacted soil produced 16-36 % of shoot dry biomass compared to the control plant after 20 days.

Root biomass production for all plant species was similar to the shoot biomass (Table 3.2). After 20 days of growth, cereal plants without PGPR treatment (No PGPR) grown in salt-impacted soil produced only 10-22% of root biomass compared to plants that were grown in control (ProMix^{TM}) soil. For grasses, untreated plants that were grown on salt-impacted soil produced 37-70 % of root dry biomass compared to the control plants.

PGPR treatment improved germination efficiency of all four plant species, especially in the case of tall wheatgrass. Plants without PGPR treatment did not germinate after 10 days on saline soil, whereas PGPR-treated plants (UW3+UW4, CMH3) had germinated (Table 3.1 and Table 3.2). After 20 days of growth, PGPR-treated plants had significantly increased dry weight production. In barley, treatment with UW3+UW4 increased shoot dry biomass by 50 % and root dry biomass by 66 %. For oats, treatment with UW3+UW4 resulted in an 18% increase of shoot dry biomass and 492 % of root biomass. Consistent plant growth promoting effect by PGPR was observed for the grasses. For tall wheatgrass, there was an 85 % increase in shoot dry biomass and a 16 % increase in root dry biomass with UW3+UW4 treatment after 20 days of growth. For tall fescue, there was a 42 % increase in shoot dry biomass and 58 % increase in root dry biomass after 20 days of growth. For all four plant species, treatments with CMH3 also showed significant improvements in shoot and root biomass production. However, in these experiments, a better growth promotion effect was generally observed with UW3+UW4 treatment compared to CMH3 treatment.

Out of the four plants species selected, only tall wheatgrass survived 70 days on salt-impacted soil. Under these strenuous conditions, the effects of PGPR on growth promotion with tall wheatgrass were visible (Figure 3.11). PGPR-treated plants had a greater shoot length and produced greater plant dry biomass than untreated plants. Compared to the untreated plants (No PGPR), plants treated with CMH3 showed an increase in dry biomass of 90% for shoots and 152% for roots on salt-impacted soil after 70 days of growth.

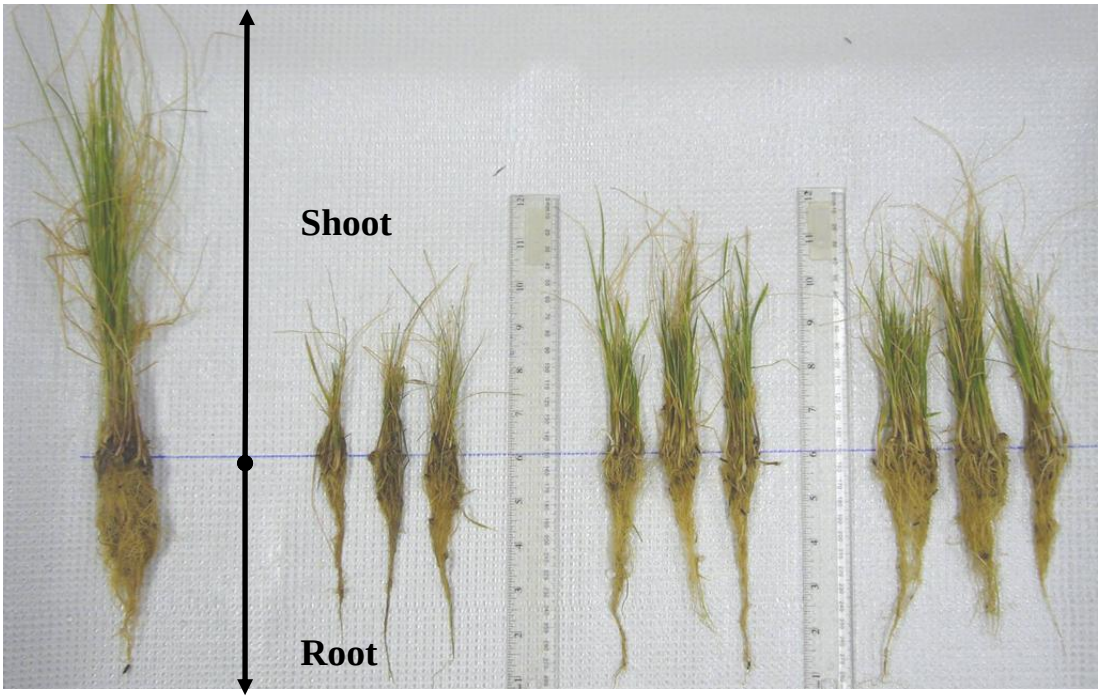


Figure 3.11. Growth of tall wheatgrass in Alameda salt-impacted soil (EC_e = 30 dS/m) and ProMix™ (EC_e < 2 dS/m) soil after 70 days treated with and without PGPR.

3.2.3 Growth of barley on diluted and undiluted Cannington Manor South (CMS) salt-impacted soil

This set of experiments was conducted to examine the plant growth promotion effects of UW3+UW4 and CMH3 treatment on barley (*Hordeum vulgare* C.V. AC ranger). Salt-impacted soil from Cannington Manor South (CMS) site (EC_e = 16.5 dS/m) was diluted with top soil (Quali-grow soil, EC_e = 2.2 dS/m) at a ratio of 2:1 (salt soil: top soil). The properties of the undiluted salt-impacted soil from CMS site and Quali-grow soil can be found in Appendix (Table 1 and Table 2). Barley seeds with and without PGPR (UW3+UW4 or CMH3) treatments were planted on control soil (Quali-grow artificial soil), as well as diluted and undiluted salt-impacted soil. After plants were grown for 21 days in the greenhouse, dry weights of shoots and roots were measured.

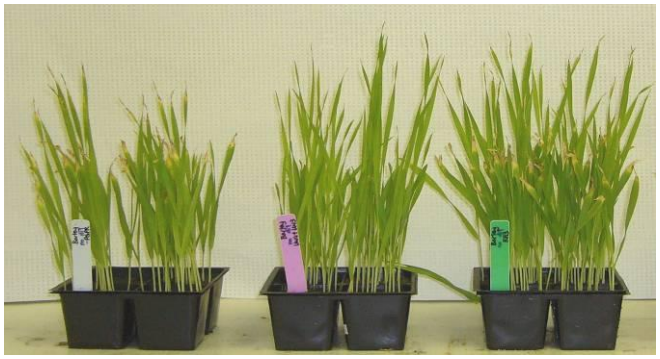
With PGPR treatment, plant growth on all three types of soils was significantly improved, particularly in soil of higher salinity (Figure 3.12). As shown in Figure 3.12, PGPR-treated plants were visibly taller in shoot length compared to the untreated plants. Comparing the dry biomass data with the untreated plants (Figure 3.13), there was 12% and 30% increase in shoot biomass production with UW3+UW4 and CMH3 treatment, respectively, in soil diluted 2:1 CMS salt-impacted soil with Quali-grow artificial soil. In undiluted CMS soil, effects of PGPR on plant growth promotion were more apparent, with 32 % (UW3+UW4 treatment) and 41 % increase (CMH3 treatment) on shoot biomass.

In these experiments, plant root growth was more responsive to PGPR treatment than shoot growth (Figure 3.13). In the case of barley, results showed that PGPR significantly increased root growth (Figure 3.13). There was 96% and 121%

(A)

(B)

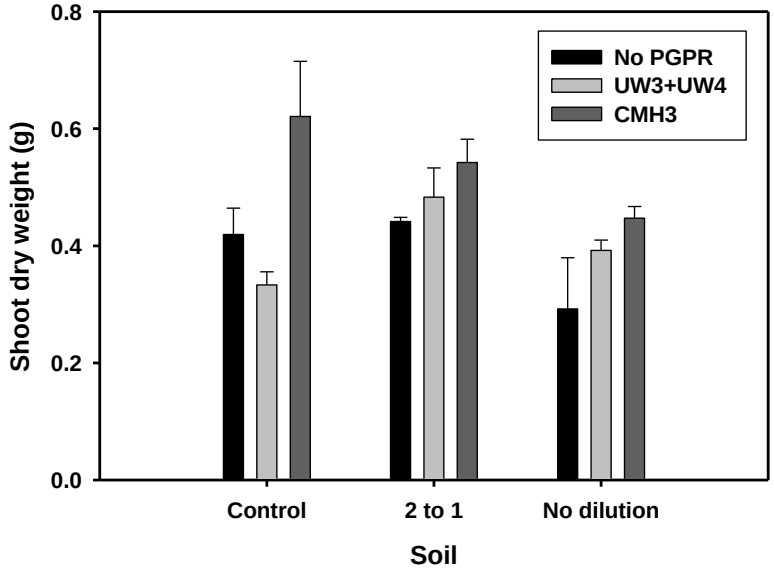
(C)



NO PGPR **UW3+UW4** **CMH3**

Figure 3.12. Growth of barley with and without PGPR treatments after 21 days in control and salt impacted soils from Cannington Manor South (CMS) research site. Three types of soils were used: Control (Quali-grow artificial soil) (EC_e = 2.2 dS/m) (A), 2:1 dilution soil (salt-impacted soil from CMS research site: Quali-grow artificial soil) (EC_e = 10.6 dS/m) (B), and no dilution soil (undiluted salt-impacted soil from CMS site) (EC_e = 16.5 dS/m) (C).

(A)



(B)

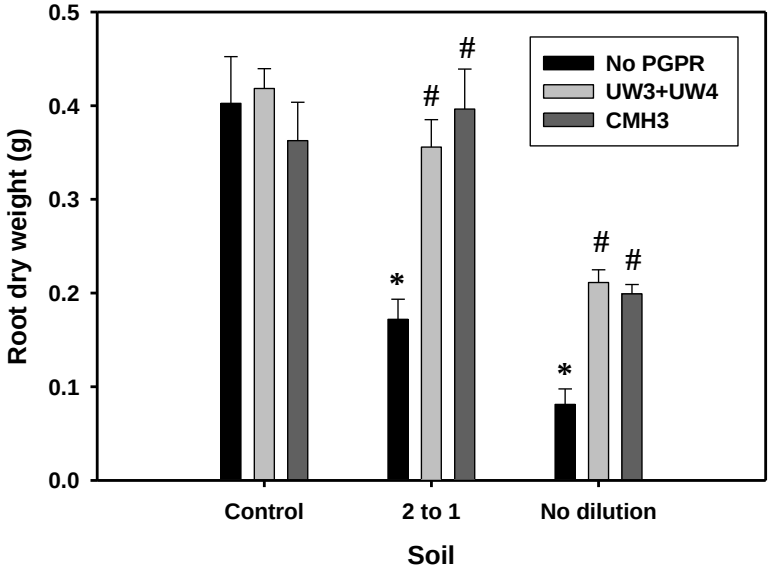


Figure 3.13. Plant dry weight of PGPR treated and untreated barley shoots (A) and roots (B) after 21 days of growth in control and salt impacted soils from Cannington Manor South (CMS) research site. Three types of soils were used: Control (Quali-grow artificial soil) (EC_e = 2.2 dS/m), 2:1 dilution soil (salt-impacted soil from CMS site: Quali-grow artificial soil) (EC_e = 10.6 dS/m), and No dilution (undiluted salt-impacted soil from CMS site) (EC_e = 16.5 dS/m). All measurements were performed in four replicates (N = 4). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences observed when comparing untreated (No PGPR) sample on control soil to all other untreated sample on various soil salinity at $P < 0.05$, # indicates significant differences observed comparing untreated versus UW3+UW4 and CMH3 treated sample on same soil salinity at $P < 0.05$.

increase on root biomass production with treatment of UW3+UW4 and CMH3, respectively, in soil diluted 2:1 CMS salt soil with Quali-grow artificial soil. In undiluted CMS soil, a 130% and 120% increase on root biomass was observed for UW3+UW4 and CMH3 treatment, respectively.

It was expected that the indigenous isolate (CMH3) may be more competitive than the non-indigenous bacteria (UW3 and UW4). However, no significant differences were found in this experiment for the growth promotion effect between UW3+UW4 and CMH3 treatments.

3.2.4 Growth of barley and oats on Cannington Manor South (CMS) salt-impacted soil at various salinity levels

In this set of experiments, salt-impacted soil from the Cannington Manor South (CMS) site (EC_e = 16.5 dS/m) was diluted with top soil (Quali-grow soil, EC_e = 2.2 dS/m) to various ratios: 1:1, 2:1, and 3:1 (x:1, where x refers to relative amount of salt-impacted soil). The properties of the undiluted salt-impacted soil from CMS site and Quali-grow soil can be found in Appendix (Table 1 and Table 2). Barley (*Hordeum vulgare* C.V. AC ranger) and oats (*Avena sativa* C.V. CDC baler) seeds with and without PGPR (UW3+UW4) treatment were planted on control soil (Quali-grow artificial soil), diluted and undiluted salt-impacted soil. After plants were grown for 30 days in the greenhouse, dry weight of shoots and roots were measured. The electrolyte leakage of plant cell membrane was also measured and will be presented in a later section.

Growth of barley was inhibited with increasing soil salinity (Figure 3.14 and Figure 3.15). Without PGPR treatment, there was more than a 40% decrease in shoot dry biomass

and 50% decrease in root dry biomass with soil EC_e > 10 dS/m compared to control (Figure 3.15). Upon PGPR treatment, growth of shoots was promoted, as indicated by increased biomass production (Figure 3.15). On average, there was a 24% increase on shoot growth upon PGPR treatment. However, there was no significant effect on root growth by PGPR in this particular experiment, possibly due to the size limitation of the small ($6 \times 6 \times 5$ cm) pots used resulting in limited root development. This may be have adversely affected PGPR performance on root growth.

Comparable effects of PGPR on plant growth were also observed for oats relative to barley (Figure 3.16 and Figure 3.17). There was a 100% increase of oats shoot dry weight production with UW3+UW4 treatment on saline soil compared to untreated plants (Figure 3.17), however, similar to the results observed with barley, there was no significant PGPR effects was observed on root biomass production.

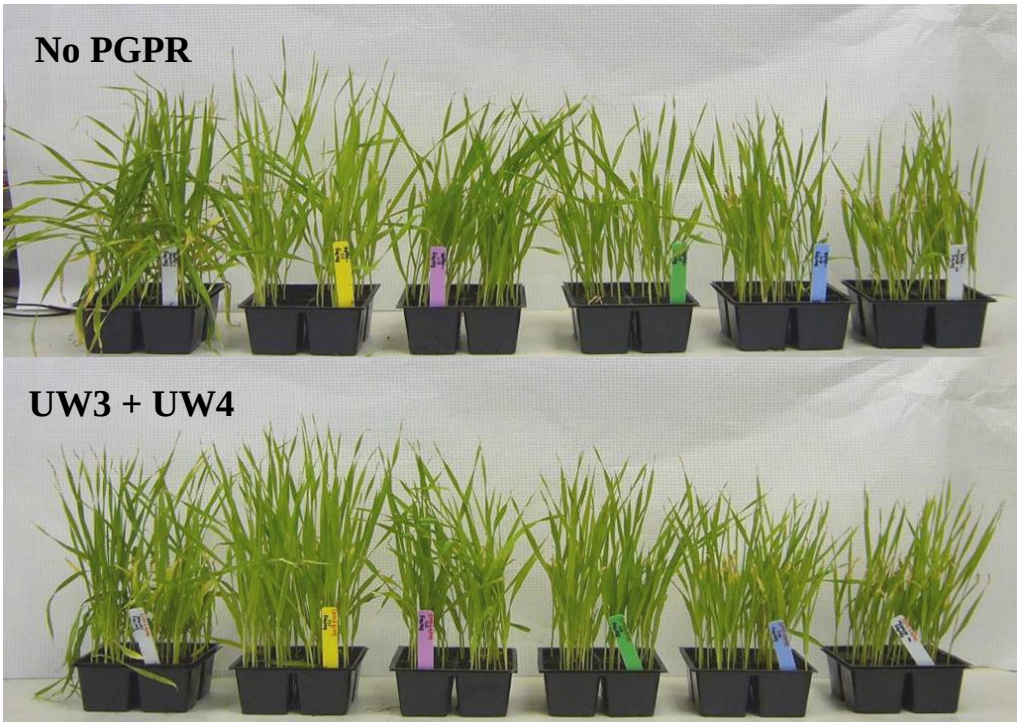
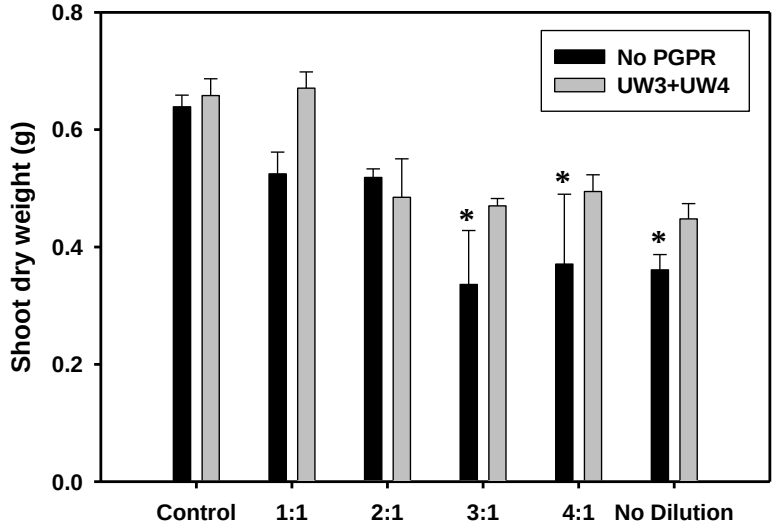


Figure 3.14. Barley after 30 days of growth in control (Quali-grow artificial) soil and salt-impacted soils from Cannington Manor South (CMS) site with various salinity. Salt-impacted soil from CMS site was diluted with Quali-grow artificial soil in ratio of x to 1, where x represents fraction of salt-impacted soil. The type of soil used in this experiment: Control (Quali-grow artificial soil) (EC_e = 2.2 dS/m), 1 to 1 (EC_e = 9.2 dS/m), 2 to 1 (EC_e = 10.6 dS/m), 3 to 1 (EC_e = 13.4 dS/m), 4 to 1 (EC_e = 14.5) and No dilution (undiluted salt impacted soil from CMS) (EC_e = 16.5 dS/m).

(A)



(B)

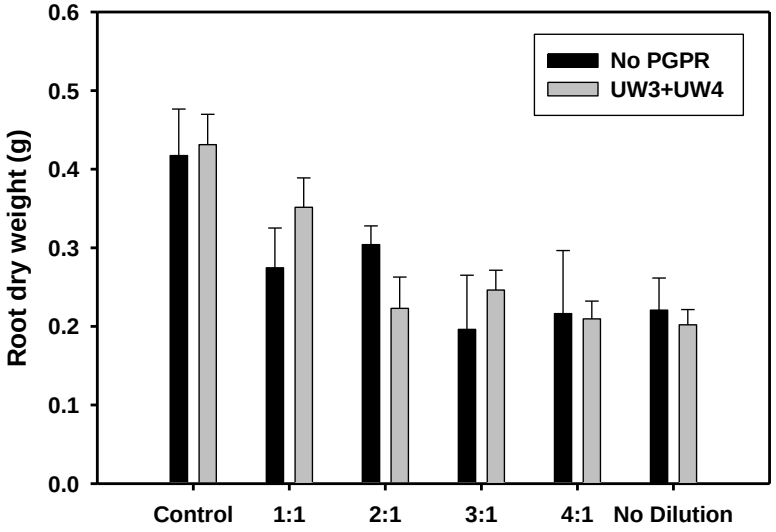


Figure 3.15. Plant dry biomass of barley shoot (A) and root (B) grown for 30 days in control (Quali-grow artificial) soil and salt-impacted soils from Cannington Manor South (CMS) research site with various salinity. Salt-impacted soil from CMS site was diluted with Quali-grow artificial soil in ratio of x to 1, where x represents fraction of salt-impacted soil. Type of soil used in this experiment: Control (Quali-grow artificial) (EC_e = 2.2 dS/m), 1 to 1 (EC_e = 9.2 dS/m), 2 to 1 (EC_e = 10.6 dS/m), 3 to 1 (EC_e = 13.4 dS/m), 4 to 1 (EC_e = 14.5) and No dilution (undiluted salt-impacted soil from CMS) (EC_e = 16.5 dS/m). All measurements were performed in four replicates (N = 4). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences comparing untreated (No PGPR) sample on control soil to all other samples without PGPR in soil of various salinity various soil salinities at $P < 0.05$.

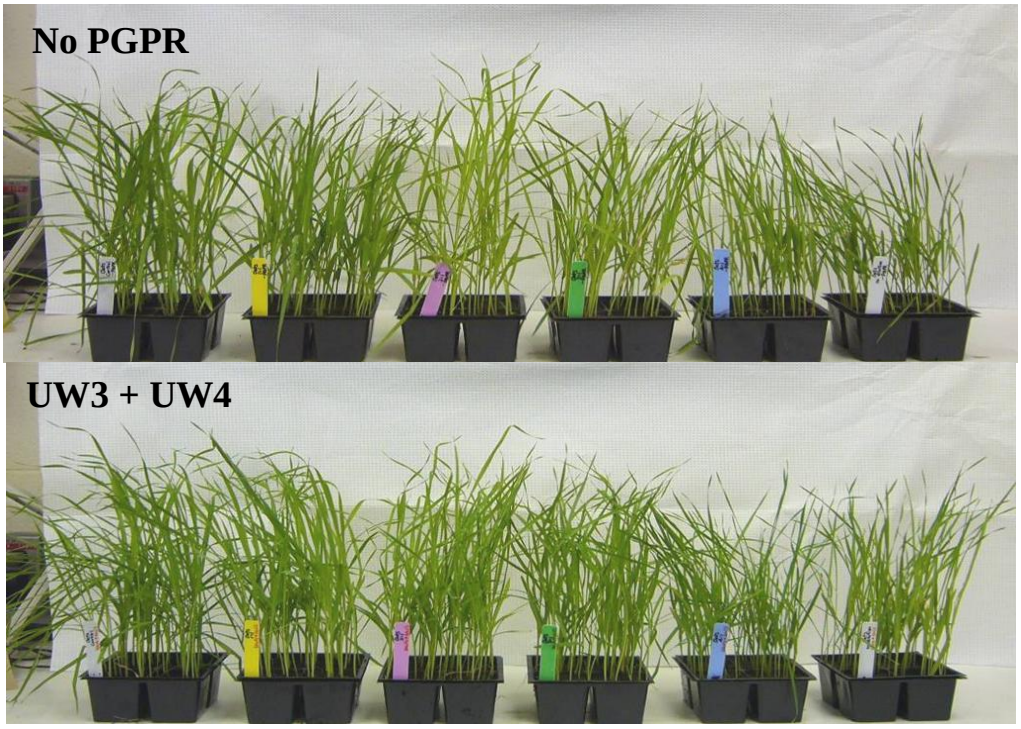
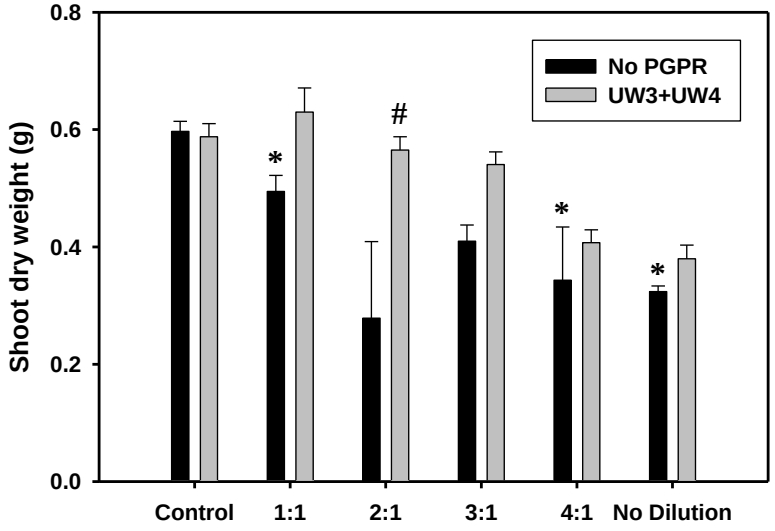


Figure 3.16. Oats after 23 days of growth in control (Quali-grow artificial) soil and salt-impacted soils from Cannington Manor South (CMS) research site with various salinity. Salt impacted soil from CMS site was diluted with Quali-grow artificial soil in ratio of x to 1, where x is fraction of salt-impacted soil. The type of soil used: Control (Qauli-grow artificial soil) (EC_e = 2.2 dS/m), 1 to 1 (EC_e = 9.2 dS/m), 2 to 1 (EC_e = 10.6 dS/m), 3 to 1 (EC_e = 13.4 dS/m), 4 to 1 (EC_e = 14.5) and No dilution (salt-impacted soil from CMS site) (EC_e = 16.5 dS/m).

(A)



(B)

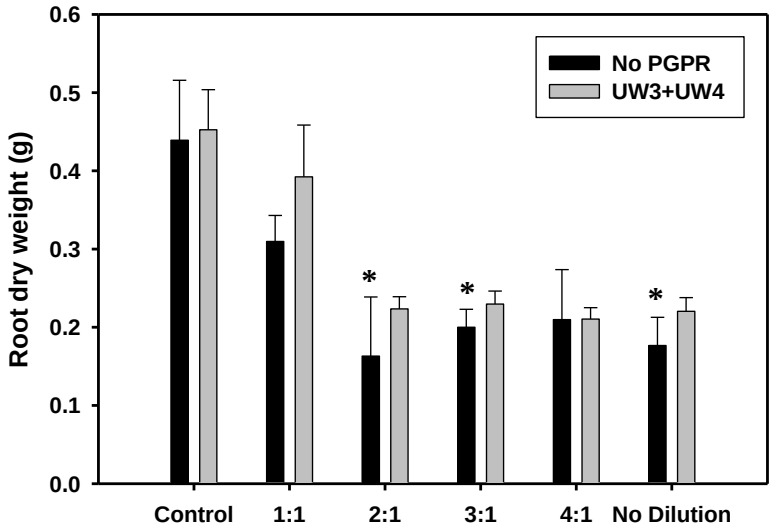


Figure 3.17. Plant dry biomass of oats shoot (A) and root (B) grown for 23 days in control (Quali-grow artificial) soil and salt-impacted soils from Cannington Manor South (CMS) with various salinity. Salt-impacted soil from CMS site was diluted with Quali-grow artificial soil in ratio of x to 1, where x is fraction of salt-impacted soil. Type of soil used in this experiment: Control (Qauli-grow artificial soil) (EC_e = 2.2 dS/m), 1 to 1 (EC_e = 9.2 dS/m), 2 to 1 (EC_e = 10.6 dS/m), 3 to 1 (EC_e = 13.4 dS/m), 4 to 1 (EC_e = 14.5) and No dilution (salt-impacted soil from CMS site) (EC_e = 16.5 dS/m). All measurements were performed in four replicates (N = 4). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test between No PGPR versus UW3+UW4 treated plants. * indicates significant differences comparing untreated (No PGPR) sample on control soil to all other samples without PGPR in soil of various salinities at $P<0.05$. # indicates significant differences observed comparing untreated versus UW3+UW4 treated sample on same soil salinity at $P< 0.05$.

3.3 Greenhouse studies to examine the effects of PGPR on plant cell membrane integrity

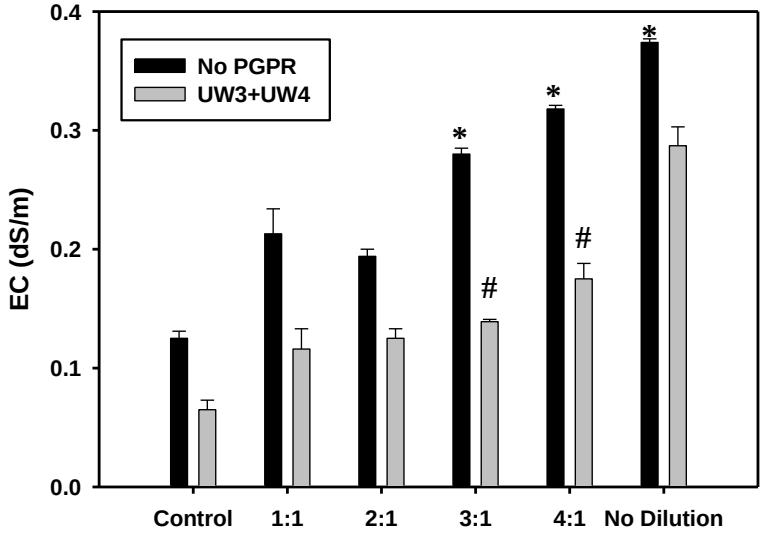
Three experiments were carried out to assess the impact of salinity on plant cell membranes, and whether PGPR has an effect on alleviating the stress by improving plant cell membrane stability.

3.3.1 Impact of increasing salinity and effects of PGPR on electrolyte leakage from plants

In these experiments, salt-impacted soil from the Cannington Manor South (CMS) site (EC_e = 16.5 dS/m) was diluted with control soil (Quali-grow soil, EC_e = 2.2 dS/m) at various ratios (x:1, where x refers to relative amount of salt-impacted soil). The properties of the undiluted salt-impacted soil from CMS site and Quali-grow soil can be found in Appendix (Table 1 and Table 2). Barley (*Hordeum vulgare* C.V. AC ranger) and oats (*Avena sativa* C.V. CDC baler) seeds with and without PGPR (UW3+UW4) treatment were planted in control soil (Quali-grow soil), diluted and undiluted salt-impacted soil. Shoots were removed from plants for electrolyte leakage analysis after 23 days of growth, and electrolyte leakage was measured as electrical-conductivity (EC) in dS/m from solutions containing ions that escaped from the plant tissue (Figure 3.18). The higher the EC (dS/m) value, the greater the amount of damage to cell membranes.

The results of the electrolyte leakage experiment showed that increasing salinity caused greater electrolyte leakage of both barley and oats (Figure 3.18). Plants grown on salt impacted soils demonstrated a significant increase in electrolyte leakage above soil salinity of 10 dS/m compared to plants grown on control (Quali-grow) soils, indicated by increase in EC value measured. For barley, the amount of electrolyte leakage from plants

(A)



(B)

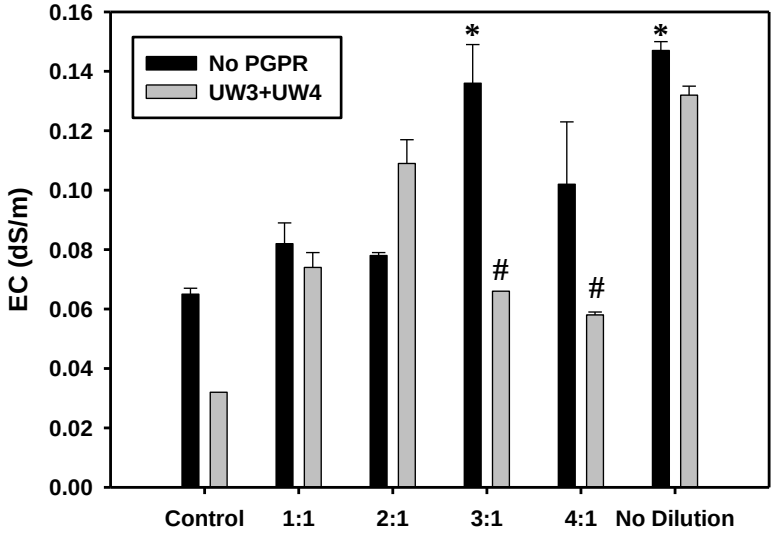


Figure 3.18. Electrolyte leakage experiment as measured by EC for barley (A) and oats (B) leaves grown for 23 days in salt-impacted soils with varying salinity. Soil from Cannington Manor South (CMS) salt-impacted site was diluted with Quali-grow artificial soil in ratio of x to 1, where x is fraction of salt-impacted soil). Six type of soil were used: Control (EC_e = 2.2 dS/m), 1:1 (EC_e = 9.2 dS/m), 2:1 (EC_e = 10.6 dS/m), 3:1 (EC_e = 13.4 dS/m), 4:1 (EC_e = 14.5) and No dilution (EC_e = 16.5 dS/m). Control soil used in this assay was Quali-grow artificial soil and salt-impacted soils were from Cannington Manor South (CMS) salt-impacted site. All measurements were performed in triplicate (N = 3). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences observed when comparing untreated (No PGPR) sample on control soil to all other untreated samples in soil of varying salinities at $P < 0.05$. # indicates significant differences observed comparing untreated versus UW3+UW4 treated samples on same soil salinity at $P < 0.05$.

grown in salt soil diluted 3:1, 4:1, and soil without dilution (No dilution) was significantly increased by 124%, 150%, and 200%, respectively, compared to plants grown on control (Quali-grow artificial) soil. Similar results were also observed in oats. The amount of electrolyte leakage from plants grown in salt soil diluted 3:1 and soil without dilution was significantly increased by 110% and 126%, respectively, compared to plants grown on control soil.

PGPR treatment with UW3+UW4 significantly reduced the amount of electrolyte leakage from plant tissue. For instance, treatment with PGPR significantly decreased electrolyte leakage from barley tissues compared to untreated plants by 50% on salt soil diluted 3:1 and 45% on soil diluted 4:1 (Figure 3.18). Similar to barley, the amount of electrolyte leakage from PGPR-treated plants significantly decreased by 50% on salt soil diluted 3:1 and 43% on soil diluted 4:1. However, it was found the effectiveness of PGPR on electrolyte leakage decreased in undiluted salt-impacted soil for both barley and oats.

3.3.2 Effect of PGPR on electrolyte leakage from plants at medium and high soil salinity

In this set of experiments, salt-impacted soil from Cannington Manor South (CMS) site (EC_e = 16.5 dS/m) was diluted with top soil (Quali-grow soil, EC_e = 2.2 dS/m) at a ratio of 1:1. The properties of the undiluted salt-impacted soil from CMS site and Quali-grow soil can be found in Appendix (Table 1 and Table 2). Barley (*Hordeum vulgare* C.V. AC ranger) and oats (*Avena sativa* C.V. CDC baler) seeds with and without PGPR (UW3+UW4, CMH3) treatments were planted on control soil (Quali-grow artificial soil), diluted and undiluted salt-impacted soil. After plants were grown for 21 days in the

greenhouse, shoots were removed from plants and the degree of electrolyte leakage from leaf tissue was measured as electrical-conductivity (dS/m).

It was found that the amount of electrolyte leakage from plant tissues increased as soil salinity was increased (Figure 3.19), suggesting that plant membrane permeability increased with salinity level. Compared to the plants grown on control soil, electrolyte leakage from untreated (No PGPR) plants grown on salt soil diluted 2:1 and undiluted soil was significantly increased by 137% and 225%, respectively.

Upon treatment with PGPR, the amount of electrolyte leakage was greatly diminished. In soil diluted 2:1 (EC_e = 10.6 dS/m), treatment with UW3+UW4 or CMH3 resulted in lower cell membrane permeability by 40% and 57%, respectively. However, there was a less apparent improvement on cell membrane permeability due to PGPR under non-saline (control soil) and highly saline (undiluted soil) conditions. This demonstrated that the effectiveness of PGPR on plant cell membrane permeability may be decreased under low stress condition or under extreme stress conditions.

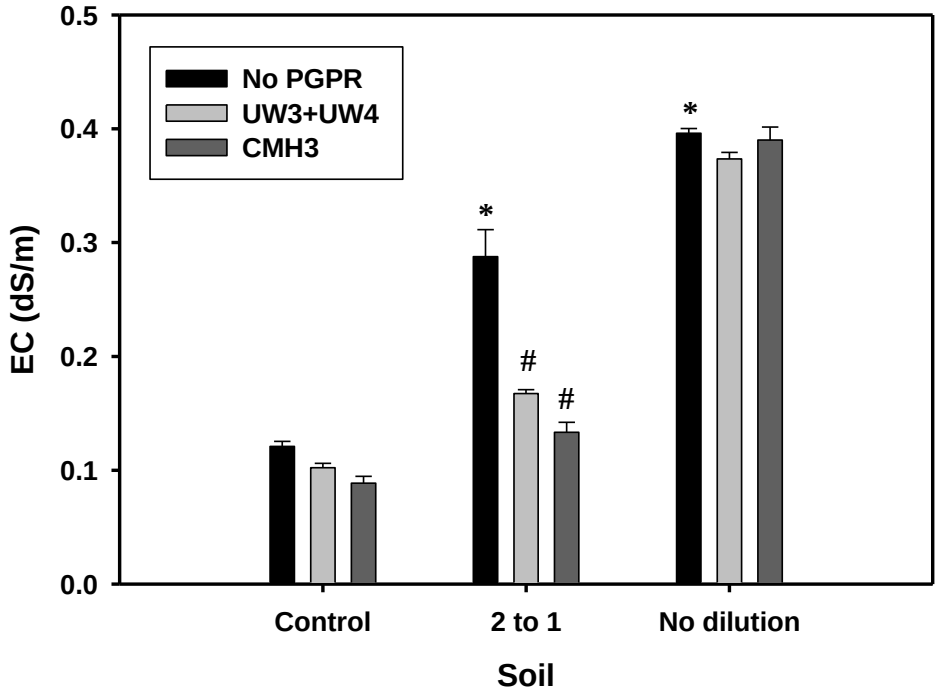


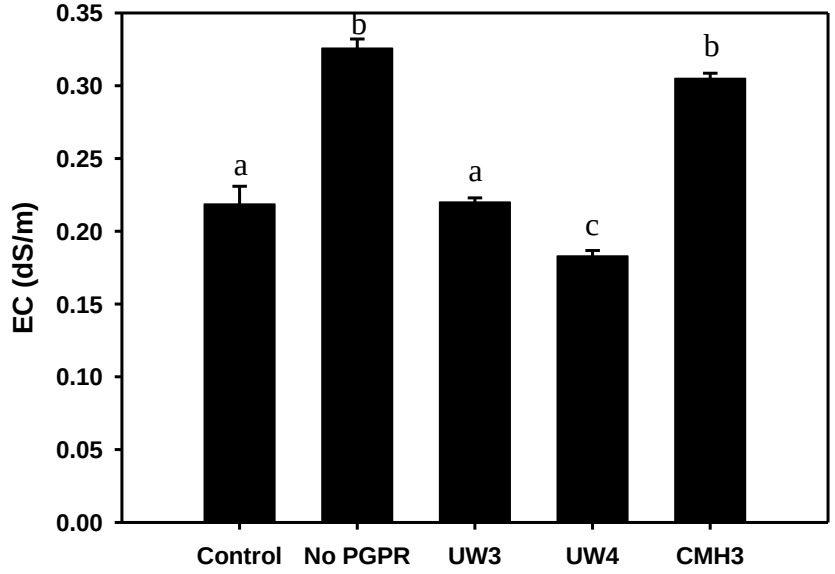
Figure 3.19. Electrolyte leakage experiment measured as EC of barley leaves grown for 21 days in three types of soils. The type of soil used were: control (Quali-grow artificial) soil (EC_e = 2.2 dS/m), 2:1 dilution soil (salt-impacted soil from CMS site: Quali-grow artificial soil) (EC_e = 10.6 dS/m), and salt-impacted soil from CMS site without dilution (EC_e = 16.5 dS/m). All measurements were performed in triplicate (N = 3). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. * indicates significant differences observed when comparing untreated (No PGPR) sample on control soil to all other untreated sample on various soil salinity at $P < 0.05$, # indicates significant differences observed comparing untreated versus PGPR treated sample on same soil salinity at $P < 0.05$.

3.3.3 Effect of various PGPR treatment on electrolyte leakage from plants

These experiments were performed using barley (*Hordeum vulgare* C.V. AC ranger) and oats (*Avena sativa* C.V. CDC baler) with three different PGPR treatments (UW4, UW3 and CMH3) after 21 days of growth. Plant seeds treated with and without PGPR were grown on salt-impacted soil from Cannington Manor South (CMS) site (EC_e = 16.5 dS/m) that was diluted with ProMix^{TM} (EC_e = 1.8 dS/m) at a ratio of 1 to 1, and the resulting EC_e of the soil mixture was 8.8 dS/m. Amount of electrolyte leakage from plant membrane was examined (Figure 3.20).

Consistent results showed that salinity increased the amount of electrolyte leakage from plant cell membrane in both plants (comparing Control versus No PGPR), suggesting that salinity makes the cell membrane more permeable (Figure 3.20). Upon PGPR treatment, plant cell membranes were found to have less electrolyte leakage. Significant improvements were found for all PGPR treatments. In barley, electrolyte leakage was significantly decreased by 44 % and 32 % withUW4 andUW3 treatment, respectively. Similar results were also found in oats. Amount of electrolyte leakage from oats was significantly decreased by 35%, 26%, and 20% with UW4, UW3, and CMH3 treatment, respectively. Most importantly, it was found that the amount of electrolyte leakage from plants grown on salt-impacted soil with PGPR treatments were similar to the amount of electrolyte leakage from plants that were grown in control soil.

(A)



(B)

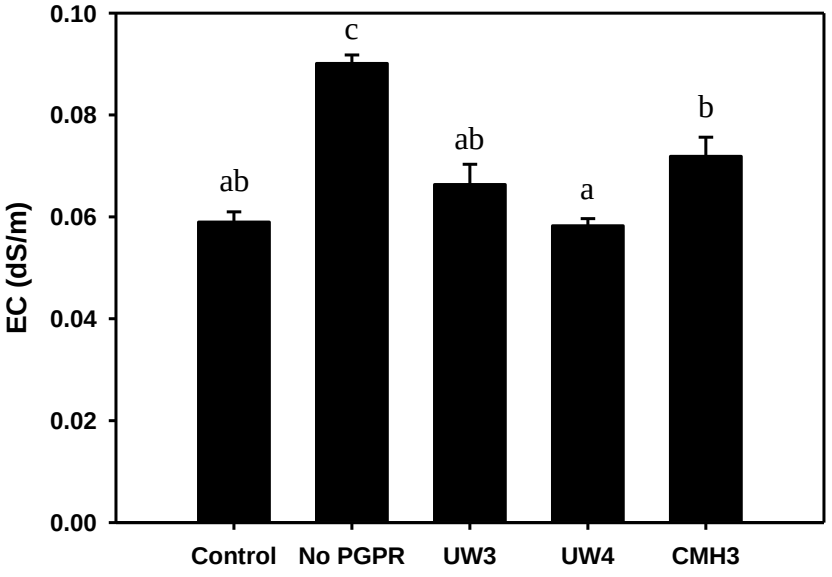


Figure 3.20. Electrical-conductivity (EC) value of membrane leakage experiment for barley (A) and oats (B) leaves grown for 21 days on Cannington Manor South (CMS) salt-impacted soils (EC_e = 16.5 dS/m) diluted with ProMix™ soil (EC_e = 1.8 dS/m) in a ratio of 1:1. The final EC_e of the soil mixture is 8.8 dS/m. All measurements were performed in triplicate (N = 3). Error bars were standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Different letters indicate significant differences observed at $P < 0.05$.

3.4 Greenhouse studies to examine PGPR effect on photosynthetic activity

Chlorophyll fluorescence is one of the few physiological parameters that have been shown to correlate with salinity tolerance (Mekkaoui et al. 1989; Monneveux et al. 1990). Two set of experiments were performed to analyze the possible changes in chlorophyll fluorescence and photosynthetic activity from plant leaves caused by incubation in saline soils. Chlorophyll fluorescence was measured with pulse amplitude modulated (PAM) fluorometry to determine if salinity would cause an impact on photosynthetic activity, and if PGPR treatment would lead to alleviation stress on photosynthetic activity.

In the first experiment, barley (*Hordeum vulgare* C.V. AC ranger), oats (*Avena sativa* C.V. CDC baler), tall fescue (*Festuca arundinacea* C.V. Inferno), and tall wheatgrass (*Agropyron elongatum*) were planted with and without PGPR treatment, in ProMix^{TM} soil (EC_e = 1.8 dS/m) and Alameda (AL) salt-impacted soil (EC_e = 30 dS/m) from research site. The properties of the salt-impacted soil from AL site and ProMix^{TM} soil can be found in Appendix (Table 1 and Table 2). Several chlorophyll *a* fluorescence parameter values for barley (AC ranger) and oats (CDC baler) after 20 days of growth on Promix^{TM} soil (EC_e < 2 dS/m) and salt-impacted soil from Alameda (AL) site (EC_e = 30 dS/m) can be found on Table 3.3. Representative PAM fluorometry induction curves for each treatment were shown in Figure 3.21 and Figure 3.22 for barley and oats, respectively.

By obtaining the minimal fluorescence in dark-adapted plant tissue (F_o) and the maximal fluorescence (F_m), the maximal quantum yield of PSII (F_v/F_m) ratio was calculated. Typical values of F_v/F_m for a healthy plant is approximately 0.8 (Björkman and Demmig 1987), and plants grown on ProMix^{TM} soil (Control) without PGPR treatment

Table 3.3. Chlorophyll *a* fluorescence parameters of two crop plants –barley (20 days) and oats (20 days) on ProMix^{TM} (ECe < 2 dS/m) and Alameda (AL) salt-impacted soil (ECe = 30 dS/m)

Cereal plants	Chlorophyll *a* fluorescence parameters	Control (ProMix^{TM})	No PGPR (Salt soil)	UW3 + UW4 (Salt soil)	CMH3 (Salt soil)
Barley (AC ranger)	**F_v/F_m**	0.791 ± 0.005 a	0.757 ± 0.009 b	0.775 ± 0.007 ab	0.788 ± 0.004 a
	Yield	0.682 ± 0.008 a	0.563 ± 0.019 c	0.635 ± 0.010 b	0.656 ± 0.007 ab
	qP	0.909 ± 0.005 a	0.834 ± 0.017 b	0.897 ± 0.006 a	0.895 ± 0.005 a
	qN	0.255 ± 0.022 a	0.423 ± 0.026 b	0.370 ± 0.017 ab	0.322 ± 0.022 a
Oats (CDC baler)	**F_v/F_m**	0.806 ± 0.002 a	0.752 ± 0.013 b	0.801 ± 0.002 a	0.803 ± 0.002 a
	Yield	0.690 ± 0.005 a	0.488 ± 0.030 b	0.711 ± 0.020 a	0.706 ± 0.022 a
	qP	0.910 ± 0.004 a	0.729 ± 0.030 b	0.865 ± 0.010 a	0.877 ± 0.006 a
	qN	0.301 ± 0.016 b	0.429 ± 0.042 a	0.358 ± 0.032 ab	0.317 ± 0.019 b

PAM measurements for barley (AC ranger) and oats (CDC baler) were performed in twelve independent replicates (N = 12). Error values were based on standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Numbers followed by different letters in a row within the same plant are significantly different at $P < 0.05$.

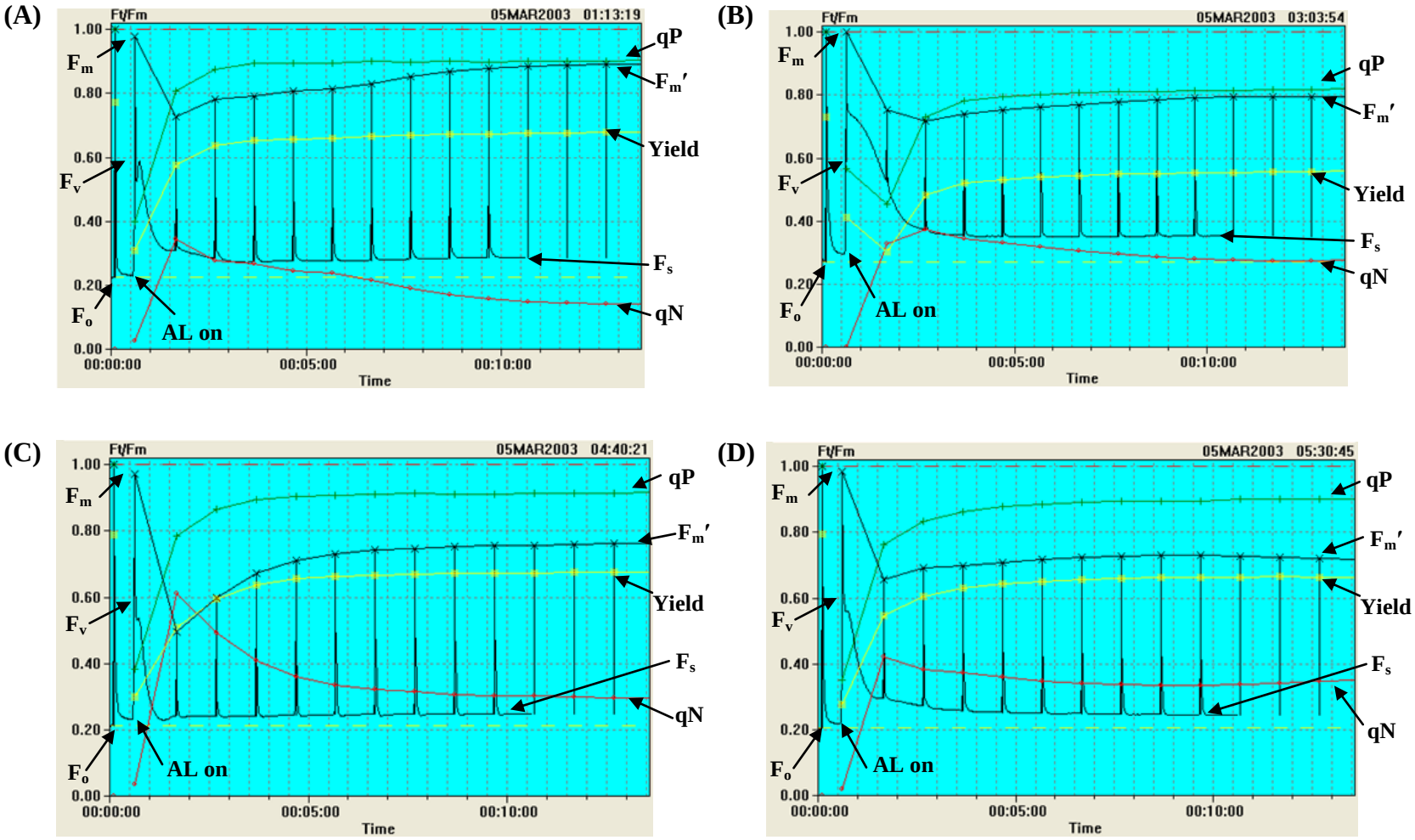


Figure 3.21. PAM induction curves of barley after 20 days of growth on AL salt-impacted soil on ProMix™ (EC_e < 2 dS/m) without PGPR treatment (A), salt-impacted soil from Alameda site without PGPR treatment (B), with UW3 + UW4 treatment (C), and CMH3 treatment (D). All treatments were performed in twelve independent replicates; however, only a representative PAM fluorescence trace was presented.

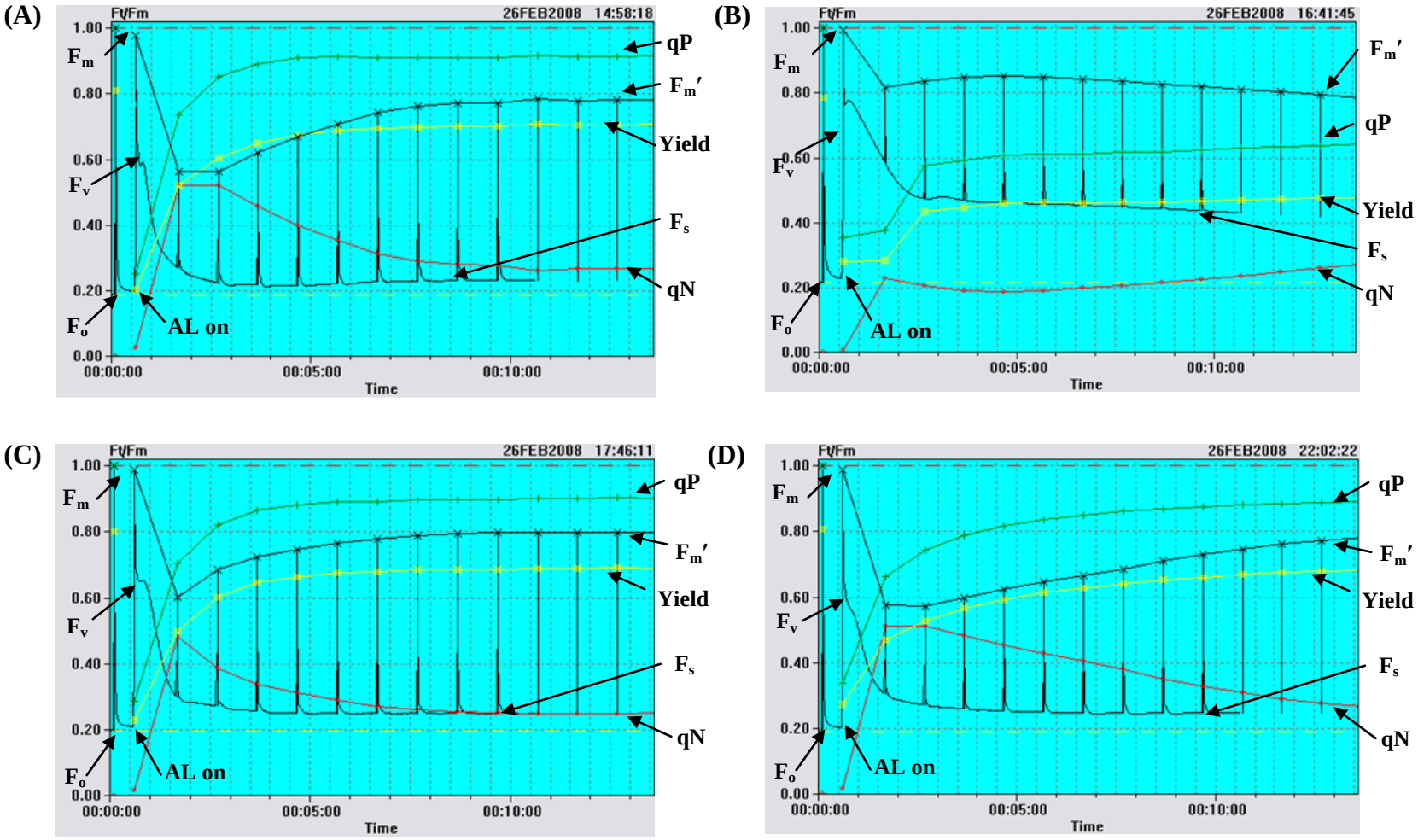


Figure 3.22. PAM induction curve of oats grown for 20 days on ProMix™ (EC_e < 2 dS/m) without PGPR treatment (A), salt-impacted soil from Alameda site (EC_e = 30 dS/m) without PGPR treatment (B), with UW3 + UW4 treatment (C), and with CMH3 treatment (D). All treatments were performed in twelve independent replicates; however, only a representative PAM fluorescence trace was presented.

yielded this typical value. When barley was grown in saline soil without PGPR treatment, a significant decrease in F_v/F_m was observed (Table 3.3). The effective quantum yield at steady state (yield) is a measure of the overall quantum yield of photochemical energy storage. In this study, the photochemical yield for untreated plants decreased 18% and 30% for barley and oats, respectively, compared to the control plants (Table 3.3). Steady-state fluorescence (F_s) can also be used to study the effect of salt stress on photosynthetic electron transport. The F_s value was found to be increased for untreated (No PGPR) plants on saline soil, which indicate possible damage to photosynthesis (Figure 3.21).

Stress response was also observed for untreated barley and oats from quenching indices, photochemical quenching (qP) and non-photochemical quenching (qN). These two parameters can range from 0 to 1. In healthy plants, qP typically stabilizes at a steady-state value which exceeds 0.8, while qN stabilizes to a much lower value, generally less than 0.6 (DeEll and Toivonen 2003). However, in this experiment, qP was higher and qN was lower than the typical values for all treatments. Based on the observation for barley and oats without PGPR treatment on saline soil (Table 3.3), qP decreased 10% to 20% compared to the plants grown on control soil, which also indicates of strains on plants.

It was found that treating barley and oats with PGPR (UW3+UW4, CMH3) can help relieve some of the stress on photonsynthesis due to salinity, as indicated by higher values of F_v/F_m, higher effective quantum yield and higher qP, as well as lower values of qN and F_s compared to untreated plants (NO PGPR) (Table 3.3, Figure 3.21, and Figure 3.22). In fact, the chlorophyll fluorescence parameters of PGPR treated plants were similar to the control values. The maximum quantum yield of PSII (F_v/F_m) was improved (closer to typical value of 0.83) in barley by 2% upon treatment of UW3+UW4 and 4% upon

treatment of CMH3 (Table 3.3). The effective quantum yield at steady-state (yield) of barley was significantly increased by 13% with UW3+UW4 and 16% by CMH3 treatment.

Improvements in the quenching indices, photochemical quenching (qP), and non-photochemical quenching (qN) were also observed with PGPR treated barley. A 7% increase was observed for qP and 24% decrease was observed for qN by treating plants with CMH3. Furthermore, a decrease of fluorescence at steady-state (F_s) was observed for PGPR plants compared to untreated plants (Figure 3.21).

Results for oats were similar to those for barley (Table 3.3 and Figure 3.22). Treatment with UW3+UW4 significantly increased F_v/F_m, yield and qP value by 7%, 46%, and 19%, respectively. The non-photochemical quenching parameter, qN, was significantly decreased by 17% with UW3+UW4 treatment. Comparable results were also found with CMH3 treatment.

In general, it was found there was greater disturbance for photosynthetic activity in oats than in barley due to salinity stress. Despite the greater stress that was observed in oats, treatment with PGPR was able to significantly alleviate the stress on plant photosynthesis, indicated by similar values of chlorophyll fluorescence parameters to the control.

Chlorophyll *a* fluorescence values for the two grass species, tall wheatgrass and tall fescue (Inferno) can be found in Table 3.4 and the corresponding representative PAM induction curves can be found in Figure 3.23 and Figure 3.24, respectively. It was found that grasses were generally more susceptible to salinity stresses in photosynthesis compared to cereal plants. Especially in the case of tall fescue, high F_s values were found for untreated plants (NO PGPR) (Figure 3.24 B) compared to the control (Figure 3.24 A).

Based on the chlorophyll fluorescence parameters obtained, consistent improvement on photosynthetic activity was found for plants that were treated with PGPR for both grass species (Table 3.4). In tall wheatgrass, treatment with PGPR significantly increased F_v/F_m, yield, and qP by 12%, 25%, 3%. Similar results were also found in tall fescue treated with PGPR. The Fv/Fm, yield, qP was significantly increased by 46%, 110%, and 28%, respectively. However, qN did not show a significant decrease with PGPR treatment in either grass plants (tall wheatgrass and tall fescue).

Table 3.4. Chlorophyll *a* fluorescence parameters of two grass plants - tall wheatgrass (35 days) and inferno tall fescue (40days) on ProMix^{TM} (EC_e < 2 dS/m) and Alameda (AL) salt-impacted soil (EC_e = 30 dS/m)

Grass plants	Chlorophyll *a* fluorescence parameters	Control (ProMix^{TM})	No PGPR (Salt soil)	UW3 + UW4 (Salt soil)	CMH3 (Salt soil)
Tall wheatgrass	**F_v/F_m**	0.799 ± 0.003 a	0.713 ± 0.008 b	0.792 ± 0.002 a	0.795 ± 0.005 a
	Yield	0.681 ± 0.009 a	0.581 ± 0.011 b	0.728 ± 0.024 a	0.727 ± 0.025 a
	qP	0.914 ± 0.008 ab	0.891 ± 0.007 a	0.923 ± 0.006 b	0.919 ± 0.004 b
	qN	0.323 ± 0.027	0.334 ± 0.013	0.328 ± 0.015	0.357 ± 0.023
Tall fescue (Inferno)	**F_v/F_m**	0.813 ± 0.003 a	0.515 ± 0.032 b	0.745 ± 0.015 ac	0.696 ± 0.037 c
	Yield	0.657 ± 0.002 a	0.276 ± 0.018 b	0.582 ± 0.018 ac	0.514 ± 0.040 c
	qP	0.880 ± 0.003 a	0.665 ± 0.040 b	0.854 ± 0.035 a	0.864 ± 0.057 a
	qN	0.389 ± 0.006 ab	0.436 ± 0.022 a	0.341 ± 0.027 b	0.451 ± 0.081 ab

PAM measurements for tall wheatgrass and inferno tall fescue were performed in eight (N= 8) and four (N= 4) independent replicates respectively. Error values were based on standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Numbers followed by different letters in a row within the same plant are significantly different at $P < 0.05$.

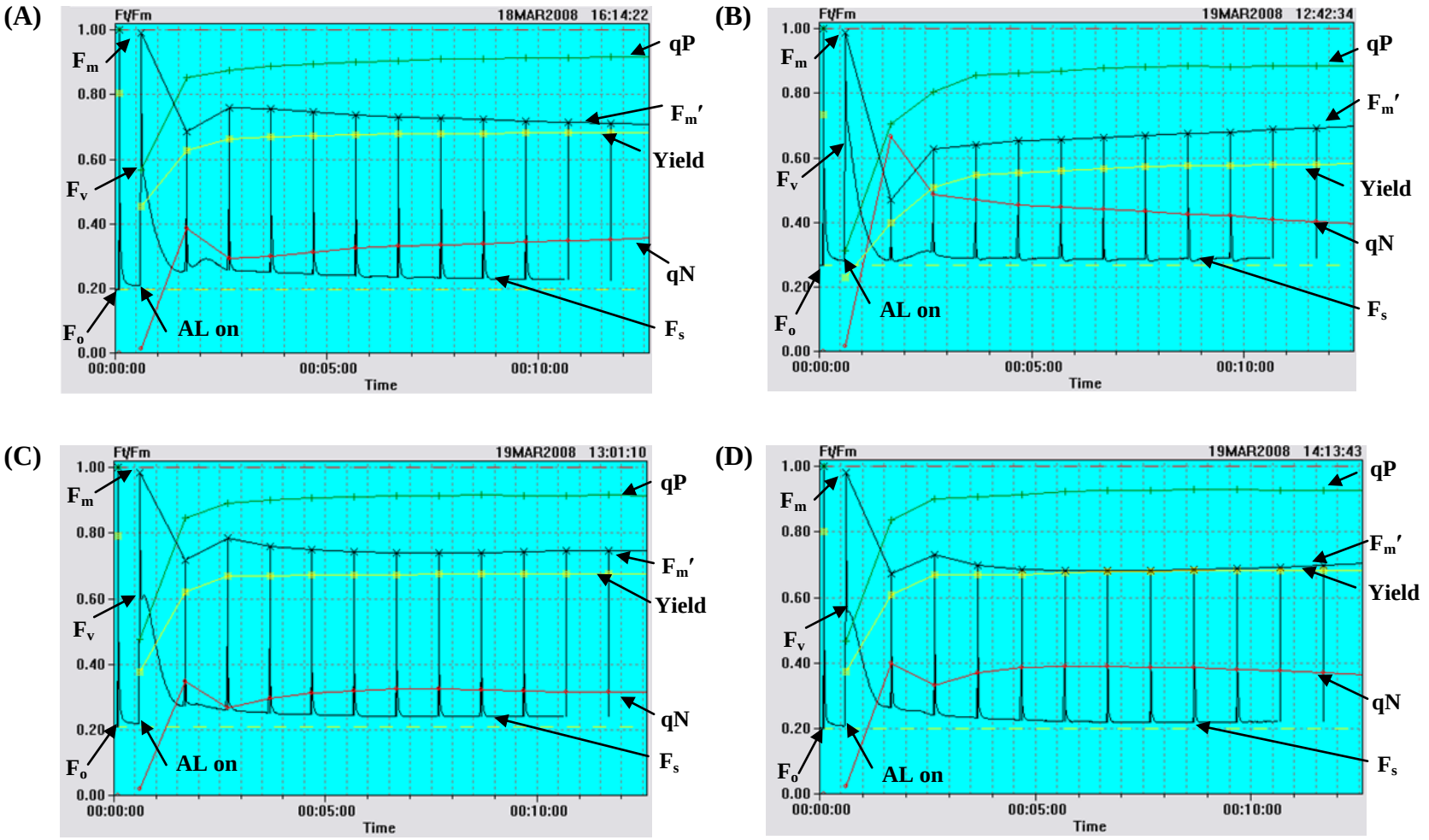


Figure 3.23. PAM induction curve of tall wheatgrass grown for 35 days on ProMix^{TM} (EC_e < 2 dS/m) without PGPR treatment (A), salt-impacted soil from Alameda site (EC_e = 30 dS/m) without PGPR treatment (B), with UW3 + UW4 treatment (C), and with CMH3 treatment (D). All treatments were performed in eight independent replicates; however, only a representative PAM fluorescence trace was presented.

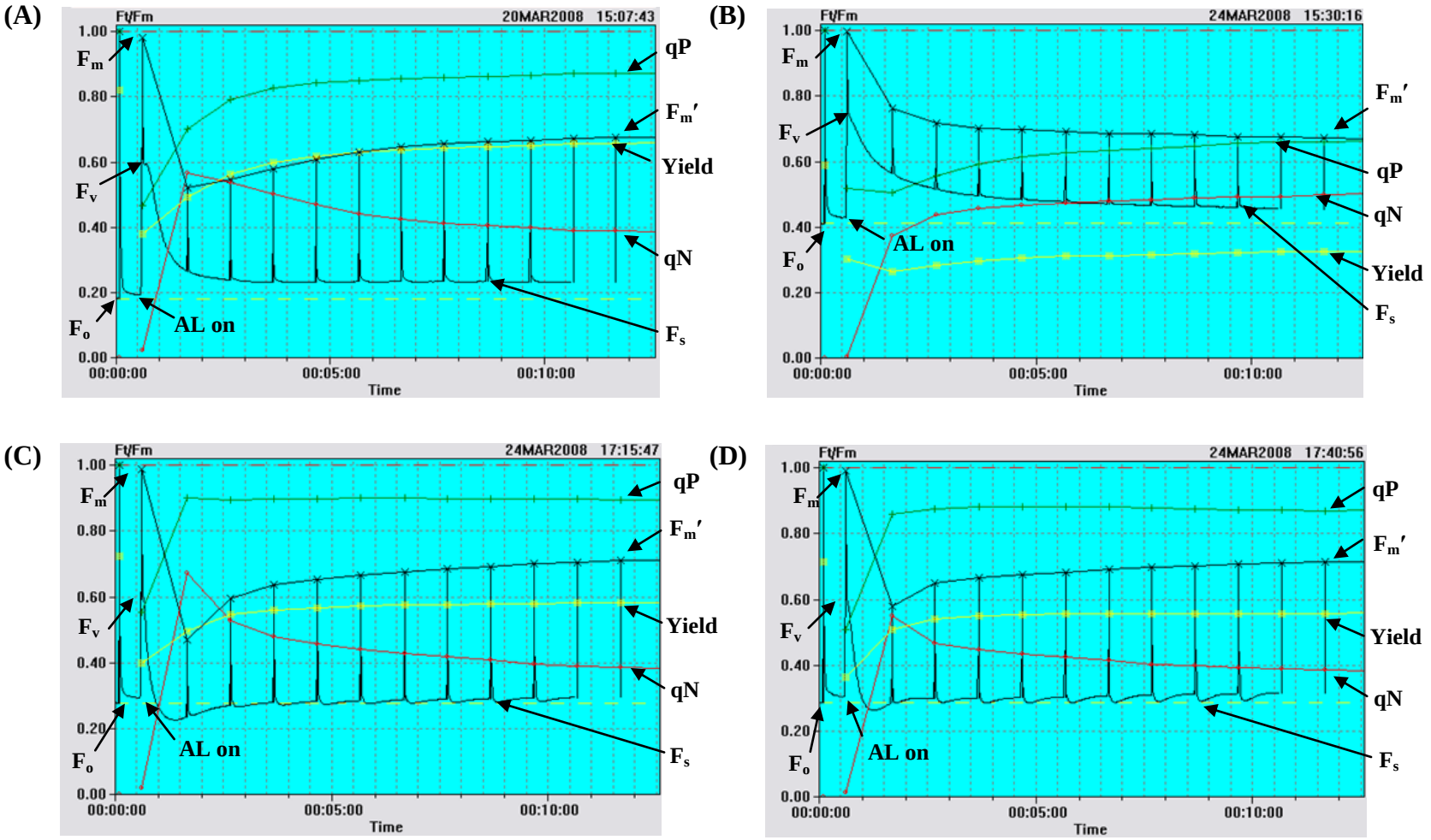


Figure 3.24. PAM induction curve of tall fescue grown for 35 days on ProMix^{TM} (EC_e < 2 dS/m) without PGPR (A), salt-impacted soil from Alameda site (EC_e = 30 dS/m) without PGPR (B), with UW3 + UW4 treatment (C), and with CMH3 treatment (D). All treatments were performed in four independent replicates; however, only a representative PAM fluorescence trace was presented.

The chlorophyll *a* fluorescence experiments with four plants, barley, oats, tall wheatgrass, and tall fescue were repeated using salt-impacted soil from Cannington Manor South (CMS) site (EC_e = 38 dS/m). The properties of the salt-impacted soil from CMS site and ProMix^{TM} soil can be found in Appendix (Table 1 and 2). It was found that the chlorophyll fluorescence parameter values of the PGPR treated and untreated plants grown on CMS salt-impacted soil resembled the chlorophyll fluorescence values of the plants that were grown on Alameda (AL) salt-impacted soil (EC_e = 30 dS/m) (Table 3.5 and Table 3.6). Comparing the plants grown on CMS salt-impacted soil without PGPR treatment to the plants grown on control (ProMix^{TM}) soil, a significant decrease in F_v/F_m, yield and qP value was observed on all four plants.

Compared to data from plants grown on AL sal-impacted soil, consistent result have found that PGPR can partially relieve stresses on the electron transport chain due to salinity. This hypothesis is supported by improved chlorophyll fluorescence parameters (F_v/F_m, yield, qP, and qN). Based on the results obtained, even though the salinity of CMS salt-impacted soil was higher than the AL soil, the degree of impact of salinity on photosynthetic activity is similar, as well as the improvement with PGPR treatments.

To summarize, a significant reduction in photosynthetic efficiency was observed on all four plants under salinity stress, as reflected by deteriorated chlorophyll fluorescence parameters (F_v/F_m, yield, qP, and qN). These results indicated that treatment with PGPR helped plants to partial relieve salt stress on photosynthesis.

Table 3.5. Chlorophyll *a* fluorescence parameters of two crop plants –barley (20 days) and oats (20 days) on ProMix^{TM} (EC_e < 2 dS/m) and Cannington Manor South (CMS) salt-impacted soil (EC_e = 38 dS/m)

Cereal plants	Chlorophyll *a* fluorescence parameters	Control (ProMix^{TM})	No PGPR (Salt soil)	UW3 + UW4 (Salt soil)	CMH3 (Salt soil)
Barley (AC ranger)	**F_v/F_m**	0.773 ± 0.006 a	0.722 ± 0.007 c	0.747 ± 0.006 b	0.783 ± 0.002 a
	Yield	0.662 ± 0.016 a	0.566 ± 0.034 b	0.623 ± 0.004 ab	0.679 ± 0.016 a
	qP	0.899 ± 0.011 ab	0.846 ± 0.022 b	0.907 ± 0.002 a	0.922 ± 0.004 a
	qN	0.225 ± 0.036	0.296 ± 0.071	0.328 ± 0.040	0.270 ± 0.077
Oats (CDC baler)	**F_v/F_m**	0.800 ± 0.005	0.727 ± 0.044	0.779 ± 0.013	0.799 ± 0.005
	Yield	0.690 ± 0.007 a	0.454 ± 0.054 b	0.665 ± 0.013 a	0.682 ± 0.010 a
	qP	0.908 ± 0.004 a	0.736 ± 0.057 b	0.905 ± 0.003 a	0.901 ± 0.007 a
	qN	0.252 ± 0.014 a	0.531 ± 0.048 b	0.273 ± 0.018 a	0.269 ± 0.011 a

PAM measurements for barley (AC ranger) and oats (CDC baler) were performed in four independent replicates (N = 4). Error values were based on standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Numbers followed by different letters in a row within the same plant are significantly different at $P < 0.05$.

Table 3.6. Chlorophyll *a* fluorescence parameters of tall wheatgrass (35 days) and inferno tall fescue (40 days) on ProMix^{TM} (EC_e < 2 dS/m) and Cannington Manor South (CMS) salt-impacted soil (EC_e = 38 dS/m)

Grass plants	Chlorophyll *a* fluorescence parameters	Control (ProMix^{TM})	No PGPR (Salt soil)	UW3 + UW4 (Salt soil)	CMH3 (Salt soil)
Tall wheatgrass	**F_v/F_m**	0.807 ± 0.001 a	0.745 ± 0.004 c	0.796 ± 0.002 ab	0.793 ± 0.004 b
	Yield	0.665 ± 0.010 a	0.585 ± 0.013 b	0.655 ± 0.009 a	0.647 ± 0.009 a
	qP	0.899 ± 0.010	0.899 ± 0.011	0.915 ± 0.005	0.906 ± 0.009
	qN	0.389 ± 0.017 b	0.460 ± 0.033 ab	0.435 ± 0.017 ab	0.427 ± 0.017 a
Tall fescue (Inferno)	**F_v/F_m**	0.813 ± 0.003 a	0.702 ± 0.026 b	0.767 ± 0.009 a	0.760 ± 0.005 ab
	Yield	0.657 ± 0.002 a	0.504 ± 0.040 b	0.600 ± 0.024 ab	0.595 ± 0.009 ab
	qP	0.880 ± 0.003 ab	0.823 ± 0.028 b	0.868 ± 0.021 ab	0.904 ± 0.013 a
	qN	0.389 ± 0.006 a	0.462 ± 0.016 a	0.406 ± 0.017 a	0.493 ± 0.025 b

PAM measurements for tall wheatgrass and inferno tall fescue were performed in four (N= 4) independent replicates. Error values were based on standard errors (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's test. Numbers followed by different letters in a row within the same plant are significantly different at $P < 0.05$

3.5 Field trials on phytoremediation of salt-impacted soils with the aid of plant growth-promoting rhizobacteria (PGPR)

The goal of this research was to assess the effectiveness of plant-PGPR remediation technology for salt-impacted soils in the field. The effectiveness of PGPR on promoting plant growth and salt uptake was assessed during and at the end of each growth season. Furthermore, the soil salinity levels for each site were monitored over two years to estimate the efficacy of the PGPR-enhanced phytoremediation technology.

3.5.1 Plant biomass production on CMS, CMN and AL sites

In this research, field studies were conducted to verify the feasibility of phytoremediation with the aid of PGPR on salt-impacted sites. Vegetation coverage after a three-month growing period was estimated on CMS, CMN, and AL site. The percent vegetation coverage was determined by visual estimation of the percent plant coverage for each subsections of the site, shown in Figure 3.25 for CMS, and Figure 3.26 for AL. The percent vegetation coverage figure was not included for CMN site because there appeared to be 100% plant growth throughout the entire site. It should be noted that soil flooding and crusting was observed on CMS and AL sites due to excessive rainfall (Figure 3.25 and Figure 3.26). Those areas were highly saline and generally had poor or no vegetation coverage. Vegetation was uneven on CMS and AL sites, possibly due to problems of waterlogging and higher soil salinity in the flooded areas. For those reasons, it was expected that there would be poor plant germination and growth on the flooded areas of CMS and AL.

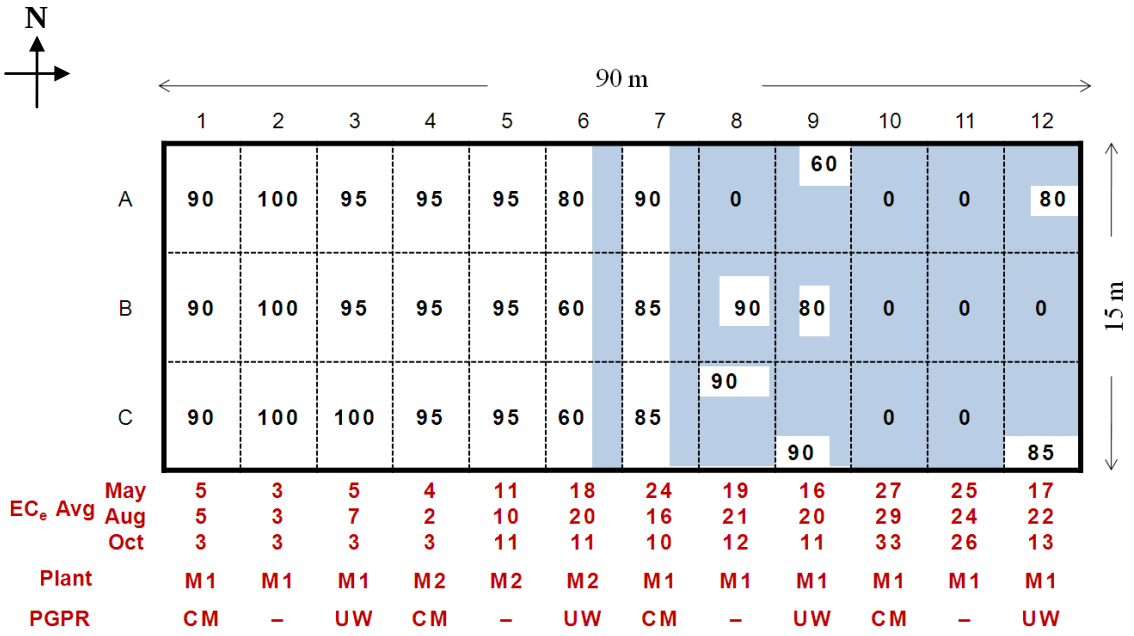


Figure 3.25. Percent (%) vegetation coverage of Cannington Manor South (CMS) salt-impacted site in 2008 after three months of growth (June-August). The EC_e Avg is the EC_e average for each plot. Flooded areas are indicated as shaded area. East side of this site was flooded and plant growth was poor and spotted in these areas.

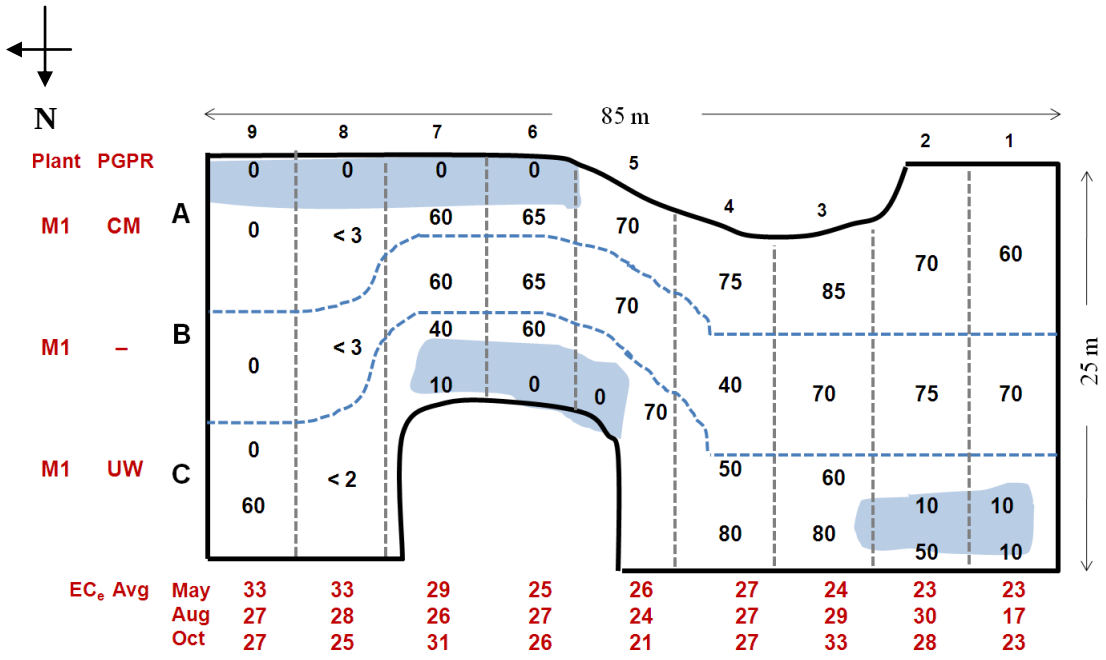


Figure 3.26. Percent (%) vegetation coverage of Alameda (AL) salt-impacted site in 2008 after three months of growth (June-August). The EC_e Avg is the EC_e average for each plot. Flooded areas were indicated as shaded area.

At the end of the growing season (5 months of growth), plants generally grew taller (30 – 40 cm for oats and 15 – 25 cm for grasses) and matured fully at the CMN site compared to the plants at CMS and AL sites which were shorter (10 – 20 cm for oats and < 15 cm for grasses) possibly due to high soil salinity. When cereals and grasses were planted as a mix on each site, the growth of oats overshadowed the grasses at first, but the grasses were able to continue growing after the oats withered after five months.

After three months (June- Aug 08) and five months (Oct 08) of growth, plant growth and plant biomass productions on area (50 cm × 50 cm) with similar vegetation coverage (> 80%) at various EC_e range plots were assessed for each of the three research sites (Table 3.7, Figure 3.27, Figure 3.28, Figure 3.29, and Figure 3.30). However, only a few plant samples were obtained from each site for analysis due to weight and space constrains in air-shipping.

PGPR effects on plant growth promotion in field trials in 2008 resembled the findings reported previously (Chang 2007). It was found that PGPR treated plants produced greater plant biomass compared to untreated plants at all three research sites (Table 3.7). In addition, the PGPR effect on plant growth was more apparent when plants were grown for longer periods on the field site, by comparing plant biomass after three months and five months of growth. Comparing plant biomass production on plot 2 and 3 in CMS site, there was a 38% increase of shoot biomass production with UW3+UW4-treated plants after three months of growth compared to untreated plants in similar soil salinity (Table 3.7). After five months of growth, both UW3+UW4 and CMH3 treatment increased shoot biomass of mixed plant species by 74% compared to untreated plants grown in similar soil salinity (EC_e ranged from 3-5 dS/m). Interestingly, PGPR also

Table 3.7. Dry weight of above-ground plant tissue treated with or without PGPR after 3 and 5 months of growth on salt-impacted sites

Site	Plot	EC_e (dS/m)	Plants	Treatment	Aug 08 Plant dry weight (g) per m^2	Increase over untreated	Oct 08 Plant dry weight (g) per m^2	Increase over untreated
CMS	2	3	Mix 1	No PGPR	160		270	
	3	5	Mix 1	UW3+UW4	220	38 %	470	74 %
	1	5	Mix 1	CMH3	160	0 %	470	74 %
	8	19	Mix 1	No PGPR	N/D		300	
	9	16	Mix 1	UW3+UW4	N/D		540	80 %
	7	24	Mix 1	CMH3	N/D		380	27 %
CMN	2	4	Oats	No PGPR	148		N/D	
	1	5	Oats	CMH3	256	73 %	N/D	
	10	9	Tall wheatgrass	No PGPR	92		N/D	
	9	8	Tall wheatgrass	CMH3	232	152 %	N/D	
AL	B-1	11	Mix 1	No PGPR	196		240	
	C-1	24	Mix 1	UW3+UW4	120	- 39 %	340	42 %
	A-1	34	Mix 1	CMH3	180	- 8 %	180	- 25 %
	B-3	24	Mix 1	No PGPR	N/D		100	
	C-3	18	Mix 1	UW3+UW4	N/D		260	160 %
	A-3	31	Mix 1	CMH3	N/D		200	100 %
	B-5	25	Mix 1	No PGPR	N/D		60	
	C-5	22	Mix 1	UW3+UW4	N/D		200	233 %
	A-5	30	Mix 1	CMH3	N/D		140	133 %

- Plant samples were taken at Aug 08 (3 months of growth) and Oct 08 (5 months of growth).
- Plant dry weight production (g) per m^2 at various EC_e range for different plant species and treatments were listed for each site.
- N/D represents plants samples were not taken from research site.
- Mix 1 is a mixture of common oats, tall wheatgrass and tall fescue in 1:1:1 ratio (v/v/v).

(A) Mixed plant species - 3 months of growth on CMS

No PGPR
Plot: 2
EC_e = 3 dS/m

UW4+UW3
Plot: 3
EC_e = 5 dS/m

CMH3
Plot : 1
EC_e = 5 dS/m

(B) Mixed plant species - 5 months of growth on CMS

No PGPR
Plot: 2
EC_e = 3 dS/m

UW4+UW3
Plot: 3
EC_e = 5 dS/m

CMH3
Plot: 1
EC_e = 5 dS/m

Figure 3.27. Plant growth of mixed plant species (oats + inferno tall fescue + tall wheatgrass) on Cannington Manor South (CMS) site after 3 months (A) and 5 months (B).

(A) Oats – 3 months of growth on CMN

No PGPR
Plot: 2
EC_e = 4 dS/m

CMH3
Plot: 1
EC_e = 5 dS/m

(B) Oats – 5 months of growth on CMN

No PGPR
Plot: 2
EC_e = 4 dS/m

CMH3
Plot: 1
EC_e = 5 dS/m

Figure 3.28. Plant growth of oats on Cannington Manor North (CMN) site after 3 months (A) and 5 months (B).

(A) Tall wheatgrass – 3 months of growth on CMN

No PGPR
Plot: 9
EC_e = 9 dS/m

CMH3
Plot: 8
EC_e = 8 dS/m

(B) Tall wheatgrass – 5 months of growth

No PGPR
Plot : 9
EC_e = 9 dS/m

CMH3
Plot: 8
EC_e = 8 dS/m

Figure 3.29. Plant growth of tall wheatgrass on Cannington Manor North (CMN) site after 3 months (A) and 5 months (B).

(A) Mixed plant species – 3 months of growth on AL

No PGPR
Plot: B-1
EC_e = 11 dS/m

UW4+UW3
Plot: C-1
EC_e = 24 dS/m

CMH3
Plot: A-1
EC_e = 34 dS/m

(B) Mixed plant species – 5 months of growth on AL

No PGPR
Plot: B-1
EC_e = 11 dS/m

UW4+UW3
Plot: C-1
EC_e = 24 dS/m

CMH3
Plot: A-1
EC_e = 34 dS/m

Figure 3.30. Plant growth of mixed plant species (oats + inferno tall fescue + tall wheatgrass) on Cannington Manor South (CMS) site after 3 months (A) and 5 months (B).

showed improvements on plant growth under high soil salinity (EC_e ranged from 16-24 dS/m). Comparing the plant biomass production on plot 7-9 in CMS site, treatment with UW3+UW4 and CMH3 increased plant dry weight production by 80% and 27%, respectively, compared to the untreated plants.

At the AL site, plant growth promotion by PGPR was even more pronounced. Comparing the plant biomass production of mixed plant species per m^2 in plot B-5 with C-5, plants treated with UW3+UW4 were able to increase plant biomass by 230% relative to the untreated plants after five months of growth in similar soil salinity (EC_e ranged from 22-25 dS/m). Interestingly, CMH3 treatment increased plant shoot biomass production by 133% in soil with a much higher salt level (EC_e of 30 dS/m) than the untreated plants.

At the CMN site, cereals and grasses were planted individually rather than as a mix. Cereal and grass plants resulted similar amount of plant biomass production. Results from field study of CMN site showed that treatment of CMH3 increased shoot biomass by 73% and 152% in oats and tall wheatgrass, respectively, after three months of growth (Table 3.7). Plant biomass production after five months of growth in CMN site was not available because plant samples were not taken from the site due to weight and space constrains in shipping.

It is clear that PGPR treatment can improve plant growth in saline soil (EC_e up to 31 dS/m). Greater plant biomass production would be beneficial for salt removal from soil because of the greater amount of salt uptake by plants, hence ultimately increased phytoremediation efficiency.

3.5.2 Salt uptake in above-ground plant tissues of the CMS, CMN and AL sites

Salt and metal ion uptake analyses in plant tissue were performed for the field studies to examine the effect of PGPR on plant salt uptake ability. In this research, a particular interest has been put into examining the accumulation of salt ions in above-ground plant tissues, therefore, only plant shoot tissue was analyzed for ion accumulation. Plant samples from field sites after three and five months of growth were analyzed for salt and metal content by ALS Environmental Inc. (Edmonton, AB) and Maxxam Analytics Inc. (Missisauga, ON) (Table 3.8 and Table 3.9). Samples were sent to two different labs due to the excessive analysis time required by ALS Environmental Inc, therefore the plant samples obtained in October were sent to Maxxam Analytics Inc. to shorten the waiting time. However, due to differences in the digestion procedure used by the two labs, the data may not be comparable.

During the 2008 growth season, NaCl accumulation in plants ranged from 10000 - 40000 mg/kg (Table 3.8 and Table 3.9). If there is an even accumulation of Na and Cl ions in plant tissues, the ratio of Cl/Na should be approximately 1.5 (accounting their atomic weight differences). However, the ratio of Cl/Na ranged from 2-5 from experimental results, indicating that the accumulation between Na^+ and Cl^- in plant tissues was uneven, and that there was a greater accumulation of Cl^- than Na^+. In terms of salt accumulation between plant species, higher salt accumulation as observed in the oats compared to tall wheatgrass (Table 3.8), suggesting that cereals may be a more suitable candidate for phytoremediation than grasses due to greater ability for salt uptake. In general, the amount

Table 3.8. Salt and metal ion uptake in plant tissue with various treatments from field sample of August 2008 (3 months of growth)

Site	Plot	EC_e (dS/m)	Plants	Treatment	Na (mg/kg)	Cl (mg/kg)	NaCl (mg/kg)	K (mg/kg)	Ca (mg/kg)	Cl/Na ratio	K/Na ratio
CMS	2	3	Mix 1	No PGPR	7270	12700	19970	20800	5940	1.7	2.9
	3	5	Mix 1	UW3+UW4	4370	11600	15970	17800	9100	2.7	4.1
	1	5	Mix 1	CMH3	8110	15500	23610	20300	9510	1.9	2.5
CMN	2	4	Oats	No PGPR	8590	19900	28490	19100	5290	2.3	2.2
	1	5	Oats	CMH3	10100	20400	30500	19900	4620	2.0	2.0
	10	9	Tall wheatgrass	No PGPR	3090	15000	18090	21500	4830	4.9	7.0
	9	8	Tall wheatgrass	CMH3	3720	17800	21520	30300	7970	4.8	8.1
AL	B-1	11	Mix 1	No PGPR	4840	15600	20440	17500	8860	3.2	3.6
	C-1	24	Mix 1	UW3+UW4	4120	22100	26220	29200	5480	5.4	7.1
	A-1	34	Mix 1	CMH3	7680	32900	40580	32600	4060	4.3	4.2

- All plant samples were analyzed by ALS Environmental Inc. (Waterloo, ON).
- Mix 1 is a mixture of common oats, tall wheatgrass and tall fescue in 1:1:1 ratio (v/v/v)

Table 3.9. Salt and metal ion uptake in plant tissue with various treatments from field sample of October 2008 (5 months of growth)

Site	Plot	EC_e (dS/m)	Plants	Treatment	Na (mg/kg)	Cl (mg/kg)	NaCl (mg/kg)	Cl/Na ratio
CMS	2	3	Mix 1	No PGPR	3300 ± 100	6100 ± 200	9400 ± 100	1.8
	3	5	Mix 1	UW3+UW4	3650 ± 550	6100 ± 900	9750 ± 350	1.7
	1	5	Mix 1	CMH3	4800 ± 700	9100 ± 1700	13900 ± 2400	1.9
	8	19	Mix 1	No PGPR	1700	8400	10100	4.9
	9	16	Mix 1	UW3+UW4	1700	9100	10800	5.4
	7	24	Mix 1	CMH3	2600	7400	10000	2.8
AL	B-1	11	Mix 1	No PGPR	3600	20600	24200	5.7
	C-1	24	Mix 1	UW3+UW4	3600	17900	21500	5.0
	A-1	34	Mix 1	CMH3	2900	26700	29600	9.2
	B-3	24	Mix 1	No PGPR	2100	27400	29500	13.0
	C-3	18	Mix 1	UW3+UW4	2100	20000	22100	9.5
	A-3	31	Mix 1	CMH3	3700	25200	28900	6.8
	B-5	25	Mix 1	No PGPR	5700	25300	31000	4.4
	C-5	22	Mix 1	UW3+UW4	4400	18600	23000	4.2
	A-5	30	Mix 1	CMH3	3000	22700	25700	7.6

- All plant samples were analyzed by Maxxam analytics Inc. (Missisauga, ON).
- Mix 1 is a mixture of common oats, tall wheatgrass and inferno tall fescue in 1:1:1 ratio (v/v/v).

of NaCl accumulation did not differ much from plants grown on varying soil salinity levels, implying that salt accumulation in plants may not correlate with soil salinity levels.

Based on the data obtained after three months of growth (Table 3.8), CMH3 but not UW3+UW4 treatment increased NaCl accumulation in mixed plants species by 18% on CMS site. At CMN, CMH3 treatment increased 7% on NaCl accumulation in oats, and 19% on tall wheatgrass. A pronounced increase of NaCl accumulation in plant shoots was observed with PGPR-treated plants at AL salt-impacted site. Treatment with UW3+UW4 and CMH3 increased NaCl accumulation in plant tissues by 28% and 99%, respectively. Data obtained after five months of growth did not show much PGPR effect on NaCl accumulation in plant shoots (Table 3.9). This may be due to the incomplete digestion extraction method used by Maxxam Analytic Inc. that did not fully extract salt ions out from plant tissues.

In field studies, K^+ ion accumulation in plant tissues was performed after 3 months of growth on salt-impacted sites (Table 3.8). The ratio of Na^+ and K^+ concentration has been shown to be related to salinity tolerance in plants, and can be use as an indicator for salinity tolerance (Gorhman et al. 1987; Houshmand et al. 2005; Saleque et al. 2005; Tester and Davenport 2003). It was found that UW3+UW4 treated plants resulted in an increase of K/Na ratios relative to the untreated plants by 41% observed at CMS, and by 97% observed at AL site (Table 3.8). Similarly, treatment with CMH3 resulted in increase of K/Na ratio by 16% in tall wheatgrass at CMN site.

Calcium has been reported to reduce the toxic effect of salinity in barley seedlings (Cramer et al. 1989). The field data showed that treatment with UW3+UW4 resulted in an increase of Ca^{2+} accumulation in plant tissues by 60% compared to the untreated plants at CMS site. Similarly, a 65% increase of Ca^{2+} accumulation in tall wheatgrass was observed at CMN site with CMH3 treatment. However, for AL site, no correlation between Ca^{2+} accumulation in plant tissues and PGPR treatment was observed.

3.5.3 Soil salinity measurements of the CMS, CMN, and AL sites

Soil salinity (EC_e) each site was measured as a regular grid assay. The data for 2008 is shown for Cannington Manor South (CMS) (Figure 3.31), Cannington Manor North (CMN) (Figure 3.32), and Alameda (AL) (Figure 3.33). Soil salinity for CMS, CMN, and AL sites in 2007 (Chang 2007) can be found in Appendix (Figures 1, 2, and 3). The CMS and AL sites were highly saline, with an average EC_e of 17.6 and 23.5 dS/m, respectively, measured at the end of the growth season in 2007 (Figure 1 and 3 in Appendix), and EC_e of 13.5 and 27.0 dS/m measured at beginning of growth season in 2008 (Figure 3.31and Figure 3.33). In contrast, soil salinity for CMN was much lower, with average EC_e of 6.5 dS/m in 2007 (Figure 3 in Appendix) and 7.1 dS/m in 2008 (Figure 3.32).

Soil salinity levels of all sites measured in 2008 before planting were similar to the salinity levels measured at the end of growth season in 2007 (Chang 2007), indicating that soil salinity levels remained relatively constant over the Winter (2007-2008) when there was no plant growth (Figure 3.31, Figure 3.32, and Figure 3.33). At the end of the second growing season, results showed that overall soil salinity levels decreased by approximately

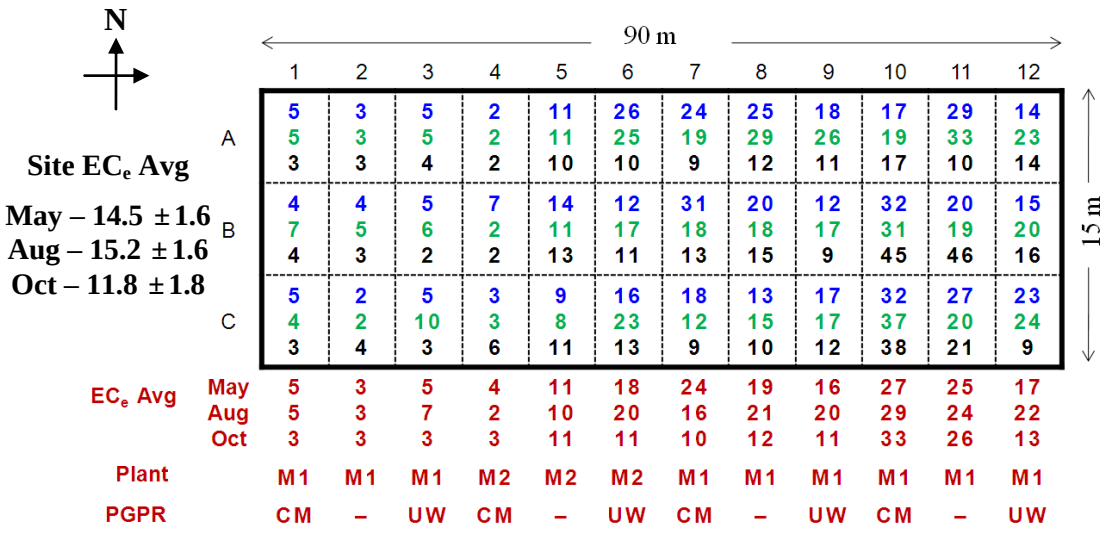


Figure 3.31. Soil salinity (EC_e) in dS/m of Cannington Manor South (CMS) salt-impacted site in 2008 growth season (May-October). The EC_e of May soil samples are shown in blue text, August soil samples are shown in green text, and October soil samples are shown in black text. The ECe Avg is the average ECe of each plot. The average EC_e of the site at the beginning, during and the end of 2008 growth season is 14.5, 15.2, and 11.8 dS/m, respectively. All stated errors are calculated as standard error (S.E.). M1: Oats + tall fescue + tall wheatgrass; M2: Oats + tall fescue; CM: CMH3; UW: UW3+UW4.

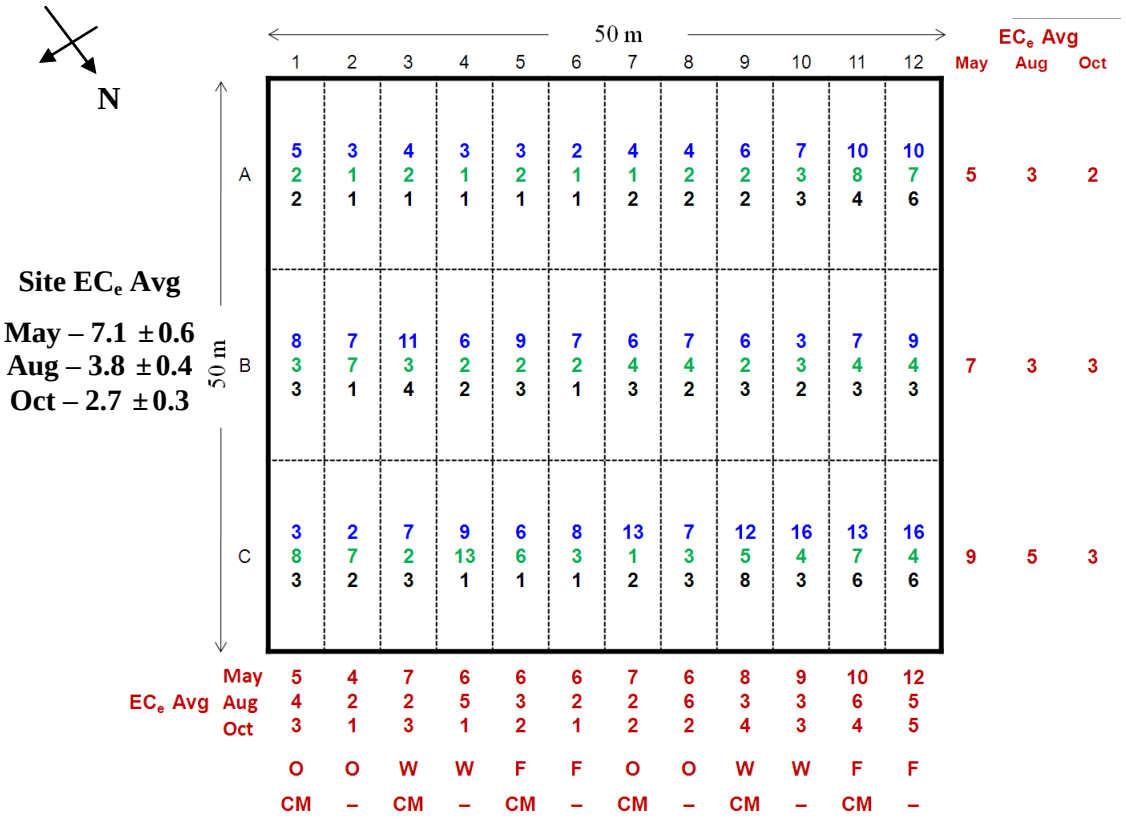


Figure 3.32. Soil salinity (EC_e) in dS/m of Cannington Manor North (CMN) salt-impacted site in 2008 growth season (May-October). The EC_e of May soil samples are shown in blue text, August soil samples are shown in green text, and October soil samples are shown in black text. The ECe Avg is the average ECe of each plot. The average EC_e of the site at the beginning, during and the end of 2008 growth season is 7.1, 3.8, and 2.7 dS/m, respectively. All stated errors are calculated as standard error (S.E.). O: Oats; W: Tall wheatgrass; F: Tall fescue; CM: CMH3.

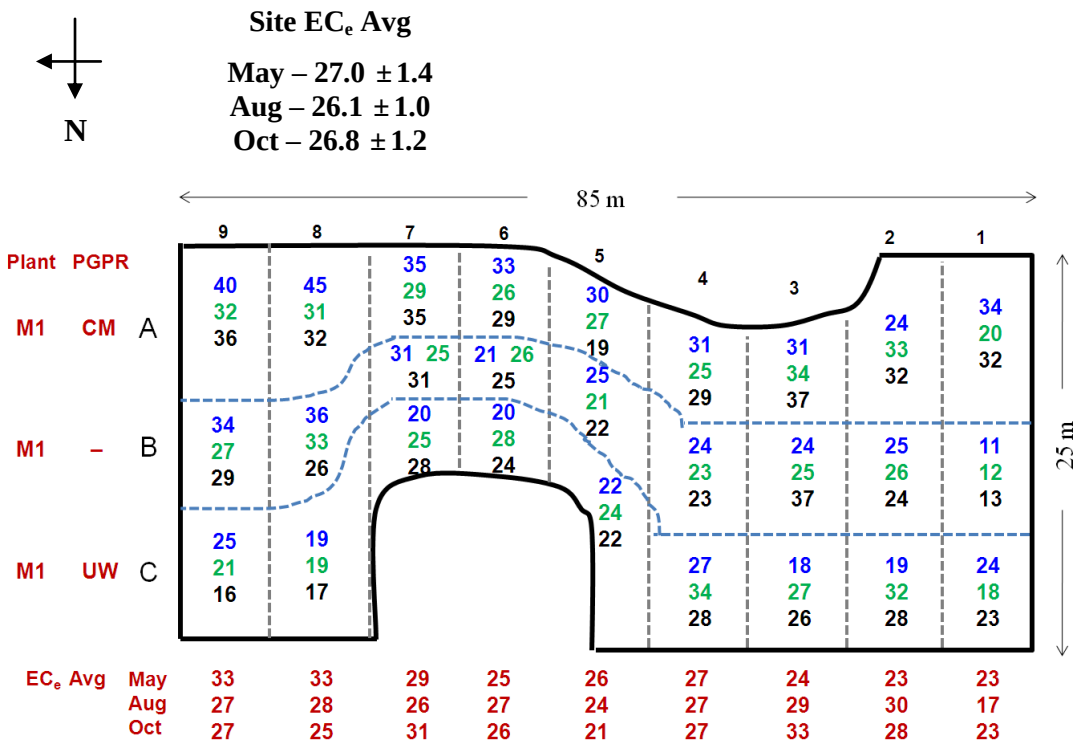


Figure 3.33. Soil salinity (EC_e) in dS/m of Alameda (AL) salt-impacted site in 2008 growth season (May-October). The EC_e of May soil samples are shown in blue text, August soil samples are shown in green text, and October soil samples are shown in black text. The ECe Avg is the average ECe of each plot. The average EC_e of the site at the beginning, during and the end of 2008 growth season is 27.0, 26.1, and 26.8 dS/m, respectively. All stated errors are calculated as standard error (S.E.). M1: Oats + tall fescue + tall wheatgrass; CM: CMH3; UW: UW3+UW4.

30% on CMS site and 60% on CMN site (Figure 3.31 and Figure 3.32). Within the growing season in 2008, the overall soil salinity level was decreased by 20% on CMS site and 60% on CMN site. However, soil salinity level did not decline in 2008 at AL (Figure 3.33).

3.6 Estimation of phytoremediation efficiency

For this field research, the top 50 cm of soil where plant roots can reach was used to estimate the phytoremediation efficiency at the CMS, CMN and AL salt-impacted sites. The estimated time required to remove 25% of salt by plants from the top 50 cm soil of each research site was calculated. This was based on the amount of plant biomass production and salt uptake from 2008 trial. To calculate salt uptake (on a plant dry weight basis), results from the CMN site was used.

Area of CMN site is 2500 m^2 (50 m $\times$ 50 m), therefore the volume of top 50 cm of soil is 1250 m^3 (2500 m^2 $\times$ 0.5 m). The density of the dry soil was 1.4 g/cm^3. Therefore, the dry weight of soil for top 50 cm soil of CMS site is 1.75 $\times 10^6$ kg (1250 m^3 $\times$ 1.4 g/cm^3 $\times 10^6$ cm^3/m^3 $\times 10^{-3}$ kg/g). The average EC_e of the CMN site was 7 dS/m at the beginning of 2008 (Figure 3.32), this is approximately equal to NaCl concentration of 4.5 g/kg (from raw data of 3000 g/kg Na^+ and 1500 g/kg Cl^-). The calculated total amount of salt of the top 50 cm of soil of CMS site is 7875 kg (1.75 $\times 10^6$ kg $\times$ 4.5 g/kg $\times 10^{-3}$ kg/g).

According to the plant biomass production from the field study 2008, up to 260 g of oat dry biomass per m^2 can be produced on CMN site with PGPR treatment (Table 3.7). The total plant dry biomass production for the entire site is then calculated to be 650 kg (0.26 kg/m^2 $\times$ 2500 m^2). With the growth rate of plants on CMN site, it would be feasible

to mow three times per growing season, therefore, the total plant biomass yield per season could be 1950 kg for the entire CMN site. Based on the salt accumulation in plant tissues, up to 30 g/kg of NaCl can be taken up by oats in three months (Table 3.8). Therefore, plants treated with PGPR can remove 59 kg of salt from CMN per season (30 g/kg $\times$ 1950 kg). The time to remove 25% of salt from CMN site is estimated to be approximately thirty years (7875 kg $\times$ 25% $\div$ 59 kg) based on data obtained from 2008 growth season.

Following similar calculations, the expected remediation time for CMS and AL site was calculated to be approximately sixty years and one-hundred years, respectively, based on the plant biomass production and soil salinity level in 2008 growth season.

Estimated remediation may vary due to specific conditions of the growing season. It was noted that the average temperature for 2008 growth season was colder than in 2007. Chang (2007) reported that up to 1400g/m^2 of barley dry biomass can be produced with PGPR treatment, and the amount of salt accumulation in barley can be up to 50g/kg from 2007 field trials at the CMN site. If the amount of plant biomass production and salt uptake were averaged from 2007 and 2008 field trials (field data of 2007 were adapted from Chang (2007)), the expected remediation time to remove 25% of NaCl for the top 50cm soil at the CMN site was calculated to be six years. Similarly, the expected phytoremediation efficiency for CMS and AL site was twelve and sixteen years, respectively, based on average data from 2007 and 2008 field trials. Whereas without PGPR treatment, the time required for 25% salt remediation for the top 50 cm soil at the CMS, CMN, and AL site was estimated to be eight, sixteen, and twenty-five years, respectively.

Chapter 4 - Discussion

Effects of salinity and PGPR on plant biomass production, membrane permeability and photosynthetic activity were examined in cereals (barley and oats) and grasses (tall wheatgrass and tall fescue). In greenhouse studies, treatments with PGPR (UW3+UW4, CMH3) promoted plant growth of both cereals and grasses under salt stress. As well, PGPR significantly relieve the damages on plant cell membrane and stresses on photosynthetic activity due to salt. In field studies, PGPR treatment increased plant biomass production at the CMS, CMN, and AL sites and increased NaCl accumulations in plant tissues. The average soil salinity level at the CMS and CMN during 2008 season decreased by 20% and 62%, respectively. However, soil salinity level did not decrease at the AL site during 2008, possibly due to insufficient plant growth for remediation. Phytoremediation efficiency is expected to increased by 30% - 60% with PGPR treatment. The estimated time required for 25% salt removal for the top 50 cm soil at the CMS, CMN, and AL site was expected to be six, twelve, and sixteen years, respectively, assuming that plant biomass production and salt uptake in plants during the remediation process are similar those in the 2008 field season.

4.1 Assessment of tolerable salt range of plants and PGPR effect on plant growth in growth pouch assays

Selection of plant species that can withstand elevated levels of salinity and capable of producing adequate biomass under salt stress is important for phytoremediation. To maximize experimental efficiency, growth pouch assays provided a quick, convenient, and space-conserving method for preliminary selection of PGPR and plant species.

In this study, experimental results showed that barley was more responsive to PGPR treatment but less tolerant to salt than canola based on germination (Figure 3.4). This may be due to the differences in plant anatomy, since barley is a monocotyledonous plant, whereas canola is a dicotyledonous plant. Results from this study indicated that canola was more tolerant to salt than barley in terms of percent germination. This is because NaCl may act as an osmoticum in dicotyledonous plants and the Na^+ ions are mainly stored in the vacuole. In monocotyledonous plants, osmotic balances are partially achieved by sugar synthesis, and they may have less Na^+ storage capacity in vacuoles than dicotyledonous plants (Flowers and Yeo 1988; Glenn et al. 1999; Reimann and Breckle 1993). This is in contrast to findings reported by the government that barley is considered a more salt tolerant plant than canola (AE 2001). This is possibly due to variation in salinity tolerance in relation to the stage of plant growth (Al-Karaki 2001). Barley was found to be vulnerable to salt stress in the growth pouch assay, which may be attributed to the fact that this assay is an early growth test. Barley is more sensitive to salt during germination and young seedling stages than more mature plants (Abu-Sharar 1988; Ayers et al. 1952). The discordance observed in salt tolerance of barley in this growth pouch assay compared to literature findings may also depend on the cultivar, since salt tolerance can vary among cultivars within the same plant species (Al-Karaki 2001; Niazi et al. 1991).

In addition to percent germination, shoot and root length of both plant species were found to be shorter at 0% NaCl than at low salt concentrations (0.1-0.2 % NaCl) (Figure 3.5 and Figure 3.6). This suggests adequate amounts of salt are necessary to maintain turgor pressure in plant cells (Jones and Gorham 2002), and other plant functions (i.e.

chloride ion is required for photosynthesis and responsible to chemically balance potassium ions during the opening and closing of stomata) (Izawa et al. 1969; Olesen and Andreasson 2003; Popelkova and Yocum 2007).

In high salt concentrations (above 0.8% NaCl), shoot and root growth of both plant species were significantly inhibited by salt (Figure 3.5 and Figure 3.6). In canola, treatment with UW3+UW4 significantly promoted root elongation at 0.6% NaCl (Figure 3.7). However, the effect of PGPR on root elongation was less pronounced at 1.0% NaCl. These results demonstrated that UW3+UW4 can significantly promote plant growth in canola exposed to a moderate concentration (0.6%) of NaCl compared to a high concentration (1.0%) of NaCl. This observation is in agreement with findings reported by Cheng et al. (2007) that inoculation of PGPR significantly increased shoot biomass of canola. In contrast to canola, the effect of PGPR on barley growth was not significant (Figure 3.6), possibly due to the roots having reached the bottom of the growth pouches by the time the root lengths were measured.

The growth pouch assay provided a quick method for early plant growth assessment. However, it is not likely to be representative of plant and bacteria performance in greenhouse experiments or field studies. As soil is a complex media, the pH of soil, presence of organic and inorganic matter, as well as existing bacterial communities could influence the growth of plants and the effectiveness of PGPR. Furthermore, plants may show growth in highly saline soil (EC> 25 dS/m ≈ 1.4% NaCl) (Chang 2007) compared to pouch assays containing lower NaCl concentrations (EC< 20 dS/m ≈ 1.0% NaCl). This difference is likely due to salt bioavailability whereby particles of soil *in situ* may temporarily trap salt ions thus limiting exposure to plants. Conversely,

in the growth pouch assays, salt ions are dissolved in solution and are readily available for uptake. Moreover, the presence of nutrients in the soil can facilitate plant growth. Conversely, nutrients was limited in growth pouch assays since Na^+ and Cl^- were the only ions available to plants. Therefore, at the same salinity, plants may show a greater toxic effect in growth pouch assay than in greenhouse or field assays.

4.2 Effects of salinity and PGPR on plant growth assessed by biomass production in greenhouse trials

Salinity can severely inhibit plant growth of many terrestrial plant species (Munns and Tester 2008; Tester and Davenport 2003; White and Broadley 2001). Results from greenhouse studies showed significant inhibition of growth of cereals (barley and oats) and grasses (tall wheatgrass and tall fescue). The inhibition of barley and oat growth upon exposure to saline soils observed for plants used in this experiment corresponded with some findings previously documented (Al-Karaki 2001; Cramer et al. 1990; Veselov et al. 2008; Zhao et al. 2007). For plants grown in highly saline soil, a decrease in shoot thickness and greenness was observed. Changes in shoot thickness due to salt stress can be attributed to reduced plant cell intercellular space (Delphine et al. 1998) as well as to an increase in epidermal and mesophyll thickness (Longstreth and Nobel 1979). The decrease in shoot greenness of untreated (No PGPR) plants grown on saline soil may indicate less chlorophyll content relative to control, as salinity can inhibit chlorophyll synthesis or accelerate chlorophyll degradation (Reddy and Vora 1986). Observation in this research agreed with findings reported in other literature where chlorophyll content was reduced for oats exposed to 250 mM of NaCl (Zhao et al. 2007).

Salinity stress can shorten the life cycle of plants in terms of days to maturity (Abdel-Ghani 2009). In this research, grasses had a longer growing period, lasting up to 70 days in high salt impacted soil, whereas cereal plant species reached the end of their growth cycle after 20 days. This implies grasses may be more salt tolerant than cereals. The grasses used in this study, tall fescue and wheatgrass may have control mechanisms such as prevention of salt uptake into plants, compartmentalization of salt ions into vacuoles, synthesis of osmolytes, and exclusion of salt ions by roots to help them survive and complete their life-cycle under saline conditions (Breckle 1990; Breckle 1995).

A mechanism of ACC deaminase –containing PGPR in promoting plant growth was proposed by (Glick et al. 1998). ACC is a precursor to the stress hormone, ethylene. The enzyme ACC deaminase can degrade ACC to ammonia and α-ketobutyrate, hence lower the levels of ethylene in plants and facilitate plant growth promotion (Glick et al. 1998). In addition, most PGPR synthesize the auxin, IAA, which also can stimulate plant growth (Patten and Glick 2002). In this study, the effects of PGPR on plant growth promotion were evident on both cereal and grass plants. The growth promotion effect of PGPR on plants occurred not only during germination and during early growth, but also helped plants to maintain growth and survive in high salinity by partially relieving stresses.

Greenhouse experiments demonstrated that PGPR effects on plant roots versus plant shoots varied. The variation in PGPR effect may be due to differences in duration of growth, since plant biomass data was obtained at varying incubation times between experiments. PGPR effects on root growth were less apparent as plants were grown for longer periods in the greenhouse. The use of small growing pots might have limited root growth and obscured to some degree the differences. This constraint on growth due to

limited growing space would not be expected to occur in field trials, since seeds would be directly planted in soil, so plant roots would have enough space to develop.

PGPR effects on plant growth were found to be plant species dependent. For instance, applying UW3 and UW4 together improved growth in oats exceeding that which was observed when using UW3 and UW4 separately (Figure 3.8 and Figure 3.9). Conversely, treatment using only UW4 produced the greatest plant dry biomass in barley. This results suggests that different plant species may exhibit different responses to varying PGPR combinations, as previously reported by Chang (2007). This is possibly due to specie-specific differences in physiology and anatomy, as well as species-specific differences in conditions required for optimal growth. As a result, the influence of different PGPR will vary depending on the plant species.

4.3 Effects of salinity and PGPR on plant cell membrane stability assessed by electrolyte leakage in greenhouse trials

Measuring ion leakage from plant tissue is an established method for assessing membrane permeability in relation to salt stress. In this study, increasing soil salinity caused resulted in damage to barley and oats, and affected plant membrane permeability, as indicated by higher ion leakage. A significant increase in electrolyte leakage was observed from plant tissues grown in soil with an EC_e above 13 dS/m. Salt toxicity can result in production of lesions on membranes, resulting in leakage of solute from plant cells (Leopold and Willing 1984). Previous experiments with water chestnut (*Trapa* sp.) also demonstrated increased membrane permeability with increasing salinity (Hoque and Arima 2000).

PGPR treatment with UW3+UW4 significantly decreased the amount of ion leakage from plant tissues, implicating PGPR in the protection of plant cell membranes, possibly by promoting synthesis of lipids, which are structural constituents of most of the cellular membranes (Singh et al. 2002). However, there was less apparent improvement attributed to PGPR under non-saline (control soil) and highly saline (undiluted salt-impacted soil) conditions (Figure 3.18 and Figure 3.19). This suggests that the effectiveness of PGPR may decrease under low stress conditions or under extreme stress conditions.

4.4 Effects of salinity and PGPR on photosynthetic activity assessed by chlorophyll fluorescence using pulse amplitude modulated (PAM) fluorometry in greenhouse trials

Salt stress is known to decrease net photosynthesis in plants (Jiang et al. 2006; Long and Baker 1986). Photosynthetic activity can be measured as chlorophyll fluorescence (Zhao et al. 2007). Pulse amplitude modulated (PAM) fluorometry is a technique that can measure chlorophyll fluorescence and used to analyze both instantaneous and steady-state photosynthesis in plants (Juneau and Popovic 1999). In this study, PAM fluorometry was used to assess the photosynthetic activity of four plants (barley, oats, tall wheatgrass, and tall fescue) under salinity stress, and evaluate the effects of PGPR on photosynthesis.

The chlorophyll fluorescence parameter, F_v/F_m, provides a measure of PSII photochemical efficiency (Maxwell and Johnson 2000). A significant decrease in F_v/F_m was observed in all four plant species under salt stress (Table 3.3, Table 3.4, Table 3.5, and Table 3.6), suggesting that photosynthesis was impaired (Naidoo et al. 2008). Steady-state

fluorescence, F_s, increased for untreated (No PGPR) plants grown in salt-impacted soils compared to plants grown in control (ProMix^{TM}) soil (Table 3.3, Table 3.4, Table 3.5, and Table 3.6), indicating possible damage to photosynthesis from salt stress and prevention of re-oxidation of the plastoquinol pool (Babu et al. 2001). The effective quantum yield at steady state, was significantly decreased for untreated plants grown in salt-impacted soils compared to the plants grown in control soil (Table 3.3, Table 3.4, Table 3.5, and Table 3.6), which suggested that there was a disturbance in photosynthetic activity and reduction in net carbon dioxide assimilation due to salt stress (DeEll and Toivonen 2003; Genty et al. 1989). This indicated that without PGPR treatment, the overall quantum yield of photochemical energy storage in plants was decreased due to salt stress. The photochemical quenching (qP) decreased and non-photochemical quenching (qN) increased for untreated (No PGPR) plants grown in salt-impacted soil compared to plants grown in control soil (Table 3.3, Table 3.4, Table 3.5, and Table 3.6).

The decrease in chlorophyll fluorescence parameters due to salt stress observed in this experiment is in agreement with Zhao et al (2007), who reported a significant decrease in F_v/F_m for oats at salt concentrations above 150 mM (EC ≈ 16 dS/m) as well as a significant decrease of qP at 200 mM (EC ≈ 22 dS/m) and 250 mM (EC ≈ 27 dS/m). Similarly, reduction of F_v/F_m was also observed for sorghum when plants were subjected to 250 mM of salt (Netondo et al. 2004b). The decreased photosynthesis associated with exposure of plants to salt may be due to stomatal closure and consequently limited carbon dioxide uptake (Brugnoli and Björkman 1992; Netondo et al. 2004b; Saqib et al. 2005; Woodwarrd 1998; Zhao et al. 2007; Zhu 2001).

PGPR (UW3+UW4 and CMH3)- treated plants were able to tolerant photosynthetic stresses due to exposure to high soil salinity, as indicated by improvements on chlorophyll fluorescence parameters (F_v/F_m, F_s, yield, qP, and qN). Treatment with PGPR resulted in higher values of F_v/F_m, yield, and qP, as well as lower values of qN and F_s compared to untreated plants (Table 3.3, Table 3.4, Table 3.5, and Table 3.6). Furthermore, plants treated with PGPR have similar chlorophyll fluorescence parameters to the plants that were grown in control soils, implying that treatment with PGPR may improve the overall rate of electron transport and the light harvesting efficiency in plants under salt stress. The improvements to photosynthesis rates in PGPR-treated plants suggest that PGPR may partially relieve salt stresses in plants by affecting structure of the photosynthetic apparatus, influencing photochemical reactions, and affecting the transport of photosynthetic intermediates between subcellular compartments (Parida and Das 2005). Moreover, the improvement to the photosynthetic rate observed for plants treated with PGPR likely enhanced growth of plants under saline conditions, as observed in greenhouse experiments. Similar relationships between growth and photosynthetic capacity were reported in *Brassica* (Nazir et al. 2001).

4.5 Effects of salinity and PGPR on plant growth in field trials

Percent vegetative coverage after a three-month growing period was estimated for CMS, CMN, and AL site. Vegetation was uneven at CMS and AL site due to soil flooding from excessive rainfall and crusting. Poor or no vegetation coverage on flooded areas at CMS and AL site was probably due to problems of waterlogging and higher salinity (EC_e > 20 dS/m). High electrical-conductivity (EC) and sodium adsorption ratio (SAR) values

can result in deteriorated soil conditions and cause dispersion of clay particles into pore spaces which affects water drainage (Richard 1954). Also, waterlogging can alter soil porosity and decrease the oxygen diffusion rate and availability, which can severely inhibit plant growth and nutrient balances of the soil as plant roots require oxygen to maintain adequate respiration (Drew 1991; Huang 2000; Kozlowski 1984).

In general, impact of salinity on plants observed in field studies resembled the findings observed for the greenhouse trials in that plant biomass production decreased with increasing soil salinity (Table 3.7). Plant dry biomass production per m^2 in 2008 field studies was 5-fold less than the plant biomass production reported in 2007 field studies (Chang 2007). The decrease of biomass production in field 2008 was attributed to a combination of multiple factors (e.g., colder weather, excessive rainfall, and animal grazing). These factors can negatively affect plant growth.

In this field study, PGPR-treated plants produced greater plant biomass compared to untreated plants at all three research sites (Table 3.7). In addition, the PGPR effect on plant growth was more evident when plants were grown for longer period in the field soils. Greater plant biomass production would be beneficial for salt removal from soil due to the greater amount of salt uptake by plants, hence ultimately increased phytoremediation efficiency.

4.6 Effects of salinity and PGPR on salt ion accumulations in plants in field trails

Phytoremediation of salt-impacted soil relies on plants to take up salt from the soil, which can accumulate in plant tissue and be subsequently removed from soil by harvesting the foliage. Therefore, the amount of salt accumulation in above-ground biomass is a

factor contributing to the efficiency of phytoremediation in addition to biomass production. Salt ions, Na^+ and Cl^-, are readily taken up from the soil by plants and transported into plant shoots via the xylem, and can only be return to the roots via the phloem (Tester and Davenport 2003; White and Broadley 2001). Only a small amount of salt ion can be transported back to the roots, suggesting that the transport of Na^+ and Cl^- is somewhat unidirectional and mainly accumulates in above-ground plant tissues (Tester and Davenport 2003; White and Broadley 2001). In this research, a particular interest has been put into examining the accumulation of salt ions in above-ground plant tissues. Therefore, only plant shoot tissue was analyzed for ion accumulation.

Considering the atomic mass differences between Na^+ and Cl^-, the ratio of Na/Cl should be 1.5 if there is an even accumulation of Na^+ and Cl^-. The ratio of Cl/Na in plant tissues ranged from 2-5 from field studies, suggesting that the accumulation between Na and Cl in plant shoots was uneven with greater accumulation of Cl than Na. Plants uptake more Cl^- than Na^+ because of Cl^- is required for photosynthesis (Olesen and Andreasson 2003) and for adjusting osmotic potential in vacuoles and the cytosol (Flowers 1988).

In this study, the NaCl accumulation did not differ much when plants were grown in soil with varying salinity levels (Table 3.8 and Table 3.9), implying that salt accumulation in plants may not correlate with soil salinity levels. This observation is in agreement with the other findings in sorghum, tissue Na^+ concentration saturated after 25 days of 150 mM (EC ≈ 16 dS/m) salt (Netondo et al. 2004a). In contrast, Zhao et al. (2007) reported that Na^+ accumulation in oats increased significantly with increasing salinity levels ranging from 0-250 mM (EC ≈ 28 dS/m) of NaCl solutions. The discrepancy may be due to differences in growth conditions (soil versus solution) that altered the availability

of salt ions for uptake into plants, since plants take up Na^+ and Cl^- more readily when grown in salt solution relative to when grown in saline soil.

It was expected that plants that have grown for five months would result in greater NaCl accumulation than plants that were grown for three months. However, this trend was not observed in experimental results because Maxxam Analytic Inc. employed a partial digestion method that may not fully extract the salt ions out from plant tissues (Table 3.8 and Table 3.9). Differences in digestion procedures used by the two accredited analytical labs generated results that are not directly comparable between time points, but can be used to indicate general trend.

PGPR-treated plants showed accumulated more NaCl (Table 3.8). In general, accumulation of NaCl in plant tissues was greater for the CMH3 treatment than the UW3+UW4 treatment. This was in contrast to the findings reported previously where no significant differences were found for NaCl accumulation in plant tissues among the different PGPR treatments (Chang 2007).

NaCl uptake can compete with the uptake of other nutrient ions, such as K^+ and Ca^{2+} by altering ion selectivity (Parida and Das 2005; Zhao et al. 2007). As such, increases in salinity can decrease uptake of K^+ and Ca^{2+} in plant cells (El-hendawy et al. 2005; Netondo et al. 2004a). A sufficient amount of K^+ is important in plant cells as it is responsible for osmotic adjustment and activation of enzymes (Carden et al. 2003; Schachtman and Liu 1999). However, Na^+ cannot replace K^+ in any of the essential cytoplasmic functions (Leigh and Storey 1991). The ratio of Na^+ and K^+ concentrations has been shown to be related to salinity tolerance in plants, hence the K/Na ratio has been used as an indicator for salinity tolerance (Gorhman et al. 1987; Houshmand et al. 2005;

Saleque et al. 2005; Tester and Davenport 2003). The treatments of UW3+UW4 and CMH3 resulted in an increase of K/Na ratios in plant tissues. This suggests that PGPR treatment may help to maintain ion balance in plants and increase its tolerance to salinity stress (Zhao et al. 2007). Also, calcium has been reported to reduce the toxic effect of salinity (Cramer et al. 1989). However, PGPR treatment did not result an increase of Ca^{+} accumulation in plants, possibly due to the fact that the plants were grown in higher salinity soil than the untreated plants, since salinity lowers Ca^{+} uptake ability (El-hendawy et al. 2005; Netondo et al. 2004a).

4.7 Changes in soil salinity in field trails and estimation of phytoremediation efficiency

Soil salinity levels at CMS, CMN, and AL sites were monitored through a two-year study (2007-2008). Soil salinity levels of all sites measured in 2008 before planting were close to the salinity levels measured at the end of growth season in 2007 (Chang 2007), indicating that soil salinity levels remained relatively constant over the Winter (2007-2008). At the end of the growing season in 2008, the overall soil salinity levels at the CMS and CMN sites were lower than the initial levels in 2007. The decrease of soil salinity levels are possibly due to the direct uptake and accumulation of NaCl by plants. Moreover, the decrease of soil salinity may partially be due to leaching of salt from the root zone through excessive rainfall (Qadir et al. 2007). It was noted that the overall soil salinity level did not decline in 2008 at the AL site, which may be due to the high levels of salt found at over one-third of AL site area that severely impacted plant growth. The decrease of plant biomass production on the AL site affected the phytoremediation

efficiency because of direct toxicity and plant biomass was simply insufficient for remediation.

Salt is usually carried upward by the capillary movement of water and accumulates at the soil surface as water evaporates (AE 2001). The thickness of soil where salt accumulates is site specific, since it depends on the soil texture and pore size of the soil (Smedema and Rycroft 1983; Talsma 1963). For the interest of this field research, only the top 50 cm of soil (the rooting zone for the plants) was used to estimate the phytoremediation efficiency.

The phytoremediation efficiency is expected to increase by 30-60% with PGPR treatment, based on the extent of improvements in plant biomass production and NaCl uptake in plants. The estimated time require to remove 25% of salt by plants of CMS, CMN, and AL site was calculated to be thirty, sixty and one-hundred years, respectively, based on the plant biomass production and salt accumulation in plant tissues obtained in 2008. However, this estimation of remediation efficiency may not be accurate. The remediation efficiency can vary due to specific conditions of the growing season (e.g., amount of rainfall, temperature fluctuation, and episodic events). These factors can affect plant growth and salt accumulation in plant tissues. For instance, the plant biomass production of cereal plants in 2008 at the CMN site was five-fold less than the plant biomass production in 2007 (Chang 2007) due to colder weather.

When averaging the plant biomass production and salt accumulation in plant tissue from 2007 and 2008 field trials, the expected phytoremediation efficiency of CMN, CMS, and AL sites was calculated to be six, twelve, and sixteen years, respectively. Nevertheless, the phytoremediation efficiency is expected to increase as the soil properties

and conditions improve during the remediation process. Plants will produce greater plant biomass in soil as salinity decreases and the rate of remediation will increase.

4.8 Conclusions

The effects of soil salinity on plant growth are complex, causing damage and inhibition to various plant physiological processes such as plant growth, plant cell membrane structure and photosynthetic activity. Inoculation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase containing plant growth-promoting rhizobacteria (PGPR) relieved plant stresses and damages attributed to exposure of plants to soil with high salinity. In greenhouse studies, treatment of seeds with indigenous (CMH3) or a combination of non-indigenous (UW3+UW4) PGPR consistently promoted shoot and root growth of cereals (barley and oats) as well as grasses (tall wheatgrass and tall fescue) grown on soils from salt-impacted sites. Plant growth promotion was likely due to plant cell membrane stabilization conferred by PGPR. Specifically, PGPR treated plants showed improvements in cell membrane stability as demonstrated by less electrolyte leakage from plant cells relative to the plants that were not treated with PGPR. Furthermore, results from pulse amplitude modulated (PAM) fluorometry studies indicated that PGPR-treated plants had increased rate of photosynthesis and the PGPR prevented salinity damage to photosystems relative to the untreated plants.

In field trials, germination and growth of plants was poor and uneven on CMS as well as AL sites due to high soil salinity (EC_e > 17 dS/m) and waterlogging problems. In contrast, plant growth flourished at CMN site, where soil salinity was much lower (EC_e = 7 dS/m). During the two-year long remediation process, the average soil salinity level at

CMS and CMN decreased by 30% and 60%, respectively. However, soil salinity did not show a decrease on AL site possibly due to severe flooding and the high initial soil salinity. On all three research sites, PGPR treatment showed increased biomass production and salt ion accumulation in plant tissues, which may enhance phytoremediation efficiency. Based on the plant biomass and salt uptake data from 2008 field study, the estimated time to remove 25% of the salt from the top 50 cm soil at CMN, CMS, and AL sites with PGPR treatment were expected to be thirteen, twenty, and thirty years, respectively. The remediation efficiency is expected to increase as the soil properties and salinity levels improve during the remediation process as a result of greater plant biomass production and salt removal. In conclusion, phytoremediation with PGPR is a feasible and cost-effective remediation technique.

4.9 Recommendation and future perspectives

In this research, ProMix^{TM} and Quali-grow artificial soil were used as control soils. However, a reference control soil that is free of contaminants of concern (e.g. NaCl) and matches as close as possible the physical-chemical characteristics to the salt-impacted soil should be used in greenhouse studies. This is because the soil structures, pH level, presence of organic and inorganic matter, as well as existing bacteria communities can influence the growth of plants and the effect of PGPR. Using reference control soils that have similar physical-chemical properties to the salt-impacted soil allows to account for these factors that can contribute to plant growth other than PGPR.

Soil waterlogging was one of the major problems that inhibited plant germination and growth of plants at the CMS and AL sites due to uneven elevation throughout the site

allowing water to accumulate in low-lying areas. Since salt ions are water soluble, and these ions would have concentrated in these flooded areas, making the surface soil highly saline. One solution to improve the flooding problem at CMS and AL sites is to use machinery (i.e. plow) to level the site, eliminating variations in soil elevation. To avoid uneven plant growth, the soil from highly saline area could be mixed with the soil from lower saline area, making the distribution of soil salinity more even on the CMS and AL sites. Evening out the soil elevation and soil salinity may help to increase phytoremediation efficiency because the soil would support more plant growth at the site.

From the setup of planting design in 2007 and 2008 field studies, the feasibility of PGPR application on phytoremediation technology can be evaluated. To better monitor the soil salinity level changes due to phytoremediation, a field control plot where no plants were planted must be included in the experimental design, thereby accounting for any factors that can contribute to the decrease in soil salinity other than phytoremediation (i.e. fluctuation in depth of the capillary fringe and salt movement in soil nearby the research sites).

The membrane leakage experiment demonstrated that treatment of PGPR treatment improved overall plant membrane structure by preventing ion leakage from plant cells due to salt stress. It would be beneficial to be able to evaluate the ultrastructural changes in plant cell morphology due to salinity stress, and whether treatment with PGPR will help plants cells maintain normal conformation. This goal can be achieved by employing the electron- or confocal- microscopy techniques to view if PGPR treatment changes the ultrastructure in plant cells, such as change in structural properties of the cell wall (Koyro

1997), mitochondria (Smith et al. 1982), and chloroplast (Keiper et al. 1998; Li and Ong 1997).

High salinity can cause osmotic stress in plants. Accumulation of osmoprotectants in the cytoplasm was an important mechanism to maintain the osmotic balance with the external medium and vacuole in plants (Rhodes et al. 2002). Proline is one of the most common osmoprotectants used by plants to regulate cellular osmotic balance. It has been observed that many halophytic plants accumulates proline in response to salinity stress (Rhodes et al. 2002). Future research could focus on determining whether PGPR treatment results in higher proline accumulation in plants when grown under saline conditions. Proline accumulation in plant cells can be determined photometrically whereby the reaction of proline with acid-ninhydrin solution to produce a characteristic red color that can be measured at 520nm (Bates et al. 1973; Chinard 1952; Troll and Lindsley 1954). Ultimately, this would allow a better understanding to how PGPR is able to promote better plant growth.

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Appendix

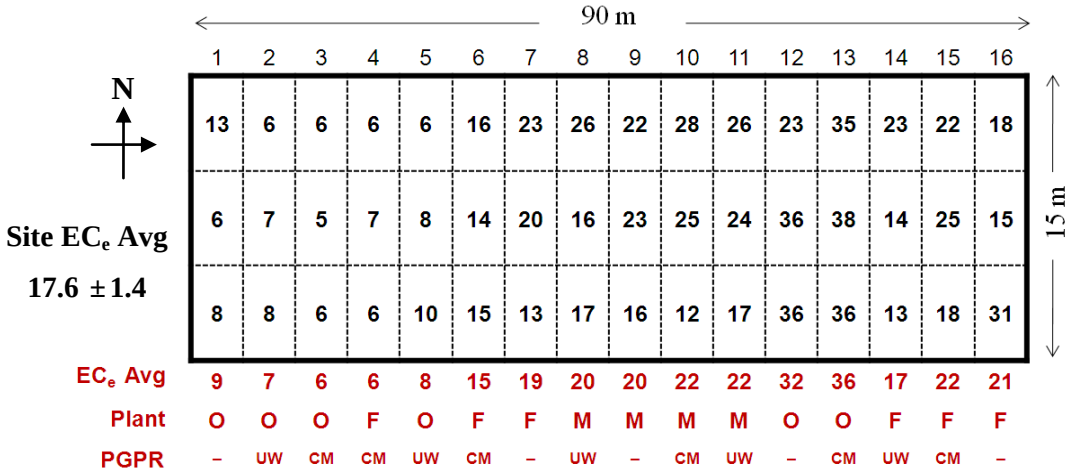

Figure 1. Soil salinity (EC_e) in dS/m of Cannington Manor South (CMS) salt-impacted site in 2007 (Adapted from Chang 2007). EC_e Avg is the average EC_e for each plot. The average EC_e for the entire site was 17.6 ±1.4 dS/m. Errors are calculated as standard error (S.E.). O: Oats; F: Tall fescue, M: Oats + tall fescue; CM: CMH3; UW: UW3+UW4.

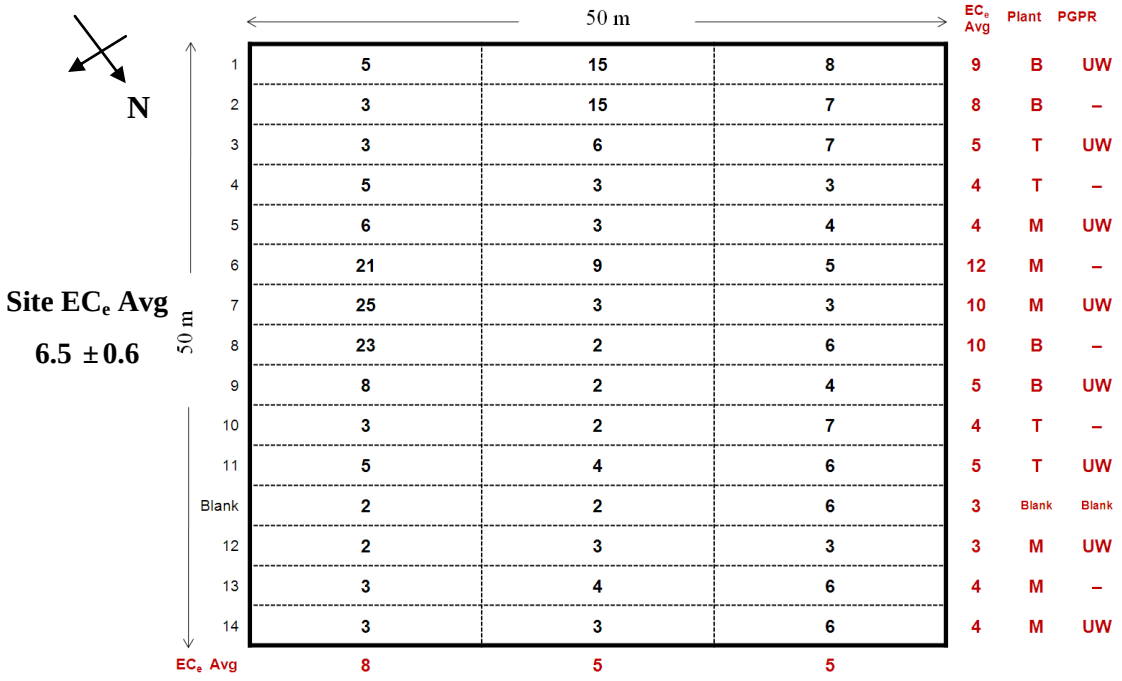

Figure 2. Soil salinity (EC_e) in dS/m of Cannington Manor North (CMN) salt-impacted site in 2007 (Adapted from Chang 2007). EC_e Avg is the average EC_e for each plot. The average EC_e for the entire site was 6.5 ±0.6 dS/m. Errors are calculated as standard error (S.E.). B: Barley; T: Rygrass (topgun), M: Barley + ryegrass; blank: Area where plants were not sown; UW: UW3+UW4.

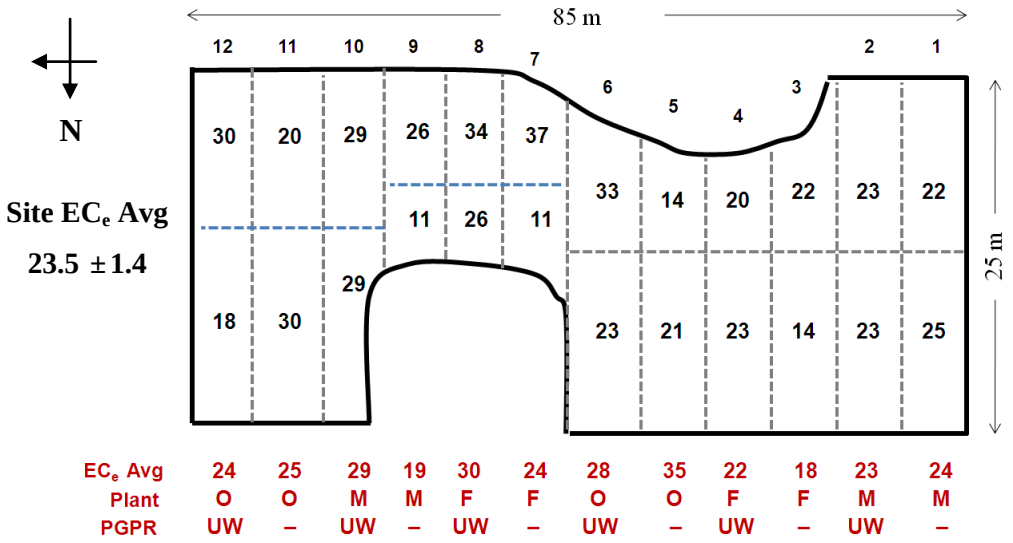

Figure 3. Soil salinity (EC_e) in dS/m of Alameda (AL) salt-impacted site in 2007 (Adapted from Chang 2007). EC_e Avg is the average EC_e for each plot. The average EC_e for the entire site was 23.5 dS/m.
Errors are calculated as standard error (S.E.). O: Oats; F: Tall fescue, M: Oats + tall fescue; UW: UW3+UW4.

Table 1. Properties of soils taken from the research sites

Soil	Alberta	CMS	CMS	AL
Sampling time	May 2006	May 2007	Aug 2007	Aug 2007
EC_e	3.2	16.5	37.8	30.0
SAR	24	10	7.0	14.0
CEC (meq/100g)	N/A	21	112	50
pH	N/A	8.1	7.7	7.7
Organic matter (%)	N/A	14.6	13.7	6.4
Sand (%)	N/A	35	32	41
Silt (%)	N/A	34	63	34
Clay (%)	N/A	31	5	25
Texture	N/A	Loam	Silk Loam	Loam
Na (mg/kg)	550	2710	3240	2500
Cl (mg/kg)	345	1400	19000	5200
Ca (mg/kg)	N/A	12900	94200	19600
K (mg/kg)	N/A	1290	980	1750
Mg (mg/kg)	N/A	19000	15800	6340

- Soil texture analysis was performed by Agri-Food Laboratories (Guelph, ON)
- Ion concentration analysis was performed by ALS Laboratory Inc. (Waterloo, ON)
- N/A indicates that the particular test was not performed for the soil sample

EC_e: Electrical conductivity of a saturated soil paste extract
SAR: Sodium adsorption ratio
CEC: Cation exchange capacity

Table 2. Major components of ProMix™ soil and Quali-grow artificial soil

Ingredient	ProMix™	Quali-grow artificial soil
Canadian *Sphagnum* peat moss	55-65%	Y
Perlite	Y	-
Dolomitic limestone	Y	-
Gypsum	Y	-
Wetting agent	Y	-
Manure	-	Y
Loam	-	Y

Y indicates the particular ingredient was one of the major components in the soil
- indicates the particular ingredient was not included in the soil