Comparative Physiology of Australian Commercial Rice Cultivars to Salinity Stress in Controlled Environment and the Field

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Barkat Rabbi

(Signature)

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Abbreviations

ABA.....Abscisic Acid
ABARES.....Australian Bureau of Agricultural and Resource Economics and Science
ABC.....ATP Binding Cassette
ACIAR.....Australian Centre for International Agricultural Research
ACC.....1-Aminocyclopropane-1-Carboxilic Acid
AFLP.....Amplified Fragment Length Polymorphism
ALMT.....Aluminium Activated Malate Channels
ATHK1.....Hybrid-Type Histidine Kinase
ATP.....Adenosine Triphosphate
CCC.....Cation-Chloride Cotransporter
CDPK.....Calcium-Dependent Protein Kinase
CLC.....Chloride Channel Conductance
COX.....Choline Oxidase
DAT.....Days After Transplant
DPI.....Department of Primary Industries
DREB.....Dehydration-Responsive Element Binding
DW.....Dry Weight
EC.....Electrical Conductivity
FAO.....Food and Agriculture Organization
FW.....Fresh Weight
GAS.....Ground-Water Associated Salinity
HKT.....High-Affinity Potassium Transporter
IAS.....Irrigation Associated salinity
IRGA.....Infrared Gas Analyser
IRRI.....International Rice Research Institute
KOR.....Out-ward Rectifying K^+ Permeable Channels
LCF.....Leaf Chamber Fluorometer
LIX.....Liquid Ion Exchanger
MIFE.....Microelectrode Ion Flux Estimation
MscS.....Mechanosensitive Channels of Small conductance
MSL.....MscS-like ion channels
MVs.....Modern Varieties
NAS.....Non-Ground Water associated salinity
NLWRA.....National Land and Water Resources Audit
NSCC.....Non-Selective Cation Channels
OECD.....Organisation for Economic Co-operation and Development
RAPD.....Random Amplified Polymorphic DNA
RGA.....Ricegrowers' Association of Australia
RIRDC.....Rural Industries Research and Development Corporation
RLFP.....Restriction Fragment Length Polymorphism
ROS.....Reactive Oxygen Species
SALTOL.....Salt Tolerance Gene
SLAC1.....Slow ion channel Associated Protein
SOS.....Salt Overly Sensitive

SSLP.....Simple Sequence Length Polymorphism
SSR.....Simple Sequence Repeats
TN.....Tiller Number
UNFPA.....United Nation’s Fund Population Activities
VDAC.....Voltage Dependent Anion Channels

Abstract

Salinity intrusion into agricultural lands in rice-producing countries around the world has become a serious threat to food security. Currently, more than 960 million hectares of productive land is adversely affected by salinity and is expected to grow in the future. This problem is exacerbated by the projected increase in world population from the current 7.2 to 9.6 billion by 2050. The cultivated rice (*Oryza sativa*) tolerates <4 dSm^{-1} NaCl, which makes this important crop the most salt sensitive cereal compared to wheat and barley. Despite many attempts, scientists have been unable to produce a rice variety that can tolerate >10 dSm^{-1}. This study used morpho-physiological methods including gas exchange measurement, microelectrode ion flux estimation (MIFE) and agronomic measurements to screen salinity tolerance levels of two Japonica (Koshihikari and Reiziq) rice genotypes and one Indica (Doongara) genotype. In addition, this is the first time that four basic ion fluxes were measured using MIFE in both glasshouse and field conditions to screen rice for salinity tolerance. The effects of salinity stress on photosynthetic activities, ion fluxes and growth parameters of the three rice genotypes under glasshouse conditions and in the field were also examined. Variations in the response of the three cultivars to salinity stress were found, thus providing evidence that morpho-physiological basis of salinity stress tolerance can be applied to improve the salinity stress tolerance of this important crop. This may allow the exploitation of salt affected marginal lands and could positively contribute to global food security.

1. Introduction

Salinity intrusion into prime agricultural lands in rice-producing countries around the world has become a serious threat to food security (Ahuja et al. 2010, Food and Agriculture Organization [FAO] 2010, Ruan et al. 2010, Tester and Langridge 2010, Mainuddin et al. 2011, Negrao et al. 2011, Bansal et al. 2014). Salinity occurs in all regions of the world however, it is more prevalent in arid and semi-arid regions in Africa, Asia and Australia, due to decreased lixiviation of salt from soil resulting in increased salt accumulation (Prakash and Chandra 1983, Schofield and Ruprecht 1989, Richter and Kreitler 1993, Ghassami et al. 1995, Mashali 1999, Funakawa et al. 2000, Marie and Vengosh 2001). Currently, more than 960 million hectares (Figure 1.1) of productive land is adversely affected by salinity (Szabolcs 1989, Martinez-Beltran and Manzur 2005, Rengasamy, 2006, Rengasamy, 2010, Ruan et al. 2010, Hoang et al. 2016) resulting in reduced yield (Eynard et al. 2005), and economical losses of up to US$ 27 billion per year (Qadir et al. 2014). Moreover, major rice-producing fertile deltaic regions in Asia are constantly shrinking as a result of seawater inundations caused by the rising sea levels, climate change and human activities (Eckardt 2009, Ahuja et al. 2010, FAO, 2010, Mainuddin et al. 2010).

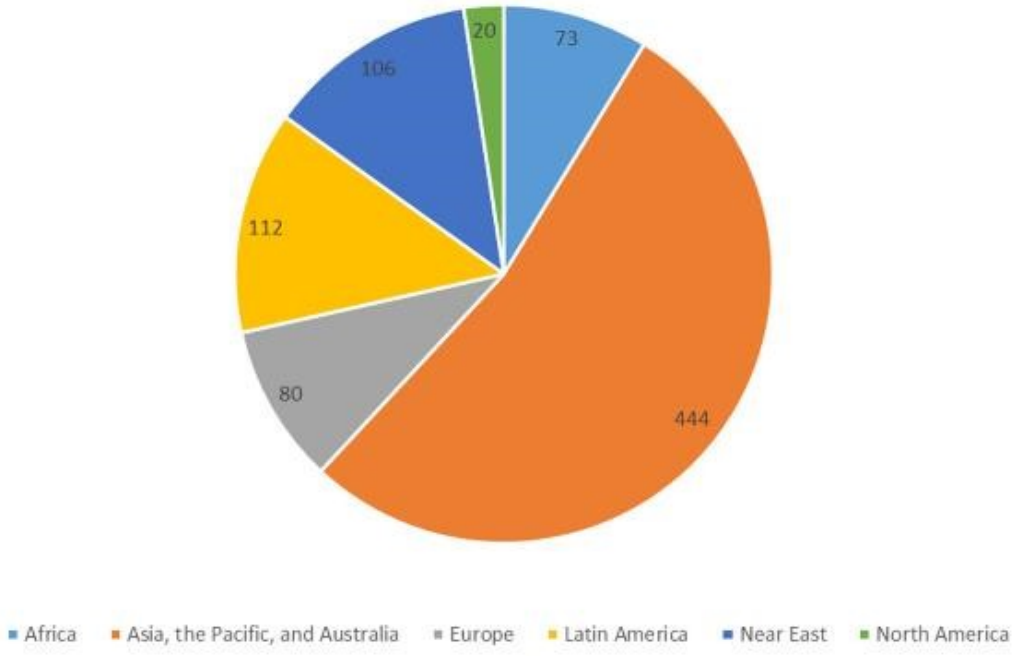

Figure 1.1: Global distribution of salinised soils (million ha) (Hoang et al. 2016)

On a global scale, the cost of salinity to agriculture is estimated to be approximately US$ 27 billion per year which is expected to increase (Qadir et al. 2014). This problem is exacerbated by the projected increase in world population from the current 7.2 billion to 9.6 billion by the year 2050 (FAO 2010, United Nations Fund Population Activities [UNFPA] 2014). Reports from India reveal a 45 % reduction in rice crop yield in the salt-affected Indo-Gangetic Basin (Tripathi 2009, Qadir et al. 2014). The cost of salinity to the Australian economy was reported to be AU$305 million per year in the Murray-Darling River Basin alone (Wilson 2003, Qadir et al 2014). In Bangladesh, more than 30 % of the net arable land lies in the coastal regions of Bay of Bengal, of which 53 % is already damaged by salinity (Petersen and Shireen 2001, Haque 2006). Therefore, to meet the demand of the growing population, rice production must also commensurate, by producing an additional 114 million tonnes (Figure 1.2) of rice by 2035 to cover the deficit (Purevdorj and Kubo 2000, Tester and Langridge 2010, Awika et al. 2011, Seck et al. 2012), International Rice Research Institute [IRRI] 2015).

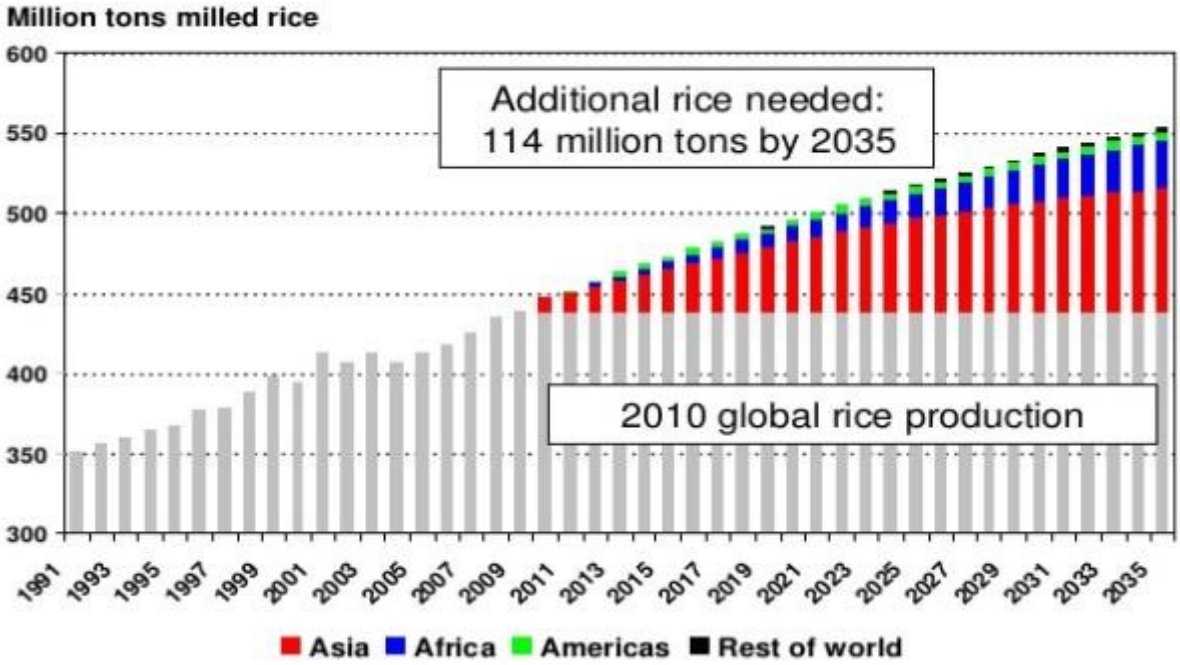

Figure 1.2: Global rice production increases needed to meet demand by 2035 (IRRI 2015)

Rice is the world's second largest crop after wheat, providing more than 20 % (Figure 1.3) of daily calorie requirements to more than half of the world's population (Khush 2005, Sweeny and McCouch 2007, IRRI 2015). Rice is also the staple food for approximately 50 % of the current world population and is cultivated in an area of 165 million hectares (Figure 1.4) in 100 countries around the world (Hossein and Fischer 1995, Khush 2005, FAO 2017).

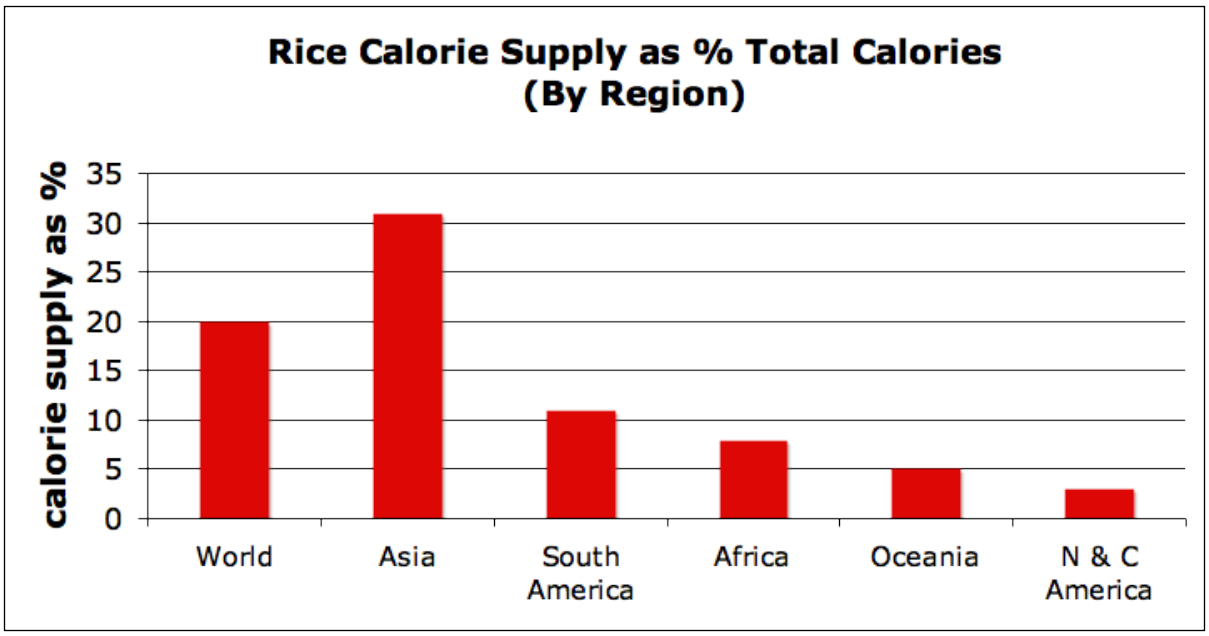

Figure 1.3: Rice calorie supply by region (IRRI 2015)

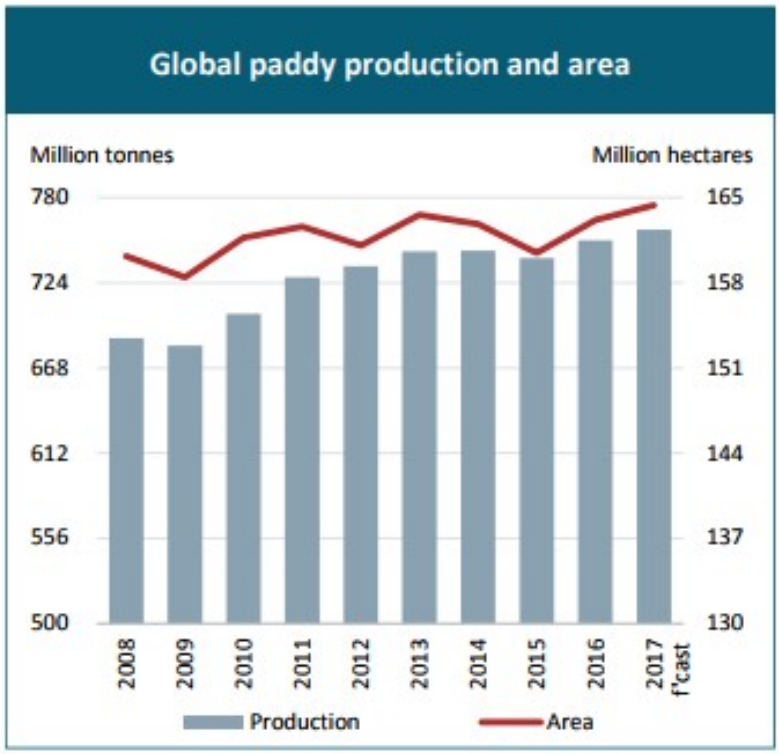

Figure 1.4: Global paddy rice production and area (FAO 2017).

In the period between 2013 and 2014 farmers around globe harvested more than 780 million (Figure 1.4) tonnes of rice (Khush 2005, FAO 2017). Although, Asia produces almost 90% of the world's combined output, the popularity of rice in many countries has been on the rise (Khush 2005). Since 1990, rice consumption per capita has increased by as much as 40 % in Africa and 46 % in South Americas respectively (Khush 2005). Furthermore, rice imports by region has almost doubled in Africa and the Far East Asia, while steadily increasing in Latin America and the Near East Asia (Figure 1.5) (FAO 2017).

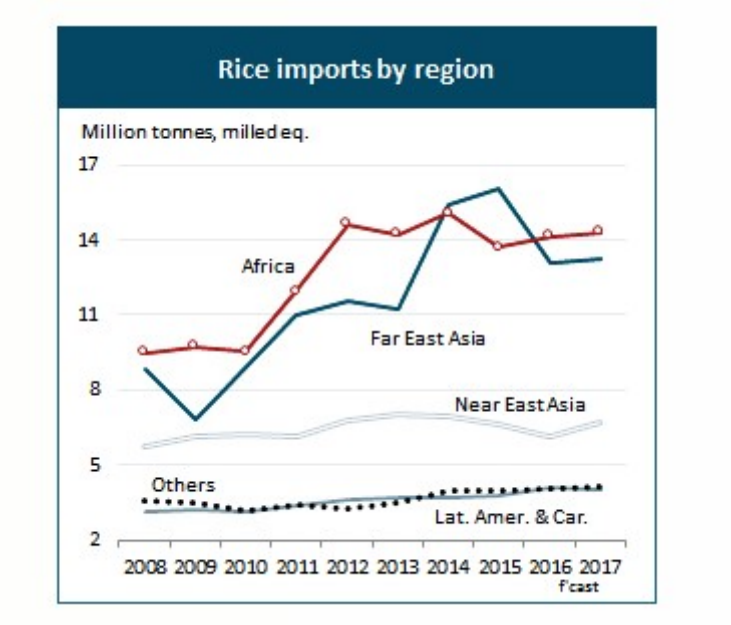

Figure 1.5: Rice import by region from 2008 – 2017(Source: FAO 2017).

1.1 Soil salinity and its origins

The underlying origins of soil salinization is a multifaceted and complex process that comes from many sources including natural and anthropogenic activities. Soil salinization may result from deforestation for agricultural purposes and dry-land irrigation, which may draw water tables close to the surface (Katerji et al. 2003, Rengasamy 2006, Munns and Tester 2008, Rengasamy 2010). Although natural processes such as rock weathering, rainfall, high tides, wind and storms are the dominant sources of salinity formation, dry land irrigation also contribute to soil salinization (Rengasamy 2006). Based on soil type and ground water processes, Rengasamy (2006) identified three major salinity types: (i) "Ground water associated salinity (GAS)", (ii) "Non-groundwater-associated salinity (NAS)", and (iii) "Irrigation associated salinity (IAS)".

GAS occurs when ground water table rises bringing dissolved salts to the surface. In general, salt accumulation is more prevalent in areas where ground water table is closer to the soil surface (>1.5 meters) (Talsma 1963). However, this depth may differ according to the prevailing climatic conditions and soil hydraulic properties (Rengasamy 2006).

NAS is more prevalent in drier climates where sodic soils are a common feature of the landscape. Although water tables in these areas are much deeper, their hydraulic properties are poor, hence allowing the accumulation of salts in surface soil and hampering agricultural activities (Rengasamy 2006).

IAS results from agricultural practices such as using poor quality or brackish water for irrigation purposes. This condition is commonly observed in areas with heavy clay and sodic soils with low hydraulic conductivity. Consequently, salt molecules in the irrigation water accumulates in the root zone because of inadequate leaching. This problem is exacerbated when the rising water table overlaps with soils in the root zone (Rengasamy 2006).

1.2 Soil salinity in Australia

The environmental, social and economic impacts of soil salinization on the Australian landscape have received a considerable attention from Government agencies, scientific communities and the public at large. In Australia, soil salinization has been dubbed as the "white death" referring to the barren white coloured salt affected areas in the greater wheat-belt and the Murray Darling Basin (Beresford 2004). In harmony with the landscape, the Australian native plants have evolved to develop deeper roots, thus preventing the rise of water table reaching both the topsoil and subsoil. Unlike deciduous shrubs and trees in temperate climates, Australian native plants tend to grow all year round, thus effectively controlling water table levels in all cyclical seasons (Beresford 2004).

However, with the advent of European settlements in Australia, extensive land clearing for agricultural and pastoral purposes saw the emergence of a novel problem unseen in magnitude (Kurlansky 2002). This equilibrium has changed, when the deep-rooted vegetation was replaced with shallow rooted exotic crops, thus causing water movement to the surface rendering large swathes of land unfit for farming and pastoral forage (Hatton et al. 2003). In a review, Rengasamy (2000) detailed the problem of dry-land salinity with focus on sodic soils in the Australian terrain. For millions of years, leaching of salts caused by natural processes have been sequestered in shallow waters or deep regolith. Although, the electrical conductivity (EC) of this saline groundwater ranged between 15─150 dS m^{-1}, most native plants were not affected because water levels were usually about 4 meters below the surface while more tolerant species thrived well in valley floors with shallower water levels (Rengasamy 2006). According to the National Land and Water Resources Audit [NLWRA] (2001), more than 5.7×10^4 km^2 agropastoral areas have the potential of becoming permanently salinised and projected to reach 17×10^4 km^2 by the year 2050, (which will be equivalent to

71% of the total land area of the United Kingdom). In addition, another 2.5×10^6 km^2 could be affected by transient salinity compounded by acidity, alkalinity, and other toxic elements including aluminium, boron and carbonates (NLWRA 2001). This is a major threat to food security here in Australia and overseas, given that Australia's total agricultural area is 7.6×10^6 km^2 approximately (NLWRA 2001). The cost of salinity to the Australian economy has been estimated to be more than AU$1.3 billion annually (Rengasamy 2002).

Rengasamy (2006) reported that groundwater samples taken from different geographical locations in Australia have similar composition as seawater. Nonetheless, results from Isotope tracing studies show that the isotopic composition of Australian saline groundwater is mostly of rainfall origin (Herczeg et al. 2001). Recent geophysical studies using airborne electromagnetics revealed occurrences of salt bulges scattered deep in the soil layers of the Australian terrain (Lawrie 2005). Moreover, the use of recycled effluent for irrigation (with salt and high pH properties) purposes have also contributed to the long-term soil salinization and sodification in the Australian landscape (Radcliff 2006).

1.3 Effects of salinity stress on plants

Salinity stress in plants can be broadly categorized into three areas namely, osmotic stress, oxidative stress and ionic toxicity (Munns 1995, Munns and Tester 2008, Shabala 2008, Horie et al. 2012). Osmotic stress in plants occurs when osmotic pressure in the soil is higher than that of plants, inhibiting the plant's ability to absorb water and other essential nutrients (Munns et al. 2006). In highly salinized environments, soil solution may reach a hyper-osmotic stage where plant roots lose more water than they absorb. This condition is described as chemical drought (Zhang et al. 2001, Apse and Blumwald 2002, Munns et al. 2005, Munns and Tester 2008) and causes cell expansion (Zhang et al. 2001).

Oxidative stress in plants occurs when excessive salinity induces, ion imbalance,

ion toxicity and reduced water potential leading to diminished CO_2 assimilation and ultimately oxidative stress (Zhang et al. 2001). Salinity increases the generation of reactive oxygen species (ROS) including Hydrogen peroxide (H_2O_2), superoxide radicle (O_2^-), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$) (Zhang et al. 2001). The increased ROS triggers phytotoxic reactions such as protein degradation, lipid peroxidation, deactivation of enzymes and denaturing DNA molecules (Zhang et al. 2001, Jiang and Zhang 2001, Bor et al. 2003).

On the other hand, ionic toxicity occurs when the sodium ions (Na^+) in the cytosol reach toxic levels, displacing potassium (K^+) (Maathuis and Amtmann 1999, Maser and Gierth 2002, Cuin et al. 2003) and calcium (Ca^+) (Zhu 2003, Shabala, 2005, Chen et al. 2007a). Moreover, ionic toxicity in plants leads to leaf senescence, photosynthesis restrictions, chlorosis, necrosis and death (Yeo and Flowers 1983, Zhu 2003, Munns 2005, Chen et al. 2007a, Shabala 2009).

1.4 Mechanisms of salinity tolerance in plants

Salinity has varying effects on different plants, depending on their biological, physiological, and molecular adaptations to saline soils. Thus, the mechanisms of salinity tolerance in plants can be classified into three inter-related main categories viz. osmotic tolerance, ion exclusion and tissue tolerance (Figure 1.6) (Flowers et al. 1977, Tuteja 2007, Munns and Tester 2008, Horie et al. 2012, Deinlein et al. 2014, Roy et al. 2014a, Munns et al. 2016). Osmotic tolerance is the first adaptive response of plants when salt concentration around the root reaches a threshold of 40 mM NaCl for most plant species (Munns et al. 2008). Although, the mechanism of osmotic tolerance remains obscure, it is believed to be regulated by rapid, long-distance signalling such as ROS, Ca^{2+} signalling, and long-distance electrical signalling (Maischak et al. 2010, Mittler et al. 2011, Hasegawa 2013, Munns et al. 2016).

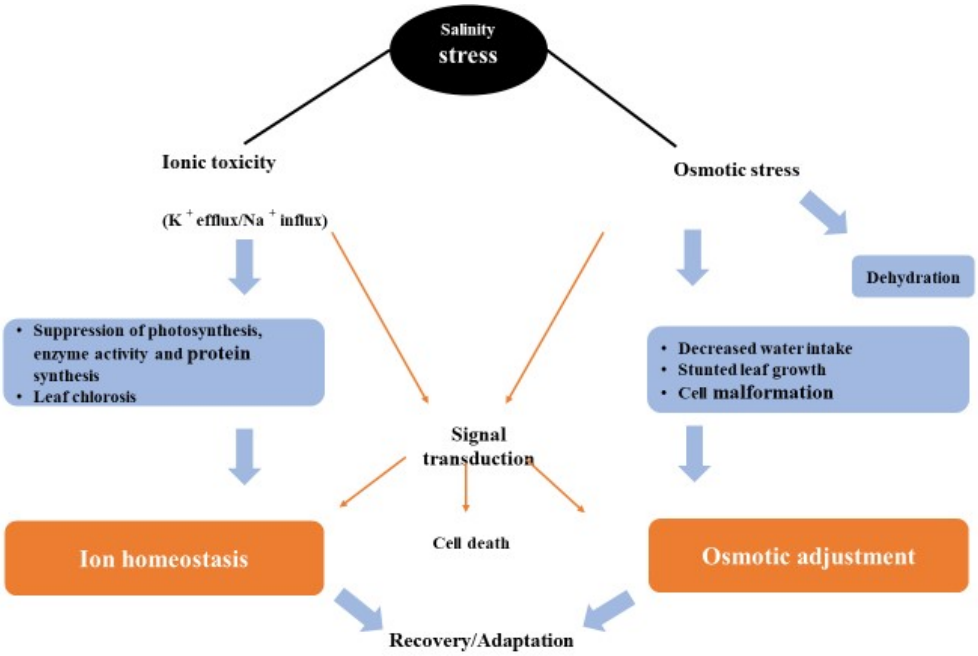

Figure 1.6: Schematic representation of effects of ionic, osmotic stress and adaptive mechanisms employed by plants. Orange coloured arrows and boxes show plant adaptive mechanisms. Bold blue arrows and boxes represent deleterious effects of salinity stress on plants (modified from Horie et al. 2012).

These root signalling processes then rapidly alter shoot function before Na^+ accumulation reaches the shoot (Munns et al. 2016). Studies from (Knight et al. 1997, Tracey et al. 2008) have shown that plants sense and respond specifically to salinity addition within seconds.

Unlike osmotic tolerance, the mechanism of ion exclusion is well understood as the traits controlling Na^+ and Cl^- transport are relatively easy to phenotype (Munns et al. 2016). Plants achieve ion exclusion by restricting the accumulation of Na^+ and Cl^- in their leaves via compartmentation of ions in vacuoles, recovering Na^+ from the xylem before effluxion of ions back to soil (Munns and Tester 2008, Zhang and Shi 2013, Deinlein et al. 2014). However, if the ion exclusion system fails and the concentration of Na^+ and Cl^- reaches toxic levels in the leaves, plants can exert tissue tolerance mechanism by compartmentation of Na^+ and Cl^- excess ions at both cellular and intracellular level (usually in vacuoles). This strategy requires precise

control over the coordination of biochemical processes and their transport, which mainly involve synthesis of compatible solutes, ion transporters and proton pumping (Tuteja 2007, Munns and Tester 2008, Petronia Carrilo 2011, Horie et al. 2012, Ahmad et al. 2013, Roy et al. 2014b, Munns et al. 2016). In plant cells, several Na^+ -permeable transporters have been suggested to mediate Na^+ uptake (Tester and Davenport 2003). In non-saline conditions, HKT ion transporters have been reported to mediate Na^+ uptake in plant cells (Horie et al. 2007, Ji et al. 2013). Phylogenetic analysis and biophysical studies have grouped HKT into two subgroups, based on their preference for class A (Na^+ uniport) or class B (Na^+ -K^+ symport) (Horie et al. 2007, Horie et al. 2009, Jabnoune et al. 2009, Yoa et al. 2010, Ali et al. 2012). When excessive salt concentrations are present in the surrounding area, Na^+ ions are thought to penetrate the symplast via plasma-membrane non-selective cation channels (NSCC), whose activities are yet to be defined, or Na^+ may enter through the root endodermis (Davenport and Tester 2000, Hasegawa et al. 2000, Demidchik and Tester 2002, Munns and Tester 2008). Jeschke (1987) reported that Na^+ exclusion mechanisms in cereals is thought to depend on several key transporters including N^+/H^+ antiporter, H^+-pump ATPases and high-affinity K^+ ion uptake.

At the cellular and organelle level, the main mechanism of salt tolerance involves the compartmentation of ions in the vacuole to protect the cytoplasm. Despite the higher concentration of Na^+ influx, halophytes and to lesser extend glycophytes can maintain basic plant functions by sequestering excess cytosolic Na^+ and Cl^- in their vacuoles. This process not only prevents the toxic effects of Na^+ in the cytosol but also enables the plant to use NaCl as an osmoticum ─ the ability to maintain osmotic pressure which drives water into plant cells (Blumwald 2000). Munns et al. (1983) found that the leaves of certain plant species were functioning normally at concentrations exceeding 200 mM of sodium chloride even though

most enzyme activities were known to be suppressed at 100 mM NaCl. Moreover, salt tolerant plants were found to sequester toxic ions in their vacuoles, while simultaneously accumulating organic solutes such as sugar alcohols, polyamines, betaine, proteins and proline (Figure A.1) in the cytoplasm to maintain osmotic pressure (Munns and tester 2008, Hasegawa et al. 2000, Blumwald et al. 2000). The production of these compatible organic solutes (osmoprotectants) has been found to be the most common plant response to salinity stress (Ashraf and Foolad 2007). However, most of the cultivated crops do not have the capability to produce osmoprotectants. Consequently, many studies have focused on identifying the genes responsible for the over-expression of osmoprotectants in plants to develop salt-tolerant crops (Bhatnagar-Mathur et al. 2008). For instance, genes involved in the biosynthesis of choline oxidase *COX* or *BetA* or *CodA* for glycine betaine have been introgressed into rice, tobacco, cabbage and *Arabidopsis* to confer salinity tolerance traits (Hayashi et al. 1997, Hayashi et al. 1998, Sakamoto et al. 1998, Holmstrom et al. 2000, Huang et al. 2000, Bhattacharya et al. 2004, Khan et al. 2015). Similarly, genes responsible for proline biosynthesis such as *AtProDH cDNA* encoding proline dehydrogenase (*ProDH*) and Δ^1-pyrolline-5-carboxylate reductase (*TaP5CR*), Δ1-pyrroline-5-carboxylatesynthetase (*P5CS*) (*P5CS129A*), *DREB* (dehydration-responsive element binding protein) have been introgressed into rice, wheat and tobacco to generate salt-stress tolerance (Nanjo et al. 1999, Sawahel and Hassan 2002, Su and Wu 2004, Cong et al. 2008, Ma et al. 2008). Likewise, genes such as Myo-inositol O-methyltransferase (*ImtI*), L-ectoine synthase (*ectC*), glucitol-6-phosphate (*GutD*), Mannitol 1-phosphate dehydrogenase (*mtlD*), trehalose-6-phosphate synthase (*TPS1*), L-myo-inositol synthase, L2,4-diaminobutyric acid acetyltransferase (*ectA*) and L-2,4-diaminobutyric acid transaminase (*ectB*) were incorporated into a number of transgenic plants producing slightly salt tolerant plant varieties (Thomas et al. 1995, Sheveleva et al. 1997, Nakayama et al. 2000, Abebe et al. 2003, Majee et al. 2004, Cortina and Culianez-Macia 2005, Tang et al. 2005, Khan et al. 2015).

Despite these advancements, there has been little success in the development of salt-tolerant crop varieties that can produce the optimum protective levels of these osmolytes. However, the mechanisms that enables plants control their osmolyte levels are essential in conferring salinity tolerance, but little is known about the exact metabolic rearrangements and the regulatory pathways controlling these osmoprotectants (Ashraf and Foolad 2007, Deinlein et al. 2014).

At the molecular level, ion transporters have been shown to play an important role in the regulation of ion homeostasis (Munns 2002). Sodium ions penetrate plant cells by competing with other cations, particularly K^+ via high affinity K^+ carriers or through other low affinity non-selective cation channels influenced by Ca^{2+} (Amtmann and Sanders 1999, Munns 2002, Hasegawa 2013, Deinlein 2014). Sodium ions can compete with potassium as both elements have similar characteristics. For example, they both have similar cation radii in their non-hydrated and hydrated forms as well as possessing the same electric charge, $1.6*10^{-19}$ coulombs, which makes difficult for transport proteins to discriminate between the two ions (Nightingale 1959, Collins 1997, Blumwald et al. 2000, Mahler and Persson 2012). Therefore, interactions between sodium and potassium ions with pockets of binding proteins, selectivity filters of proteinaceous ions, active centres of proteins and amino acid are similar in action with few differences (Volkov 2014, Volkov and Beilby 2017). Some plant species evolved to exclude Na^+ from their cytoplasm by compartmentalising into the vacuole via N^+/H^+ antiporters which in turn is regulated by pH gradient across the plasmalemma and tonoplast, respectively (Blumwald 2000). However, the transporters responsible for ion homeostasis in the mitochondria and chloroplast have not been yet established (Blumwald 2000, Hasegawa 2013, Adams and Shin 2014, Maathuis 2014).

At whole plant level, the mechanism of salt tolerance involves both the cellular and molecular levels, this process starts with the selective uptake of NaCl by the roots through the epidermis and endodermis layer, followed by xylem loading and unloading, and loading of the

phloem to the final stage of excreting excess salts via specialised bladders and salt glands. This complex process of controlling the uptake, transport and excretion of salt is some of desired agronomic traits in halophytic plant species. Unlike halophytes, glycophytes lack the well-developed anatomical and physiological tolerance mechanisms, however, studies show that these plants employ all three mechanisms but to a lesser degree (Pitman 1984, Garcia et al. 1997, Munns et al. 2002, Flowers and Colmer 2008).

1.5 Ion homeostasis in plants

Maintaining an ionic homeostasis is one of the coping mechanisms employed by plants to survive and thrive under salt stress (Hasegawa et al. 2000, Pardo et al. 2006, Maurel et al. 2008, Ward et al. 2009, Horie et al. 2012). Homeostasis is therefore an important trait as it allows plants to maintain low sodium concentration (1-10 mM), while simultaneously maintaining optimum levels of potassium (100-200 mM) in their cytosol (Binzel et al. 1988, Blumwald 2000, Maser et al. 2001, Zhu 2001, Maser and Gierth 2002, Very and Sentenac 2003, Munns and Tester 2008). In saline environments, ion homeostasis depends on transmembrane transport proteins that regulates ion fluxes such as Ca^{2+} -ATPases, H^+ translocating ATPases and pyrophosphates, channels and secondary active transporters (Figure 1.7) (Niu et al. 1996, Sze et al. 1999, Binzel and Ratajczak 2000, Blumwald et al. 2000, Hasegawa et al. 2000). Although many of the transport proteins involved in the regulation of Na^+, Cl^{-1}, Ca^{2+} and K^+ have been identified in yeast mutants, it is now evident that similar proteins are also active in plants (Dreyer et al. 1999). Many studies have reported that H^+ electrochemical potentials gradients generated by H^+ pumps are involved in driving electrophoretic fluxes across the plasma membrane as well as the tonoplast and secondary active transport (Zhen et al. 1997, Luttge and Ratajczak 1997, Palmgren and Harper 1999, Hasegawa et al. 2000).

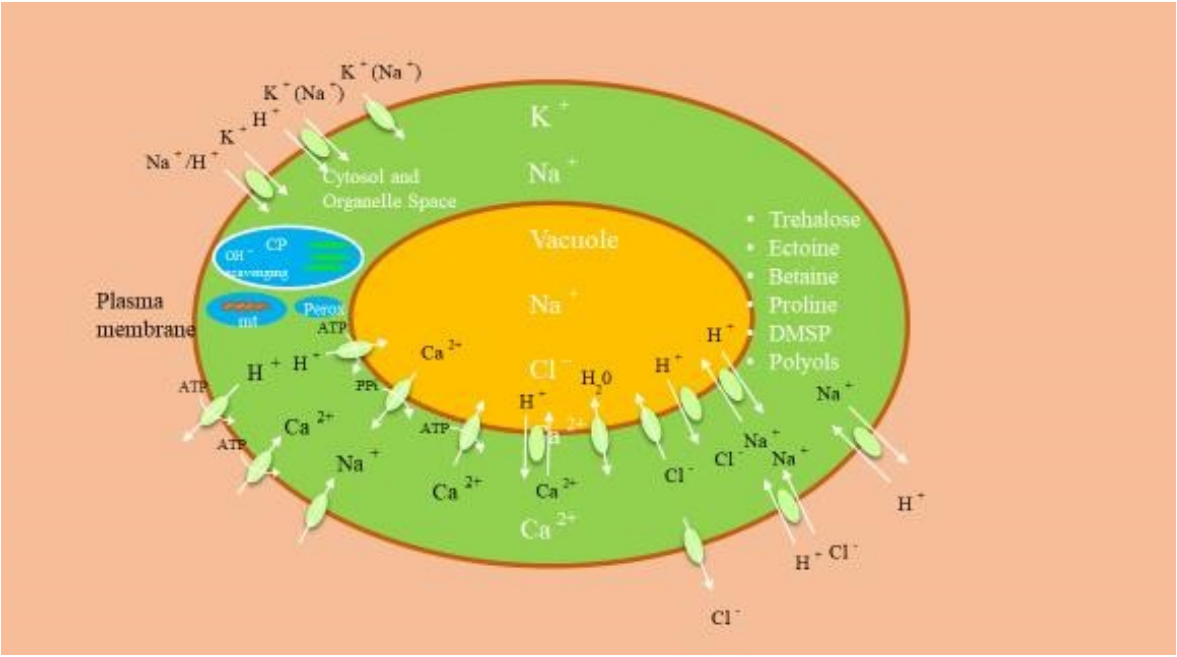

Figure 1.7: Schematic representation of cellular homeostasis and adaptation after salinity exposure. Protein transporters involved in Na^+ and Cl^- homeostasis, ROS scavenging osmolytes, water channels, tonoplast, compartmentation spaces and organelles including mitochondria (mitmt), chloroplast (chlcp) and peroxisomes (perox) are also shown (modified from Hasegawa et al. 2000)

To understand how plant cells maintain ionic homeostasis scientists have used direct and indirect methods to study ion fluxes of plants exposed to salinity stress and under control conditions. These techniques include the use of electrophysiological methods, kinetic measurements of ion concentrations, ion-selective fluorescent dyes, non-fluorescent indicators, Nuclear magnetic resonance (NMR) spectroscopy and microelectrode ion flux estimation methods (MIFE). Most of these methods except MIFE are said to be technically challenging, require longer training and are costly to operate (Flowers and Hajibaghari 2001). However, MIFE technique has been touted as an alternative, given that it is non-invasive, quicker and yet simpler method to measure net fluxes of some the basic ions such as Na^+, Cl^-, K^+ and H^+ for both salt-sensitive and salt-tolerant cultivars (Chen et al. 2005, Chen et al. 2007a, Chen et al. 2007b, Chen et al. 2007c, Cuin et al. 2008, Cuin et al. 2012).

Moreover, MIFE methods provide reliable temporal resolution of seconds and spatial resolution (within tens of a micron) which influences ion fluxes moving in and out of the target cells (Newman et al. 1987, Newman 2001, Kunkel et al. 2006, Shabala 2006, Sun et al. 2009, Shabala and Bose 2013). Similar studies have also used MIFE to study specific ion channels (Shabala et al. 2005, Demidchik et al. 2010), physiologically active molecules (Cuin and Shabala 2007, Shabala et al. 2009, Pandolfi et al. 2010, Demidchik et al. 2011, Ordonez et al. 2014), ions along the root zone of many crops (Garnett et al. 2001, Chen et al. 2005, Pang et al. 2006), ROS generation from salinity stress (Cuin and Shabala 2007, Demidchik 2010) and ion movement in cell biology (Lew et al. 2006, Valencia-Cruz et al. 2009, Demidchik et al. 2010). Based on this enormous literature about the kinetics and physiological profiles of ion fluxes, MIFE measurements are fast, and reliable method that can be used for screening of salt tolerance in many plant species. However, the ion-selective electrodes used in MIFE measurements are not totally fool proof and can be affected by physiologically active compounds and other interfering ions (Knowles and Shabala 2004, Chen et al. 2005) therefore, some controls are needed to offset these limitations.

1.6 Ion transport and salinity tolerance in plant cells.

Physio-physical forces such as the differences ion concentrations, and differences in electrical potential are the driving force linked to ion transport in living cells (Volkov 2014). Ions move from high electrochemical potential to low down a concentration gradient, therefore, when their total electrochemical potential gradients are measured, then their net fluxes (mol m^2 s^{-1}) can be obtained by applying Nernest equation described in Newman (2000). In plant cells, movement of most ions including K^+ and Na^+ occurs through two pathways viz. ion selective proteinaceous pores of ion channels ("gated"). The other pathway has slower transport rate and goes through the proteinaceous transporters. Since ions carry electrical charges, ions passing through ion channels are therefore electrogenic.

1.7 The role of ion fluxes in plant salt tolerance

1.7.1 Na^+ fluxes

Salinity tolerance is a cumulative process that requires several physiological characteristics such as maintaining low Na^+ and high K^+ ratio in root and shoot, extrusion of Na^+ from shoot, vacuolar sequestration, ion homeostasis and Na^+ exclusion (Munns and Tester 2008, Ward et al. 2009, Horie at al. 2012). Therefore, the uptake, transport and compartmentation of Na^+ are vital for plants to survive and thrive under saline conditions. Besides the exclusion of Na^+ influx, there are two basic ways to mitigate Na^+ toxicity in the cytosol; enhancing vacuolar compartmentation through tonoplast Na^+/H^+ antiporters and increasing the Na^+/H^+ antiporters in the plasma membrane (Horie et al. 2012). High affinity K^+ transporters (*HKTs*) were suggested to play an essential part in the regulation of Na^+ in certain plant species (Uozumi et al. 2000, Horie et al. 2001, Golldack et al. 2002, Maser and Gierth 2002, Platten et al. 2006, Byrt et al. 2007, Davenport et al. 2007, Hauser and 2010, Ali et al. 2012, Horie et al. 2012). Garciadeblas et al. (2003) described nine *HKT* homologues (*OsHKT 1— OsHKT 9*) genes involved in the regulation of ion homeostasis and Na^+ transport in rice plants. Furthermore, according to their sequence and transport analysis, there are two distinct HKT subgroups class I and II that act as Na^+ -selective transporter and as Na^+/K^+ cotransporter, respectively (Sunarpi et al. 2005, Horie et al. 2007, Munns and Tester 2008, Horie et al. 2009, Deinlein et al. 2014).

1.7.2 Cl^- fluxes

Cl^- toxicity has been found to be more deleterious than Na^+ toxicity in certain woody plant species such as *Vitis* and *Citrus* (Munns and Tester 2008). Cl^- is also the most dominant anion in most saline soils (Teakle and Tyerman 2010). Despite that, the responses of Cl^- fluxes under salinity stress and their transport mechanisms are less well understood in comparison to other cation transport systems. Although, Cl^- is considered to be toxic to most plants at high concentrations, it is also an essential micronutrient that plays a role in the

regulation of enzymes in the cytoplasm, turgor and pH regulation, a vital co-factor in photosynthesis, and stabilises membrane potential by acting as counter anion (Tyerman 1992, Teodoro and Lado 1998, Xu et al. 2000, White and Broadley, 2001, Teakle and Tyerman 2010, Marschner 2012). Considering that both Na^+ and Cl^- are metabolically toxic to plants, it is interesting to note that for some species such as rice and wheat, Na^+ concentrations in the shoot and not Cl^- have shown to be negatively correlated with salinity tolerance (Kinraide 1999, Lin and Kao 2001, Husain and Munns 2004, Plett and Moller 2009). In contrast, leaf Cl^- concentration in soybean was found to be negatively correlated with salinity tolerance (Luo et al. 2005), whereas the exclusion of both Na^+ and Cl^- were negatively correlated with salinity tolerance in *Medicago truncatula* (Aydi and Abdelly 2008) and *Hordeum marinum* (Islam et al. 2007).

Salinity stress induced Cl^- effluxes were observed to be genotypic dependent, for example salt tolerant *Populus euphratica* exhibited significant Cl^- efflux under salinity stress, whereas salt-sensitive relative *Populus popularis* displayed no Cl^- efflux (Sun et al. 2009). Similarly, Huang and Van Steveninck (1989) found that salt-tolerant barley cultivar were more effective in excluding Cl^- from their mesophyll cells when compared to more sensitive barley cultivars. Moreover, other studies have also detected Cl^- efflux in salt stressed transgenic *Arabidopsis* (Lorenzen et al. 2004) and in bean mesophyll cells (Shabala 2000). As mentioned earlier, the mechanisms of Cl^- transport associated with salinity stress tolerance are less well understood, however several candidate genes thought to be involved in Cl^- transport have been identified (Teakle and Tyerman 2010). In the last decade, several possible candidates involved in Cl^- homeostasis in plants exposed to salinity stress have been revealed using transcriptome, functional and mutant analysis (Teakle and Tyerman 2010). These include voltage dependent anion channels (VDAC) (Brumos et al. 2009, Lee et al. 2009, Yan et al. 2009),

mechanosensitive channels of small conductance (MscS) like MSL (Haswell 2007, Haswell et al. 2008) chloride channels (CLC) (Jentsch et al. 2002, Miller et al. 2006, Nakamura et al. 2006, De Angeli et al. 2007, De Angeli et al. 2009), aluminium activated malate channels (ALMT) (Motoda et al. 2007, Pineros et al. 2008, Rudrappa et al. 2008), cation-chloride cotransporter (CCC) (Lorenzen et al. 2004, Colmenero-Flores et al. 2007, Munns and Tester 2008, Brumos et al. 2009), ATP binding cassette (ABC) (Davies and Coleman 2000, Rea 2007, Lee et al. 2008), nitrogen transporter (NRT) (Segonzac et al. 2007, Tsay et al. 2007, Brumos et al. 2009), and slow ion channel associated protein (SLAC1) (Negi et al. 2008, Vahisalu et al. 2008). This highlights the need for further studies (using MIFE and other techniques) focusing in deciphering Cl^- transport mechanism in plants under salinity stress. However, measurements of Cl^- in MIFE could be negatively affected by the low signal-to-noise ratio for liquid ion exchanger (LIX) when used in high concentration.

1.7.3 K^+ fluxes

Potassium is an important micronutrient required by plants for growth and development (Ahmad and Maathuis 2014). It plays an important role in various cellular and physiological processes including cation-anion balance, stomata and osmotic regulation (Marschner 2012). Excessive cytoplasmic Na^+ reduces K^+ concentrations resulting changes of osmotic pressure, turgor pressure, membrane potential, ROS and calcium signalling (Marschner 2012). K^+ homeostasis plays a vital role in conferring salinity tolerance in plants cells (Shabala and Cuin 2008, Shabala and Potossin 2014). Under salinity stress, salt-tolerant plants usually maintain higher K^+ in their cellular and tissue levels (Zhu, 2001, Carden et al. 2003, Chen et al. 2005). Studies using MIFE technique have established a very strong correlation of >0.80 between K^+ and the level of salinity stress tolerance in different barley cultivars (Chen et al. 2005, Chen et al. 2007a). The stark differences of salinity-stress induced

K^+ efflux exhibited by both sensitive and tolerant varieties were remarkable, given that salt sensitive cultivars displayed higher K^+ efflux, in comparison to tolerant cultivars (Chen et al. 2005). Similar studies on ion fluxes in halophytic *Thellungiella halophila* and the glycophytic *Arabidopsis thaliana* showed lower Na^+ and higher K^+/Na^+ in their roots under salt treatment (Volkov and Amtmann 2006, Amtmann 2009). Comparable results were also reported for barley (Wu et al. 2015), wheat (Cuin et al. 2008, Cuin et al. 2012), and *Brassica* species (Chakraborty et al. 2016). This vast literature underlines the importance of K^+ uptake measurement as a fast and simple test for screening plants for salinity tolerance (Chen et al. 2005). It also gives credence to MIFE as a non-invasive tool for screening plant root and leaf mesophyll for K^+ fluxes and comparing variations in their response to salinity-stress induced K^+ efflux (Chen et al. 2005).

1.7.4 H^+ fluxes

Despite the presence of several types of secondary transport mechanisms located at the plasma membrane, such as Na^+/H^+ antiporters, H^+/K^+ symporters, and H^+/Cl^- symporters, H^+ flux measurements offer a direct evidence of ion exchange coupling with H^+ inside and along the electrochemical gradient (Tuteja 2007). Hence, H^+ selective microelectrode is a quicker and more convenient approach when dealing with NA^+/H^+ antiporters driven by H^+ ATPases (Qiu et al. 2002). Moreover, it has been suggested that salinity stress triggers H^+ ATPases to move Na^+ from the cytoplasm into the apoplast to maintain low Na^+ concentrations in the cytosol, thereby creating favourable pH and electric potential gradient across the vacuole which in turn triggers secondary transporters required for metabolite and ion uptake (Serrano 1989, Sussman 1994, Michelet and Boutry 1995, Palmgren 1998, Shabala 2006, Baisakh et al. 2012). An increase in net H^+ efflux has been reported in salt-stressed mesophyll tissues of salt sensitive broad beans (*Vacia faba*), however H^+ efflux in salt-shocked plants have shown to be species-specific (Shabala 2000). For example, a significant H^+ effluxes were reported in the

root apex in both mutant (*SOS1*) and wild (*SOS2*, *SOS3*) *Arabidopsis* exposed to salinity treatment (Shabala 2005).

1.7.5 Ca^{2+} fluxes

Ca^{2+} plays a critical role in various cellular functions involved in signalling and other adaptive mechanisms against biotic and abiotic stresses (Sanders et al. 2002, Gao et al. 2004, Henriksson and Henriksson 2005, Chen et al. 2010, Wang et al. 2016). Previous studies on *Arabidopsis* have shown that higher NaCl concentrations significantly reduced Ca^{2+} levels in root cells (Cramer and Jones 1996, Halperin et al. 2003). Similar effects were also reported on corn (Lynch and Lauchli 1988). Furthermore, many studies have reported that an increase in Na^{+} concentration around the roots triggers a flux of Ca^{2+} into the cytosol via the plasma membrane and into the tonoplast (Kiegle et al. 1997, Knight et al. 1997, Moore et al. 2002, Tracey et al. 2008). Although, changes in the Ca^{2+} are regulated by various cellular events, Na^{+} have been reported to elicit transient changes in Ca^{2+} levels (Tracey et al. 2008). Zhu et al. (2002) referred to the increase in Ca^{2+} as the "best- characterized signalling pathway to salinity stress". However, other studies have reported that the increased Ca^{2+} induced by salinity exposure depends on plant species and cell type (Cramer and Jones 1996). Moreover, the changes in Ca^{2+} fluxes caused by salinity stress creates an imbalance between Ca^{2+} and pH homeostasis thus overwhelming cells with the increased monovalent ions such as H^{+} (Gao et al. 2008). Furthermore, Chen et al. (2010) reported that Cytosolic free concentration of Ca^{2+} ($[Ca^{2+}]_i$) responds to abscisic acid (ABA) which in turn induces an increase in $[Ca^{2+}]_I$ in guard cells, which precedes stomatal closure. In addition, the elevation of $[Ca^{2+}]_I$ interfere with the membrane transport resulting in net ion flux and decreased turgor leading to reduction in stomata aperture (Chen et al. 2010).

1.8 Effects of salinity on rice morphology and physiology.

Salinity tolerance in rice is a complex process controlled by various genetic and environmental factors. Mechanisms found to be influencing salinity tolerance in rice such as Na^+ uptake restriction and exclusion from shoot have been reported in many studies (Munns and Tester 2008, Platten et al. 2013, Ismail and Horie 2017). Similarly, a considerable amount of literature has been published on genes conferring salinity tolerance via regulating growth accelerators, osmoprotectants and ion movement (Munns and Tester 2008, Horie et al. 2012, Ismail and Horie 2017). Based on the results of these studies, salinity tolerance genes (*SALTOL*) and suitable donors for these agronomically desirable traits have been identified (Garg et al. 2002, Islam et al. 2008). However, understanding the role of these mechanisms and their precise functions at the cellular, molecular and at the whole plant level under controlled condition and in the field, conditions are yet to be determined. Therefore, exploring the physiological response of cultivated and wild rice genotypes at different levels within the plant may contribute in speeding the efforts for developing high-yielding salt-tolerant rice varieties (Horie et al. 2012). Physiological responses at the whole plant scale involve adjustments of water status, stomatal conductance, reduction in photosynthesis (Figure 1.8) and ion and nutrient imbalance (Horie et al. 2012). Munns et al. (1995) classified these physiological responses as short-term implying the initial water deficit effect and ion toxicity as the long-term response. Such responses minimise or sequester toxic NaCl ions to shield newly formed shoots and the reproductive parts, thus protecting the photosynthesis apparatus and other vital physiological mechanisms required for growth and development (Radanielson et al. 2018).

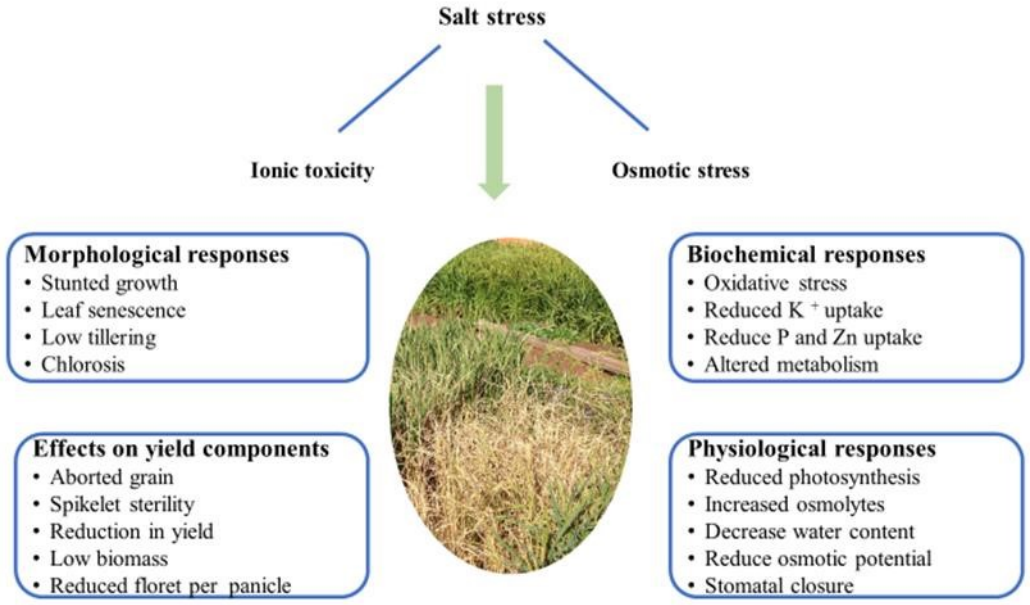

Figure 1.8: Effects of osmotic stress and ionic toxicity induced by salinity stress on rice plants. Blue boxes represent the morpho-physiological, and biochemical responses as well as effects on yield (modified from Hasanuzzaman et al. 2012)

Furthermore, salinity interferes with the process of cell expansion and division causing significant decrease in leaf area index and ultimately hampering photosynthetic processes (Netondo et al. 2004). However, Moradi and Ismail (2007) pointed out that variations in photosynthetic activities and the concentration of Na^+ accumulation in rice plants are genotypic dependent.

In addition, several studies have reported that rice genotypes exhibiting salinity tolerance during vegetative and reproductive phase are shown to have higher yield in comparison with the more salt-sensitive genotypes (Moradi et al. 2003, Singh and Flowers 2010). In rice, salinity limits sink size and grain filling as typically reflected by stunted panicles and sterile spikelets. Moreover, the senescence and death of older leaves further decrease assimilates allocated for grain filling (Flowers et al. 1985, Zeng and Shannon 2000,

Radanielson et al. 2018). At high concentrations both Na^+ and Cl^- induce osmotic and ionic effects in rice plants, however, it is difficult to categorise the effects of their ions separately. Although, many studies have focused on the effects of Na^+ on plants, in some species including barley, Citrus and grape, Cl^- is more potent by inducing toxicity when compared to Na^+ (Moya et al. 2003). The dominance of Na^+ transport and uptake studies rather than Cl^- could be explained by the fact that the mitigation of Na^+ induced stress is more complex and costs more energy to transport than Cl^- (Moya et al. 2003). Due to the negative electrical potential of cells, Cl^- plays a role in stabilising the depolarisation of membranes resulted from NaCl presence. Therefore, restricting Na^+ uptake and transport is a not only a costly manoeuvre in terms of energy but also creates other problems relating to ion selectivity. For example, Na^+ can displace K^+ and therefore, more damaging to species that are unable to restrict Na^+ movement, given that K^+ also regulates many enzymes in the cytoplasm and plays a major role in osmotic regulation activities (Moya et al. 2003).

1.9 Responsive mechanisms of rice under salinity stress

As mentioned earlier the mechanisms of salinity stress tolerance in rice is a complex trait which is controlled by many factors. Therefore, studying rice response to salinity stress provides a vital insight in understanding the underlying morpho-physiological mechanisms related to the activation of defence mechanisms during salinity stress. Responses of rice under salinity stress can be divided into three categories; morpho-physiological response, response at the biochemical level, and response at the molecular level.

1.9.1 Morpho-physiological response of rice to salinity stress

In rice, the effects of salinity stress start with the osmotic stress characterised by decreased osmotic potential and followed by ionic effects resulting in ionic toxicity (Gosh et al.2016). Physiological studies in rice plants subjected salinity stress have shown that chloroplast and mitochondria are the most vulnerable organs compared to other organs (Rahman et al. 2000). Damage to these two organs will affect the chlorophyll content,

chlorophyll fluorescence and membrane permeability which will ultimately lead to a decrease in photosynthetic efficiency (Netondo et al. 2004, Baker 2008). Likewise, salinity stress has been shown to reduce leaf area and alters leaf architecture of rice plants grown in glasshouse and in-vitro (Bahaji et al. 2002, Wankhade et al. 2010, Wankhade et al. 2013). Rahman et al. (2000) observed various inhibitory effects of salt stress on rice leaf structure such as swelling of the thylakoids which in turn causes disruptions in chloroplastids. Similarly, salinity stress also exerts detrimental effects on the mesophyll tissue as well as the vascular bundles (Rahman et al. 2000, Wankhade et al. 2013). Moreover, studies in rice root revealed the influx of NaCl ions increases the rate of vacuolation and vesiculation resulting a decrease in Mucilage production in rice plants subjected to salinity stress when compared the control variables (Flowers and Yeo 1981, Rahman et al 2000, Rahman et al. 2001). Many studies have shown the existence of a strong correlation between K^+ and Na^+ ratio and sodium content in rice plants exposed to salinity stress (Akita and Cabuslay 1990, Khatun et al. 1995, Lutts et al. 1995). Therefore, the evaluation of several rice genotypes at morpho-physiological levels may reveal defence mechanisms operating in rice plants during salinity stress.

1.9.2 Responses of rice to salinity stress at the biochemical level

The effects of salinity stress in rice can divided into osmotic effects and ionic effects (Munns 1995, Munns and Tester 2008, Horie et al. 2012). Osmotic stress which is characterised by decreased water potential is the initial osmotic effect caused by excessive salt accumulation. Plants response to stress by adjusting their osmotic potential via production and accumulation of low molecular weight sugars, polyols, organic acids and nitrogen containing compounds such as proteins, amino acids, imino acids, quaternary ammonium compounds, and amides (Jones 1981, Lutts et al. 1996, Ali et al. 1999). Bandurska (1991) reported that proline accumulation in rice plants exposed to salinity stress acts as an osmo-protectant by regulating osmotic potential, protecting enzymes and membranes while providing nitrogen and sugars as energy (Cram 1976, Perez et al. 1993, Bundurska 1993). An increase in shoot and root sugars

in rice has been shown to play a major role in osmotic adjustments (Popp and Smirnoff 1995, Hurry et al. 1995, Sakamoto 1998, Dubey and Singh 1999, Amirjani 2011). Similarly, glycine betaine accumulation in rice has been associated with enhanced osmotic adjustments, nitrogen storage and the fortification of cellular macromolecules which in turn plays a role in the detoxification of cells, scavenging for reactive oxygen species (ROS) and to balance the cellular pH (Popp and Smirnoff 1995, Hurry et al. 1995, Sakamoto 1998, Dubey and Singh 1999, Amirjani 2011). Furthermore, the accumulation and storage of proteins in rice plants exposed to salinity stress also plays an active role in osmotic adjustments. A positive correlation has been observed in the production and accumulation of soluble proteins in salt tolerant rice seedlings to their control variables (Akbar and Yabuno 1975, Singh et al. 1987, Jha and Singh 1997).

1.9.3 Responses of rice to salinity stress at the molecular level

Understanding salinity tolerance at the molecular level is one the most important pillars in the development of salt tolerant crops. Previous studies have shown that response of rice to salinity stress is genotypic dependent (Akbar et al. 1972, Bonilla et al. 2002). Scientists using molecular marker methods including; Restriction Fragment Length Polymorphs (RFLP) (Botstein et al. 1980), Simple Sequence Repeats (SSR microsatellites) (Tautz 1989), Random Amplified Polymorphic DNAs (RAPD) (Williams et al. 1990), Simple Sequence Length Polymorphism (SSLP) (McDonald and Potts 1997), Amplified Fragment Length Polymorphism (AFLP) (Zabeau and Vos 1993), have screened various rice genotypes for salinity stress tolerance (Kanawapee et al. 2011, Ali et al. 2014). To understand the nature of inheritance in salinity tolerance in rice, researchers have conducted experiments using conventional methods including “insertional mutagenesis” (Rabbani et al. 2003, Salvi and Tuberosa 2005), and “positional cloning” (Bechtold et al. 1993, Ron and Weller 2007). Several genes such as *Saltol, catalase* and few *denovo* genes involved in conferring salinity tolerance in rice have been identified (Urao et al. 1999, Horie et al. 2012, Hoang et al. 2016). Plants have

evolved to sense salinity stress using osmo-sensing adaptations based on signalling, which can be measured phenotypically via quantification of Na^+ and K^+ ratio (Xiong and Yang 2003). Studies on responses of plants to salinity stress at the molecular level have identified several important signalling pathways in various mutants of Arabidopsis thaliana including histidine kinase, candidate osmosensor, and ATHK1 (Xiong et al. 2003). Similarly, CDPKs and OsCDPK7 which is activated during salinity has been identified in rice (Xiong et al. 2003). In addition, MAPKs and OsMAPK5 have been identified in rice whose suppression caused salinity hypersensitivity in rice plants (Zhu 2002). Other genes in the Salt Overly Sensitive pathway such as SOS1, SOS2 and SOS3 have been identified in rice plants under salinity stress (Haq et al. 2008). This shows the complexity of the genes responsible for regulating responses to salinity stress in plants. These studies have initiated the work to isolate the Quantitative Trait Loci responsible for salt tolerance genes in rice. Gregorio (1997) became the first scientist to identify and map '*SALTOL*" on chromosome 1 in an F8 recombinant Pokkali crossed with IR29 using AFLP.

To improve grain yield of rice grown under saline conditions, it is important to first understand the basic mechanisms of salt tolerance in rice. As mentioned earlier, salt tolerance is a quantitative trait which is regulated by a multitude of genes (Chinnusamy et al. 2005, Garg et al. 2013). Studies have reported that rice is more susceptible to salinity stress at the reproductive stages but exhibits some tolerance at seedling stages (Lutts et al. 1995, Singh 2004, Todaka et al. 2012). Comparisons of biomass production percentage have been recommended as a viable method when assessing salinity tolerance in rice (Munns et al. 2002).

1.10 Rice plants

1.10.1 Taxonomy

Rice is part of the Poaceae or Gramineae (true grass) family which belongs to the genus *Oryza* (Table 1.1). There are 25 *Oryza* species of which 23 are wild and the remaining two, viz *Oryza sativa* and *Oryza glaberrima* are cultivated (Morishima 1984, Vaughan et al. 2003, Brar and Khush 2003). *O. sativa* is the most cultivated rice in Asia whereas *O. glaberrima* is mainly cultivated in Western and Eastern parts of Africa but is being replaced by *O. sativa* (OECD 2006, Oka 2012). Rice is grown in the tropical and subtropical regions of Africa, Asia, south and central America and Australia (Chang 1986).

Table 1.1: Classification of rice plants from Kingdom Plantae down to species *sativa*

Name	Rice
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Gramineae or Poaceae
Tribe	Oryzeae
Genus	*Oryza*
Species	*Sativa*

1.10.2 Geographic origin and domestication

Over centuries, rice has supported many successive civilisations in Southern, Eastern, and South Asia and Africa (Bray 1986, Scott 2009, Molina et al. 2011). The geographic origin of rice has been extensively researched in China, India and Southeast Asia and other parts of the world (Oka 1988, Chang 1989, Yasuda 2002, Vaughan et al. 2008, Zhang and Hung 2010). Research shows that the domestication of rice was completed in the middle of Holocene

between 6,000 and 3,000 BC (Molina 2011). Based on archaeological evidence, *Oryza rufipogon* and *Oryza nivara* (Figure 1.9) are considered to be the progenitors of the present-day rice *O. sativa* L. (Khush 1997, Sweeney and McCouch 2007, Kovach et al. 2009).

However, the African rice *Oryza galaberrima* Steud. (Figure 1.9) was independently domesticated from its progenitors *Oryza longistaminata* and *Oryza Barthii* around 3,000 years ago, 6,000 to 7,000 years after *O. sativa* (Molina et al. 2011). *O. sativa* has two cultivated distinct subspecies viz. Japonica which is grown in temperate regions, whereas Indica is popular in tropical countries (Chang 2003, Kovach et al. 2009). Gross and Zhao (2014) explained the possible domestication scenarios of both Japonica and Indica from their progenitor *O. rufipogon* and their hybridisation before final domestication (Figure 1.10).

Although, earlier studies have indicated that both species were domesticated independently (Vitte et al. 2004, Londo and Chiang 2006, Gao and Innan 2008). However, contemporary research shows that both subspecies were first domesticated in China (Molina et al. 2011, Silva et al. 2015).

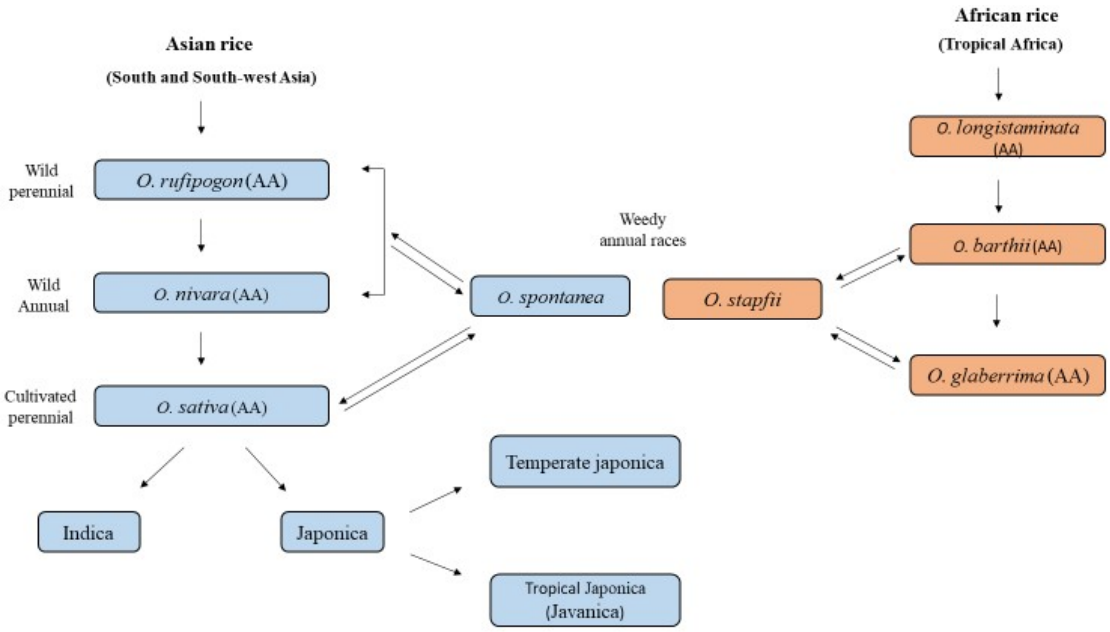

Figure 1.9: Schematic representation of the evolutionary pathways of the origin of *O. sativa* and *O. glaberrima*. (Modified from: Chang 1976)

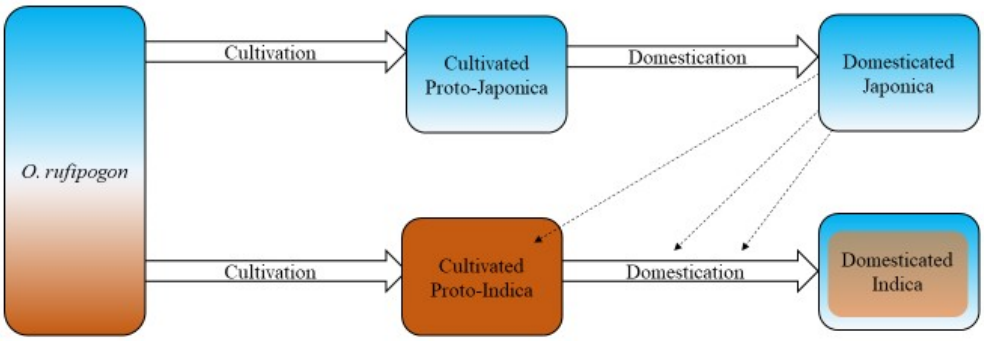

Figure 1.10: Schematic representation of the likely scenario for the origin of Japonica and Indica indicated by different colours. Colours in the domesticated Indica represent an introgression from Japonica shown in blue, while the light brown represents contributions from *O. rufipogon*. Broken lines represent the possible timing of hybridisation between Japonica and Indica (Modified from: Gross and Zhao 2014).

1.10.3 Genome evolution and allelic variation in salinity tolerance

O. sativa is model species for monocot cereals with a relatively small tractable genome of about 380 Mb (n=12) compared to wheat ─15,000 Mb (3n=42) (Paterson et al. 2005, McCouch et al. 2016). The two major cultivated rice species, viz Indica and Japonica are mainly diploid (2n=2×=24) with genome AA (Chang 2003, Paterson et al. 2005). Conversely, their wild relatives under *Oryza* genus carries both diploid and tetraploid (2n=2×=24), (2n=4×48) respectively. Wild *Oryza* varieties contain 10 types of genomes: AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK (Vaughan et al. 2003). In addition to that, *O. sativa* has the largest single-species germplasm stock in the world which is publicly available (Jackson 1997). Moreover, rice is the first crop in human history to be fully sequenced (IRRI, 2005) with over 3,000 re-sequenced varieties (Huang et al. 2012, Xu et al. 2012, The rice genome project 2014, Duitama et al. 2015). These genome sequences will not only give scientists an insight into the architecture and function of rice plant but will also enable them to decipher the framework of other important cereals including wild relatives (Paterson et al.

2004, Paterson et al. 2005, Devos 2005).

Due to the considerable allelic variations in salinity tolerance among rice germplasms, scientists were able to identify QTLs responsible for salinity tolerance (Collard et al. 2005, Yu et al. 2012, Ashraf and Foolad 2013, Shahbaz and Ashraf 2013, Bansal et al. 2014). Similar QTLs have been isolated in different crops for abiotic stresses tolerance including salinity tolerance (Byrt et al. 2007, Xue et al. 2009, Genc et al. 2010, Thomson et al. 2010, Ul Haq et al. 2010), heat tolerance (Y ang et al. 2002, Mason et al. 2010), chill tolerance (Andaya et al. 2006, Baga et al. 2007, Kuroki et al. 2007, Lou et al. 2007) and drought tolerance (Quarrie et al. 2006, Mathews et al. 2008, Von Korff et al. 2008, Peleg et al. 2009, Chen et al. 2010). Therefore, the availability of natural variations of species will help widen the genetic pool and improve the abiotic resistance by combining desired agronomical traits from various sources.

1.10.4 Classification of Oryza gene pool

The genus Oryza has been classified into four main complexes (Table 1.2) including *Sativa*, *Officinalis*, *Myeriana* and *Ridley's*. The *sativa* genus comprises of two of the most cultivated species *O. sativa* and *O. glaberrima* and their wild relatives viz, *O. barthii*, *O. rufipogon*, O. *longistaminata*, *O. sativa f. spontana* and *O. nivara* (Table 1.2)

Table 1.2: Oryza species complex with chromosome numbers, genome symbols and their geographical distribution (Source: Brar and Khush 2003).

	Species Complex	**Chromosome Number**	**Genome**	**Geographical Distribution**
I	***Sativa* complex**			
	1. *O. sativa* L	24	AA	Worldwide: originally South & Southeast Asia
	2. *O. nivara* Sharma et Shastry	24	AA	South & Southeast Asia
	3. *O. rufipogon* Griff.	24	AA	South & Southeast Asia, South China
	4. *O. meridionals* Ng	24	AA	Tropical Australia
	5. *O. glumaepetula* Stued.	24	AA	Tropical America
	6. *O. glaberrima* Steud.	24	AA	Tropical West Africa
	7. *O. barthii* A Chev et Roehr.	24	AA	West Africa
	8. *O. longistaminata* A. Chev et Roehr			Tropical Africa
II	**Officinalis complex/latifolia complex**			
	9. *O. punctata* Kotschy ex Steud	24	BB	East Africa
	10. *O. rhizomatis* Vaughan	24	CC	Sri Lanka
	11. *O. minuta* J.S. Pesl. Ex C.B.Presl.	48	BBCC	Philippines, New Guinea
	12. *O. malamphuzaensis* Krishn. et Chandr.	48	BBCC	Keral & Tamil Nadu
	13 *O. officinalis* Wall. Ex Watt	24	CC	South & Southeast Asia
	14 O. *eichingeri* A. peter	24	CC	East Africa & Sri Lanka
	15 O. *latifolia* Desv.	48	CCDD	Central & South America
	16 O. *alta* Swallen	48	CCDD	Central & South America
	17 *O. grandiglumis* (Doell) Prod.	48	CCDD	South America
	18 O. *australiensis* Domin.	24	EE	Northern Australia
	19 O. *schweinfurthiana* Prod.	48	BBCC	Tropical Africa
III	**Myeriana complex**			
	20 O. *granulata* Nees et Arn. ex Watt	24	GG	South & South Asia
	21 O. *myeriana* (Zoll. Et Mot. ex Steud.) Baill	24	GG	South Asia
IV	**Ridley's complex**			
	22 O. *longiglumis* Jansen	48	HHJJ	Indonesia & New Guinea
	23 O. *ridleyi* Hook f.	48	HHJJ	Southeast Asia
V	**Unclassified**			
	24 O. *brachyantha* A. Chev. et Roehr.	24	FF	West & Central Africa
	24 O. *schlechteri* Pilger	48	HHKK	Indonesia & New Guinea

1.10.5 Growth phases of rice

The cultivated *Oryza* species is mostly an annual crop with 90 to 180 days of life cycle depending on the cultivar, cultivation region, ecological conditions and season (Dingkuhn and Asch 1999). Mature rice plant consists of primary stem and tillers that produce effective and ineffective panicles. The morphological development of rice can be divided into three growth phases namely, vegetative (germination to panicle initiation), reproductive (panicle initiation to flowering) and Ripening (flowering to mature grain stage) (Maclean et al. 2002, IRRI 2007) (Table 1.4).

Table 1.3: Growth phases and stages of rice

Growth phase	Stage
1. Vegetative	0. Germination
	1. Seedling
	2. Tillering
	3. Stem elongation
11. Reproductive	4. Panicle initiation
	5. Heading
	6. Flowering
111. Ripening	7. Milk grain stage
	8. Dough grain stage
	9.Mature grain stage

1.10.6 Cultivation of rice in Australia

Low-scale farming of rice in Australia began in the 1850's with the arrival of Chinese migrant workers during the gold rush in Queensland (Ricegrowers' Association of Australia [RGA] 2014). However, the first commercial trial began in 1906, when the Victorian Government allocated 200 acres of flood-prone area near Swan Hill on the banks of Murray River to a Japanese immigrant Jo Takasuka (Figure 1.11) (SunRice 2004, RGA 2014).

Figure 1.11: Japanese immigrant Jo Takasuka who pioneered first commercial rice cultivation in Australia and his wife Ichiko in their rice field located near Swan Hill on the banks of Murray River (Source: RGA 2014)

After facing initial problems of drought and floods, He succeeded to produce the first commercial quantities by 1914. Following the success of that project, the New South Wales Government acquired California rice seeds to be trialled at the Murrumbidgee Irrigation Area near Griffith and Leeton in 1922, producing commercial quantities by 1924 (SunRice 2004, RGA 2004). Since then, there has been other trials, notably in Northern territories (Humpty Doo), Western Australia (Camballin and Kununurra) and Queensland (Burdekin Irrigation Area), however these trials ended in failure due to soil nutrient deficiencies and unsuitable rainfall patterns (McDonald 1979). Currently, commercial rice cultivation is confined in the Murrumbidgee and Murray valleys in NSW (Figure 1.12) where more than 99 % of Australian rice is produced (Figure 1.13) (Australian Bureau of Agricultural and Resource Economics and Sciences [ABARES] 2015, NSW Department of Primary Industries [DPI] 2017).

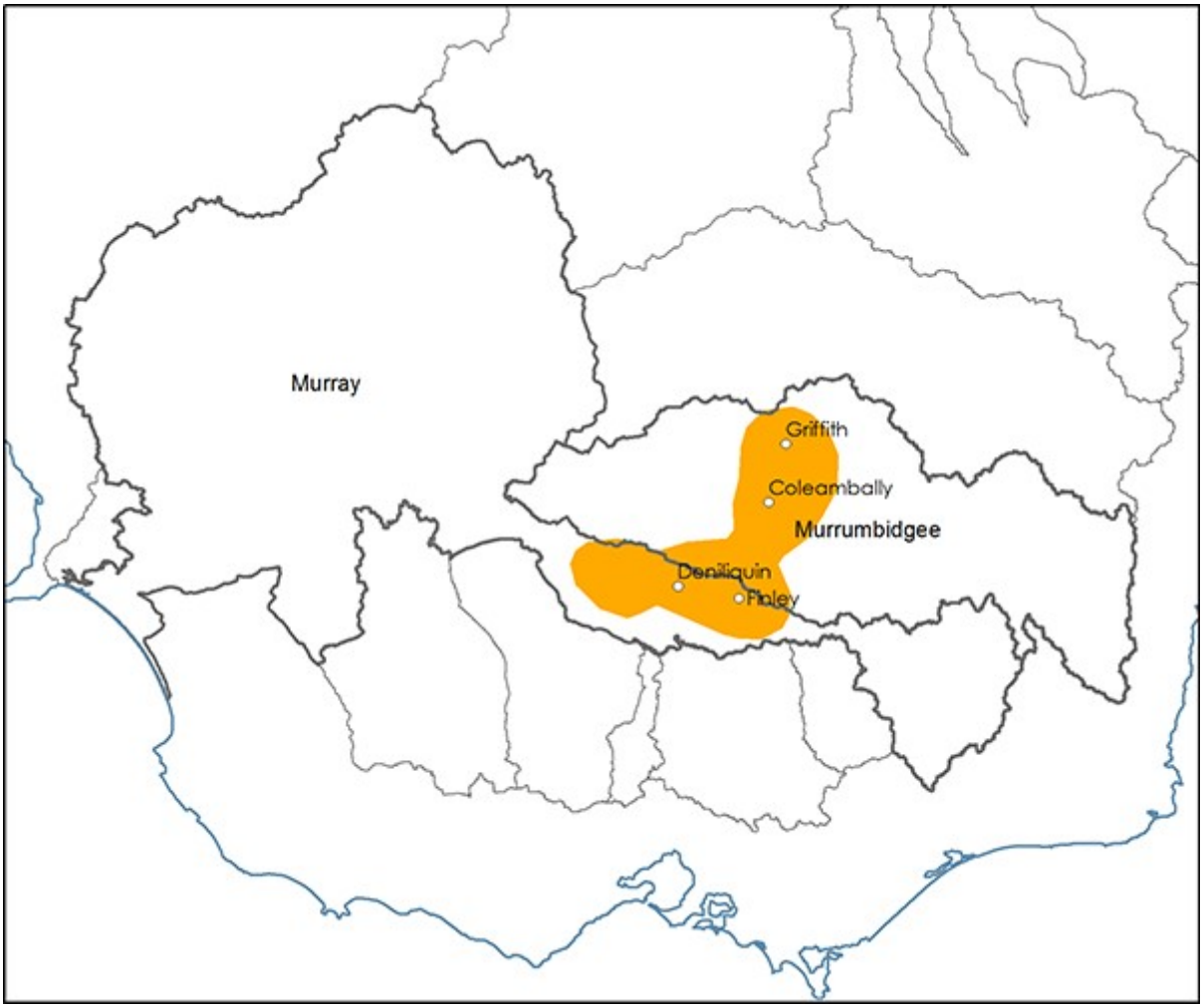

Figure 1.12: Rice growing areas in NSW (Source: ABARES Murray–Darling Basin Irrigation Survey 2015)

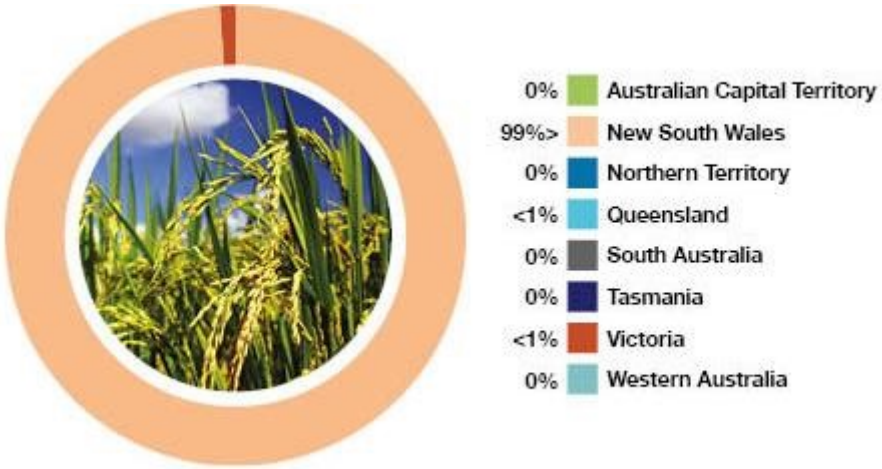

Figure 1.13: Distribution of rice-producing regions in Australia. NSW produces more than 99 % of rice production in Australia (Source: DPI 2017)

Over the years, the total rice cultivation in Australian has been fluctuating from a peak of 113,000 hectares to a very low 27,000 hectares, because of recurrent droughts, for example, rice production has significantly increased between 1980 until the year 2000, before severe droughts significantly reduced production in 2002 – 2003 (DPI 2017). In 2010, rice production increased again only to decline in 2013 – 2014 and 2015 – 2016 due to unfavourable weather conditions. Nonetheless, Australia rice production per hectare are the highest among rice growing countries at 10 – 14.5 t/h using less than 60 % water per kilogram production compared to global averages (DPI 2017). It is worth to note, that yield between the same genotypes differ from area to another within the Murrumbidgee and Murray valleys (Table 1.4) (DPI 2017). More than 80 % of rice cultivated in Australia is medium grain Japonica cultivars, while the rest are Indica varieties including Doongara (Table 1.5) (DPI 2017).

Table 1.4: Yield comparison (tonnes per hectare) between the three experimental genotypes grown in four different locations within the Murrumbidgee and Murray valleys (Source: DPI 2017)

Cultivar	Murrumbidgee Irrigation Area (MIA)	Coleambally Irrigation Area (CIA)	Eastern Murray Valley (EMV)	Western Murray Valley (WMV)
Reiziq	14.5	11.70	11.00	11.60
Koshihikari	n/a	n/a	9.20	7.6
Doongara	10.80	12.00	9.8	n/a

Table 1.5: Description of the major Australian grown rice cultivars (Source: DPI 2017)

Genotype	Description
Doongara	Semi-dwarf long grain
Koshihikari	Tall-strawed, short grain, low yielding
Reiziq	Semi-dwarf medium grain, high yielding
Kyeema	Tall-strawed, long grain
Opus	Semi-dwarf, short grain, high yielding
Amaroo	Semi-dwarf, medium grain, high yielding
Langi	Semi-dwarf, long grain
Illabong	Semi-dwarf, 'arborio' medium grain
Jarrah	Semi-dwarf medium grain, short season
Quest	Semi-dwarf medium grain, short season

1.11 Problem statement, research questions and objectives

The cultivated rice (*O. sativa*) is one of the most salt sensitive among cereal crops with a threshold of less than 4 dSm^{-1} (Munns and Tester 2008, United States Department of Agriculture [USDA] 2013). Moreover, in *O. sativa*, the sodium uptake into the shoots is relatively very high under saline conditions (Yeo et al. 1987, Yadav et al. 1996, Ochiai and Matoh 2002). Unlike the salt-sensitive commercial varieties (Indica and Japonica), some traditional rice landraces (e.g. Pokkali) have shown the capacity to reduce their uptake of toxic sodium chloride ions by maintaining a favourable cytosolic (Na^+: K^+) ratio (Greenway and Munns 1980, Gorham et al. 1987, Maathuis and Amtman 1999, Chen et al. 2008). However, a wild rice *O. coarctata* which grows in coastal estuaries of India, Pakistan and Bangladesh shows to tolerate inundation of sea water of 20 to 40 dSm^{-1} twice a day (Bal and Dutt 1986,

Sengupta and majumder 2010). Thus, to address the increasing salinity problems and the impending global food shortage, there is an urgent need for agricultural scientists and rice researchers to develop rice varieties that can tolerate higher salinity levels. Most previous attempts were concentrated on understanding the mechanisms of sodium uptake by the roots and delivery to the shoots (Ashraf and Akram 2009). Although some improvements were made, researchers were unable to produce a rice variety that can tolerate more than 10 dSm^{-1} of salinity to date (Ashraf and Akram 2009). Since physiological and molecular responses of rice to salinity stress are multifaceted and complex, the development of salt tolerant rice has become a difficult task to design and interpret (Gregorio et al. 2013). This is supported by similar studies (Moeljopawiro and Ikehashi 1981, Bartels and Sunker 2005, Chinnusamy et al. 2005, Sahi et al. 2006).

Mechanisms found to be influencing salinity tolerance in rice such as Na^+ uptake restriction and exclusion from shoot have been reported in many studies (Munns and Tester 2008, Platten et al. 2013, Ismail and Horie 2017). Similarly, a considerable amount of literature has been published on genes conferring salinity tolerance via regulating growth accelerators, osmoprotectants and ion movement (Munns and Tester 2008, Horie et al. 2012, Ismail and Horie 2017). Based on the results of these studies, salinity tolerance genes (*Saltol*) and suitable donors for these agronomically desirable traits have been identified (Garg et al. 2002, Islam et al. 2008). However, understanding the role of these mechanisms and their precise functions at the cellular, molecular and at the whole plant level under controlled condition and in the field, conditions are yet to be determined (Radanielson et al. 2018). Therefore, exploring the morphophysiological response of cultivated rice genotypes at different levels within the plant may contribute in speeding the efforts for developing high-yielding salt-tolerant rice varieties. Moreover, screening and breeding crops for salinity tolerance may contribute in the

identification of new salt tolerant crops that will help turn marginal and salinised lands into a productive farmland.

The aim of this project is to test the effects of salinity stress on various morpho-physiological parameters including photosynthesis activities, net fluxes of basic ions, biomass, plant height and tiller count of three commercially grown Australian cultivars viz, Doongara (Indica variety) and Reiziq and Koshihikari (Japonica varieties) which could reveal their tolerance status as well enhancing our knowledge of salinity tolerance mechanisms employed by rice genotypes with different genetic backgrounds.

Therefore, this study poses the following research questions:

1. Are there genotypic differences in salinity effects on morpho-physiological parameters of the three Australian rice cultivars?
2. Are there correlations between the controlled environment (glasshouse) and the field for leaf photosynthesis and ion fluxes of the three genotypes in response to salinity stress?

These research questions will be answered by pursuing the following objectives:

1. To investigate and assess morpho-physiological response at the cellular, tissue and the whole plant level of three different rice genotypes to salt stress.
2. To test the correlation between phenology, photosynthesis and ion fluxes of both Control and treatment.

1.12: Justification for Methods

Improving yield and increasing resistance to abiotic stresses are some of the parameters desired to achieve when screening salinity tolerance traits for plants. However, the conventional screening methods used in previous attempts have seen little success (Ashraf and

Akram 2009, Hauser and Horie 2010). In addition, traditional screening methods such as backcrossing are a time-intensive process that may take 10 – 15 years to develop a variety (Ashraf and Akram 2009). Another drawback for conventional backcrossing is the "linkage drag", where undesirable genes are transferred into the novel variety (Ashraf and Akram 2009). Recently, Marker Assisted Selection (MAS) techniques have been employed to boost the salinity tolerance of some crops; however, due to the difficulty of identifying the key genes underlying the Quantitative trait loci, the transfer of salt tolerant genes is often associated with unwanted traits from the donor. Therefore, to circumvent some of the above-mentioned shortcomings, this study used morpho-physiological methods including gas exchange measurements (Chen et al. 2005), microelectrode ion flux estimation (MIFE) (Shabala et al. 1997, Newman 2001, Shabala 2003, Chen et al. 2008), and agronomic measures (Chen et al. 2008) to determine the effects of salinity stress on key morpho-physiological parameters of three different rice genotypes two Japonica and one Indica cultivars. In the last two decades, numerous studies have used this non-invasive ion flux technique to explore plant responses to varying stresses including salinity stress (Shabala 2000, Babourina et al. 2001, Shabala and Van Vollkenburgh 2003, Chen et al. 2005, Pang et al. 2006, Chen et al. 2007, Chen et al. 2008), chilling (Shabala and Newman 1997, Shabala and Shabala 2002), plant injury (Hush et al. 1992), ROS generation (Demidchik et al. 2003) and osmotic stress (Shabala and Newman 1998, Shabala et al. 2000, Shabala and Lew 2002).

2: Materials and methods

2.1: Rice varieties

Koshihikari is a Japonica rice variety (*O. sativa*) that is widely grown in many countries including Australia, Japan and the United States of America. It is tall-strawed, premium short grain with good favour but low yielding when compared to other high yielding Japanese rice varieties (Koga et al. 1987, Uehara et al. 1995, Yamauchi 2001). However,

Koshihkiari's nitrogen use efficiency is higher than that of Indica─IR64 and is cold tolerant (Namai et al. 2009, Sawada and Kohno 2009, Champagne et al. 2010, Miyamoto et al. 2012).

Doongara is a premium, long grain, semi-dwarf Indica rice cultivar exclusively developed and grown in Australia (SunRice 2004). Launched in 1998, along with Koshihikari, it is tropical rice growing genotype which is sensitive to low temperatures. According to a study conducted by the Rural Industries Research and Development Corporation [RIRDC] (2002) on cold tolerance in rice, found that at low temperature of 18ºC/day and 13 ºC/night, Doongara produced 17 % less biomass compared to its control variables.

Reiziq is premium, long grain with a high market value, mainly exported to the Middle Eastern countries. This variety has the highest yield among the three experimental genotypes, at more 14.5 t/h compared to Doongara and Koshihikari at 12 and 9.20 t/h respectively (DPI 2017)

2.2: Glasshouse experiments

Pot experiments were carried out in S 35 greenhouse at the Western Sydney University, Hawkesbury campus (33.62º S, 150.75º E) between September 2017 and December 2017. Temperature was adjusted to 28º C/22º C day/night with supplemental lights provided by four 400 W sodium lamps (General Electric Lighting, Smithfield, NSW, Australia) with timers set to provide 16 hours of light and 8 h of dark. Humidity was kept between 70 – 75. For continuous monitoring and recording of temperature and relative humidity, a data logger (Tinytag TGP – 4500, Hastings Data Loggers, Port Macquarie, NSW, Australia) was installed at the start of the experiments (see appendix 4, Figures A10).

Seeds of three rice genotypes (*Oryza sativa* L. Doongara, Koshihikari, and Reiziq) were kindly supplied by SunRice, Leeton NSW, Australia. Seeds were surface sterilized in 4% bleach solution for 10 minutes and thoroughly rinsed with deionised water. Seeds were then

placed in a conical flask wrapped with aluminium foil, then filled with deionised water and kept in an Orbital Mixer Incubator (Ratek Instruments Pty Ltd, Boronia, Victoria, Australia) at 28º C until the radicle was visible. As a precaution, water was replaced every day to prevent rotting and contamination. The germinated seeds were sowed in a sandy soil until the emergence of the fully expanded second leaf. Seedlings were then transferred to 9 L bucket filled 6 litres of soil media consisting of 70 % loamy sand and 30 % potting mix. A total of 72 pots were arranged in a randomised complete block design with each cultivar represented by 12 control pots (0 NaCl) and 12 treatment pots (100 mM NaCl). Initially, four biological seedling replicates were sown in each pot for each cultivar, which were later thinned to the two healthiest seedlings per pot after three Weeks of the date of transplant (DAT). To acclimatise and prevent shock plants were grown for a further 7 days before Aquasol fertilizer (Yates, Padstow, NSW, Australia) consisting of (N: P: K: 23:3.95:14) was incorporated into the soil (1:100). A supplement of Manutec Trace elements (Manutec Manufacturing Technologies, SA, Australia) containing Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Sulphur (S), Zinc (Zn), Boron (B), Copper Cu), and Molybdenum (Mo) was also added. Water level was kept 1 cm above the soil and continued watering with tap water daily for eight Weeks. At the reproductive phase additional NPK was also administered before the start of salinity application. Salinity was added incrementally for 7 days before reaching the required target of 100 mM NaCl at a dose of 0.80 g of NaCl was for each pot. The EC of the water and soil was also measured before and after salinity each application.

2.3: Field experiments

Field experiments were conducted in an experimental plot specifically assigned for this study at the Western Sydney University horticultural field, Hawkesbury Campus (33.62º S, 150.75º E) between January 2018 to May 2018. Field preparation began with digging two parallel trenches L10 × W2 × D1.5 metres with the help of Backhoe Loader hired for this

project (see appendix Figure A2, A3 and A4). After the completion of the trenches, a double layer 100-micron thick black plastic sheeting were lined in both trenches to prevent water seepage, sodium chloride leaching to the soil and to maintain water and nutrient levels constantly (see appendix, Figure A4). The treatment plot was chosen according to field gradient to prevent unexpected heavy rainfall run-off reaching the control plot. Each plot was divided into three equal sections and randomly assigned three replicates of each genotype. Seeds were germinated following the same procedure used for pot experiments. A basal fertilizer consisting of nitrogen (60 kg ha^{-1} N as urea), phosphorous (30 kg ha^{-1} P as single superphosphate), potassium (40 kg ha^{-1} K as potassium chloride) and zinc (5 kg ha^{-1} as zinc sulphate heptahydrate) were added 24 h before transplanting. Seedlings were transplanted 14 days after germination in the field at a spacing of 0.2 × 0.2 m with two seedlings per hill. A further two doses of 45 kg/ha^{-1} of nitrogen was also administered at mid-tillering and at panicle initiation. Pest and weed control management was also routinely carried out during the experiment. Salt application commenced 28 days after transplant with an incremental dose of 6 – 8 kg per day. A total of 40 – 45 kg of premium refined salt (Cheetham Salt, Melbourne, VIC, Australia) was used until the target of 100 mM NaCl was reached.

Table 2.1: Shows field plot layout and the random allocation for each cultivar.

	Control	Salinity
Block 1	Koshihikari	Reiziq
	Doongara	Koshihikari
	Reiziq	Doongara
Block 2	Doongara	Reiziq
	Koshihikari	Koshihikari
	Doongara	Doongara
Block 3	Reiziq	Koshihikari
	Koshihikari	Reiziq
	Koshihikari	Doongara

2.4: Measurement of morphological parameters and agronomical traits

Plant heights were measured Weekly starting from the date of transplant for 4 Weeks before salinity treatment commenced. During the first four Weeks, measurements of ten replicates from the 3 genotypes were taken. The same procedure was repeated for the following 4 Weeks for both the non-stressed (0 NaCl) and stressed (100 mM NaCl). Tiller count and biomass were recorded after harvesting the plant 8 weeks after transplanting. For biomass and tiller count, ten randomly selected hills for each genotype were hand-harvested and weighed as fresh weight. Plants were then kept in paper bag and oven-dried at 60º C for 72 hrs. The dried biomass was then weighed and recorded as dry weight, whereas the tiller numbers on the primary, secondary and the main stem were separated and recorded as tiller numbers.

2.5: Measurement of photosynthetic parameters

Measurements of photosynthetic parameters such as photosynthesis rate (P_N), stomatal conductance (gs), transpiration rate (Tr), and intrinsic water use efficiency (WUEi) as outlined in (Mak et al 2014, Liu et al. 2017) were carried out in controlled conditions on young fully extended first leaves of three rice genotypes. The temperature and relative humidity in the measuring chamber were maintained at 30º C and 70% respectively. While the light intensity was set at 1000 PAR m^{-2} s^{-1} at the leaf surface. Flow rate and CO_2 mixer references were set at 380 and 400 ppm respectively. The photosynthetic parameters of three cultivars were measured using (LI-COR 6400XT, Lincoln, Nebraska USA) infrared gas analyser (IRGAs) with LCF leaf chamber which provides 0 to 2500 µmol m^{-2} s^{-1}, on both glasshouse and field trials four Weeks after transplanting (Figure 2.1). Measurements of 8 replicates of each genotype were performed on control plants (0 NaCl) and the exposure variables (100 mM NaCl) between 9:00 to 11 am, during the peak of photosynthetic photon flux density, and just before air temperature becomes a limiting factor for C_3 photosynthetic-mechanism (Feistler and Habermann 2012). In

this experiment, LI-COR 6400 protocols described in Evans and Santiago (2014) were used to operate the instrument and measure the photosynthetic parameters of the three rice cultivars.

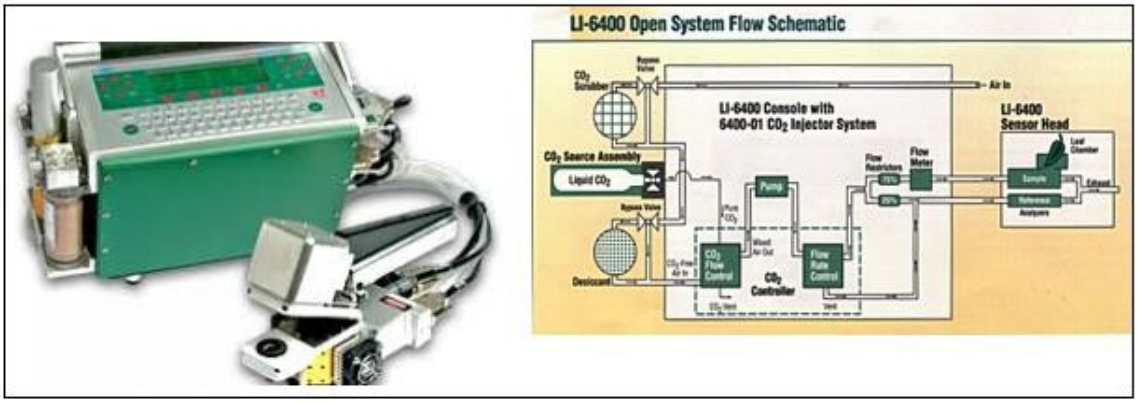

Figure 2.1: LI-COR 6400 XT portable photosynthesis system unit and system flow schematic chart (Source: https://www.licor.com/env/products/photosynthesis/LI-6400XT/)

2.6: Ion flux measurements

Net fluxes of basic ions such as Na^+, Cl^-, K^+ and H^+ were measured using microelectrode ion flux estimation (MIFE) (Figures 2.2 and 2.3) (the MIFE^{TM} technique; University of Tasmania, Hobart Australia) outlined in (Shabala et al.1997, Newman and Morris 1997, Shabala and Newman 1999, Shabala 2000). Borosilicate glass capillaries (GC150-10, Harvard Apparatus, Kent, UK) were pulled using Flaming Brown Micropipette Puller (Model P-78, Sutter Instruments, Novato, CA, USA), then baked for 5 hours at 230ºC before silanising with 70µL of tributylchlorosilane (Sigma-Aldrich, Castle Hill, Australia). The objective behind silanising is to create a hydrophobic surface ensuring LIX cocktail is confined inside the tip of the electrode (Newman 2001). The blank electrodes were then removed from the oven and allowed to cool before being back-filled with specified solution listed in Table 2.2. The electrode tips were then filled with Liquid Ion exchanger (LIX) ionophores cocktails (Fluka, Buchs, Switzerland) shown in Table 2.2. Reference electrodes were prepared by immersing pulled Borosilicate glass capillary (GC150-10) with KCl in 2 % agar solution, air bubbles were removed by shaking before solidifying the electrode in the freezer. A silver (AgCl) coated wire was then oxidised in a chlorine-based household bleach for 24 hours. The microelectrodes used

for this experiment comes with a resistance of between 0.5 – 5GΩ. The bleached electrode was then inserted into the agar-filled electrode before wrapping it with a paraffin film. The top end of the wire was left uncovered for better conductivity and contact with the MIFE reference port. The tip of the four electrodes were then carefully trimmed under a microscope to a diameter of 2 – 3 µm as outlined in Chen et al. (2007). The ion selective electrodes were then attached to the manipulator and focused using the attached microscope giving the electrodes a space of between 2 - 3µ.

The four electrodes were then calibrated in a buffer solution K^+ (KCl 1000 µM, 500 µM, 200) while Na^+ and Cl^- (NaCl 200, 500 and 1000 µM) and pH from 7, 6 and 5. Any electrode with a response of < 50 mV and a correlation value of < 0.999 was not used in this experiment. Leaf segments were excised from flag leaf of NaCl treated plants and immediately bathed in a MIFE standard solution (0.1 mM $CaCl_2$ + 0.5 NaCl). Small segments of about 6 – 8 mm were cut to expose the mesophyll as described in (Shabala and Shabala 2002) and positioned in a Perspex chamber filled with a MIFE standard solution (Figure 2.4) and mounted into the manipulator after incubating the sample in the same solution for about 50 - 60 minutes. The distance between the sample was set at 40 µm from the surface of the leaf. To start measuring the ion fluxes, the manipulator motor was turned on moving the electrodes in 10 second cycle from position A to B, with the difference used to convert to electrochemical potential by applying the Nernst slope of the calibrated electrodes.

Table 2.2: Fabrication profiles of ion-selective microelectrodes used in MIFE experiments

Measuring Ion	LIX (Fluka catalogue number	Back-filling solution (mM)	Calibration range
Na+	71178	500 NaCl	0.2-0.5-1.0 mM
Cl-	24902	500 NaCl	0.5-1.0-5.0 mM
K+	6003	200 KCl	0.1-0.2-0.5 mM
H+	95297	15 NaCl + 40 KH_2PO_4	5.1-6.4-7.8 pH

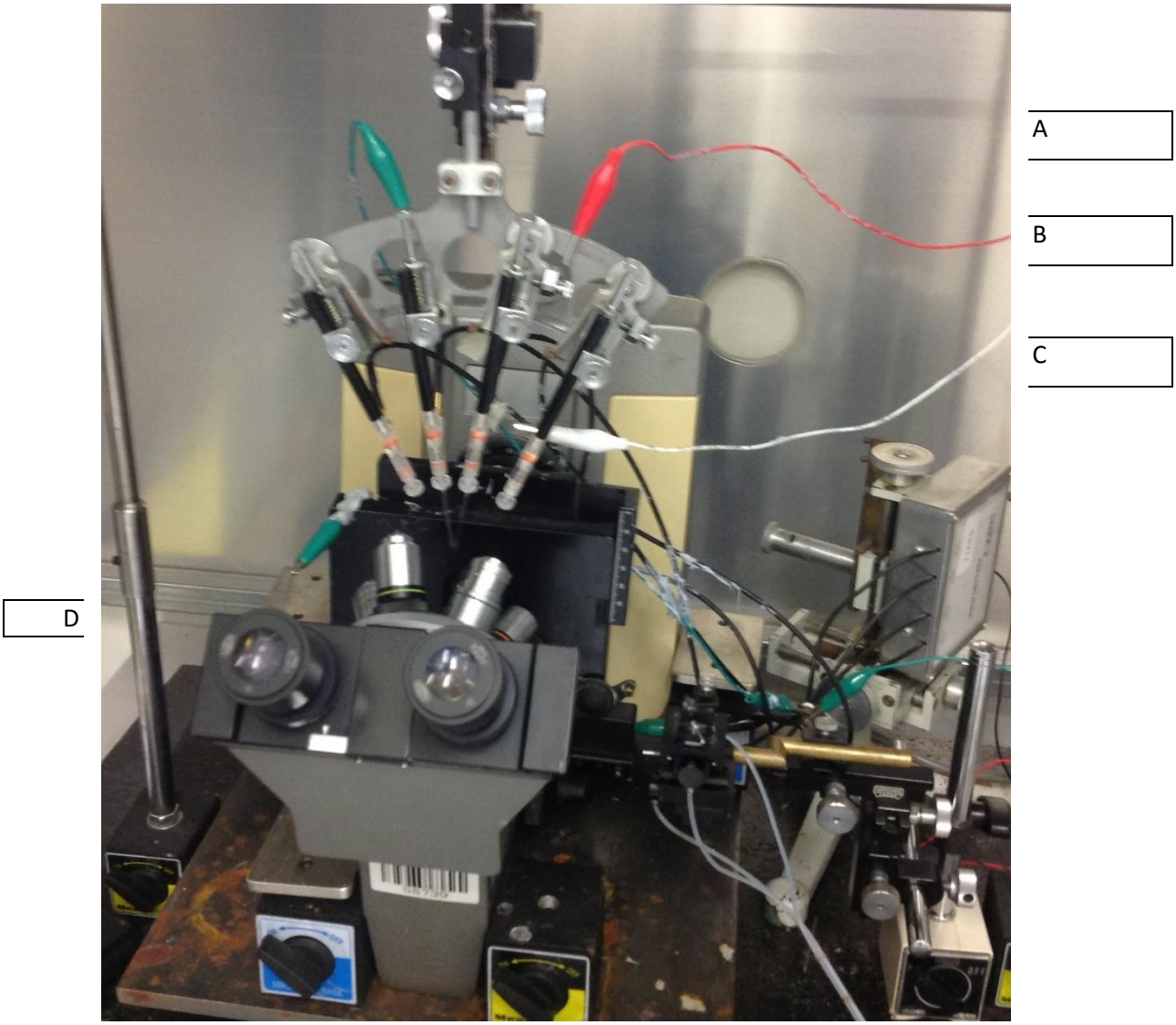

Figure 2.2: MIFE unit with four electrodes attached (A), reference electrode (B), Microscope (C) and the stepper motor (D).

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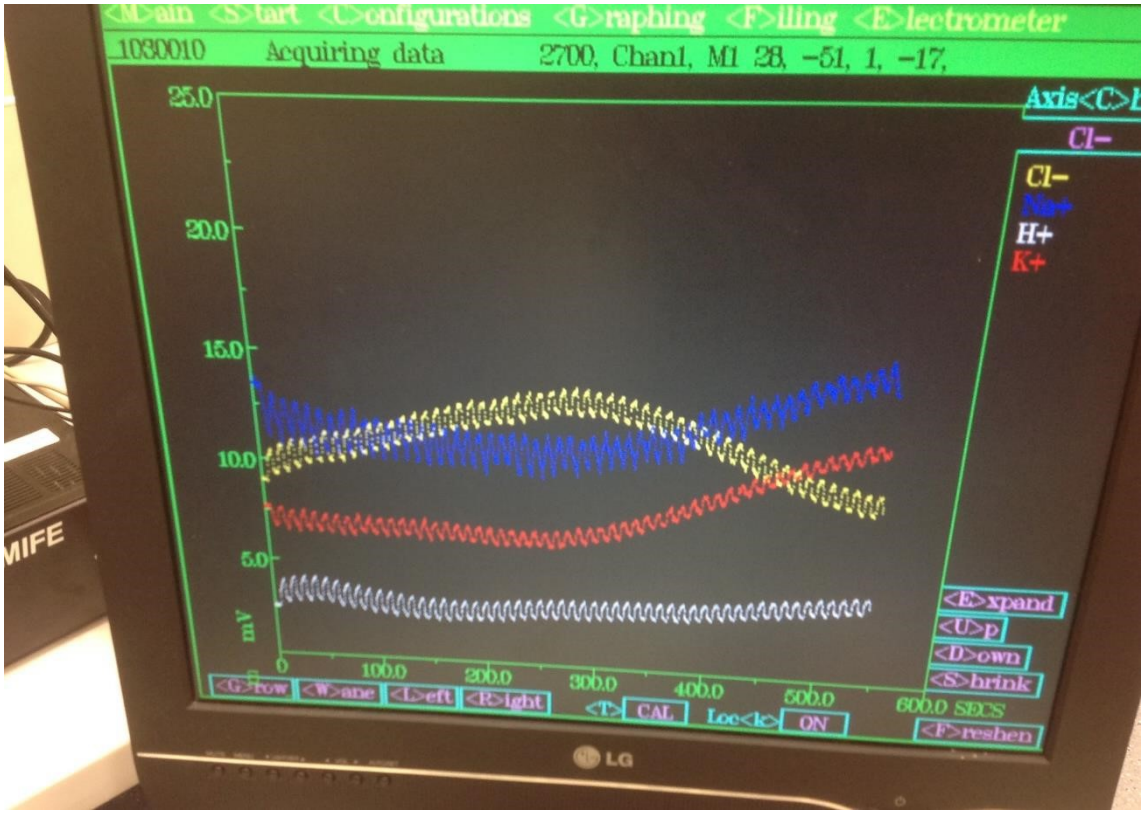

Figure 2.3: MIFE screen interface displaying changes in fluxes of four basic ions. Yellow represents Cl^-, blue represents Na^+, red represents K^+, whereas white represents H^+.

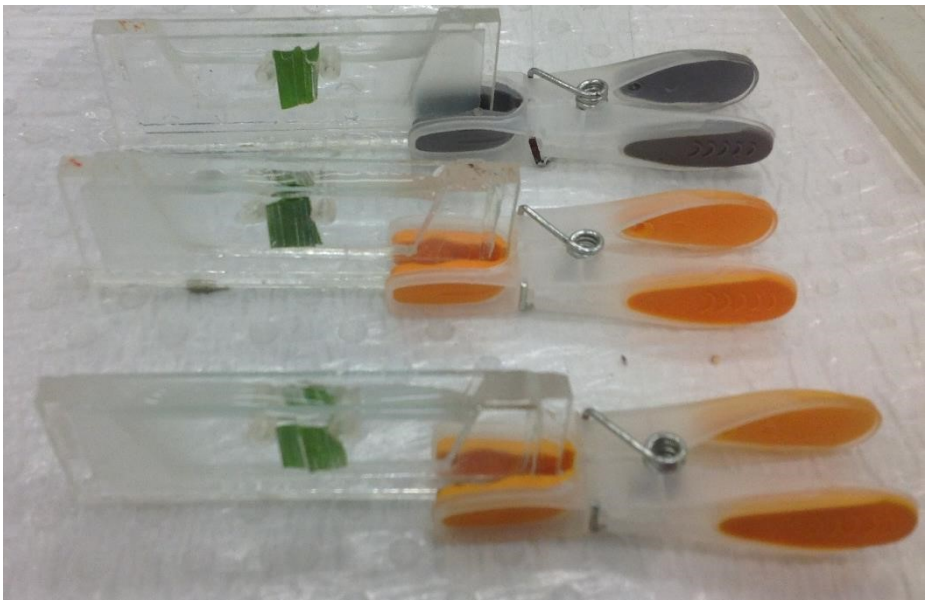

Figure 2.4: Rice leaf samples in Perspex chamber filled with MIFE standard solution.

2.7: Statistical analysis

Statistical analyses were performed using R version 3.4.2 (The R foundation for Statistical Computing, 2017). Linear models (*lm*) specifying the parameter of interest were used to test for main effects and interactions using two-way or three-way ANOVA when applicable. Image outputs were generated using '*ggplot2*' and '*ggpubr*' packages. Means were ranked using Tukey's post hoc at alpha equals 0.05. The strength of linear correlations (r) alongside with respective p-values are presented when applicable.

3: Results 3.1: Effects of salinity on plant phenology in both glasshouse and field

As mentioned earlier, salinity may inhibit plant development in two ways; first, salinity reduces plant capacity to take up water from the soil, which is termed as osmotic stress. Second, accumulation of excessive salinity may injure cells thus creating a condition known as ionic effects or ionic stress thereby hindering photosynthesis and leading to reductions in growth, leave senescence and death (Greenway and Munns 1980). In this study, the accumulation of toxic NaCl in rice cells which manifested in the form of chlorosis, leaf senescence, necrosis and death in older leaves observed in the treatment groups (Figures 3.1, 3.2, 3.3, 3.4 and Appendix 3, Figures A5 – A9). Furthermore, both FW and DW were greatly reduced by salinity stress among the three cultivars, irrespective of where grown in the glasshouse or in the field (*p <0.0000*, Table 3.1, Figure 3.7 B, Figure 3.8 B). At both the control and treatment groups, Doongara and Koshihikari have significantly higher DW in comparison to Reiziq (Figure 3.7 B, Figure 3.8 B).

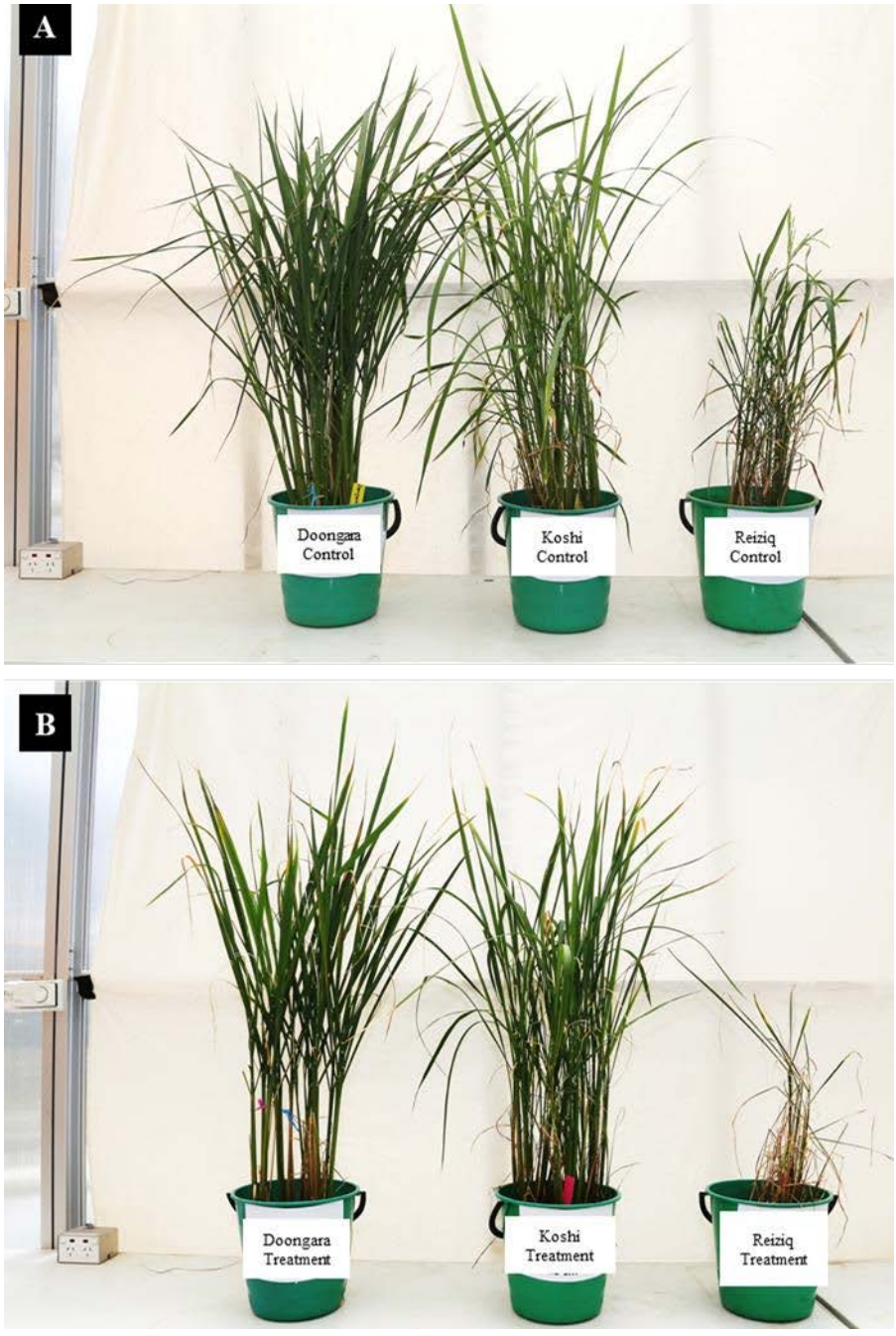

Figure 3.1: Comparison between the control group (A), and the treatment variables shown in (B) three Weeks after 100 mM NaCl treatment. Note, the yellowing and drying of leaf-tips in the treatment, while the control shows no leaf die-backs.

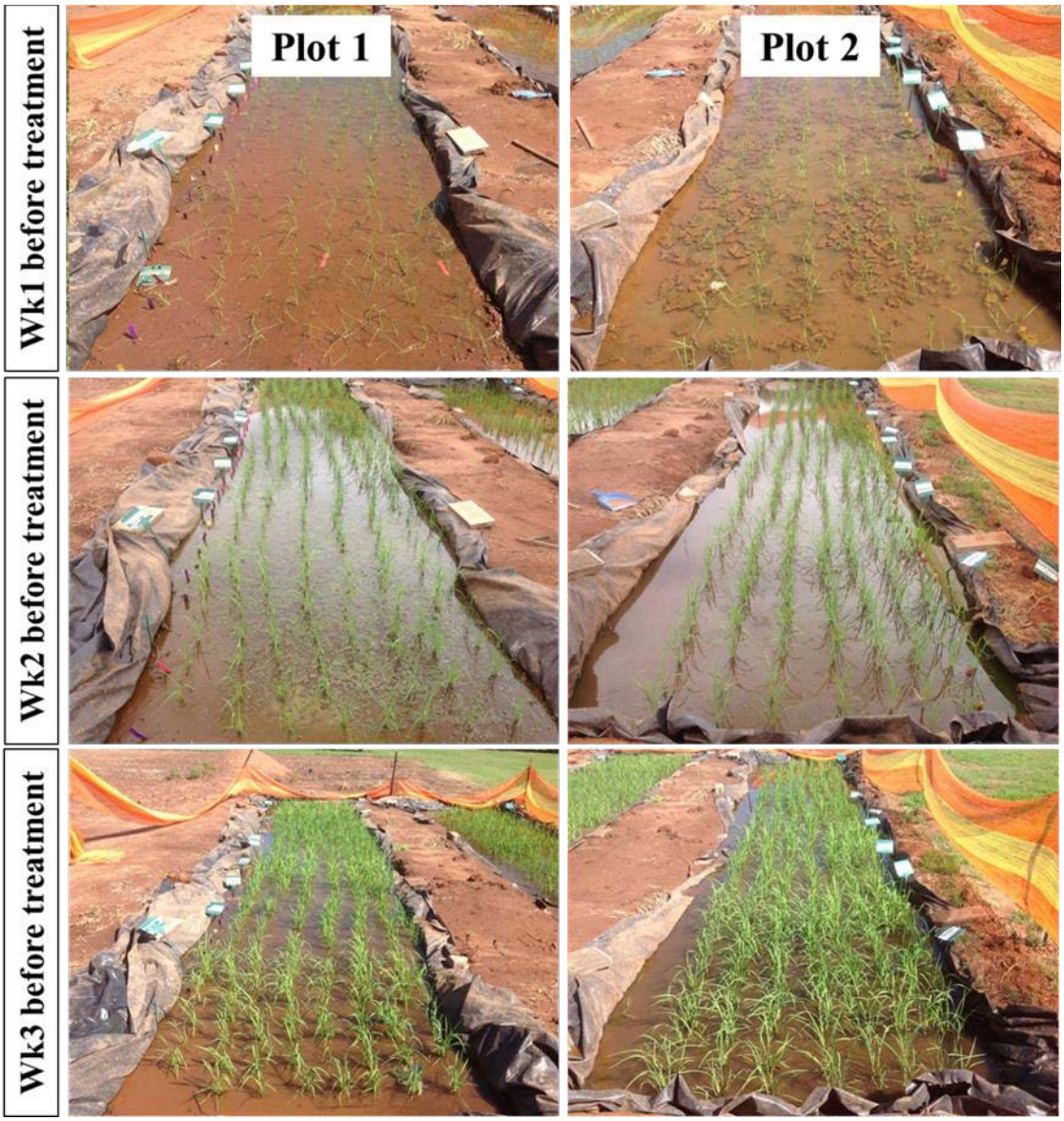

Figure 3.2: Growth performance of the three seedlings before salinity application. Plot one is designated for control, while plot 2 is designated for treatment variables.

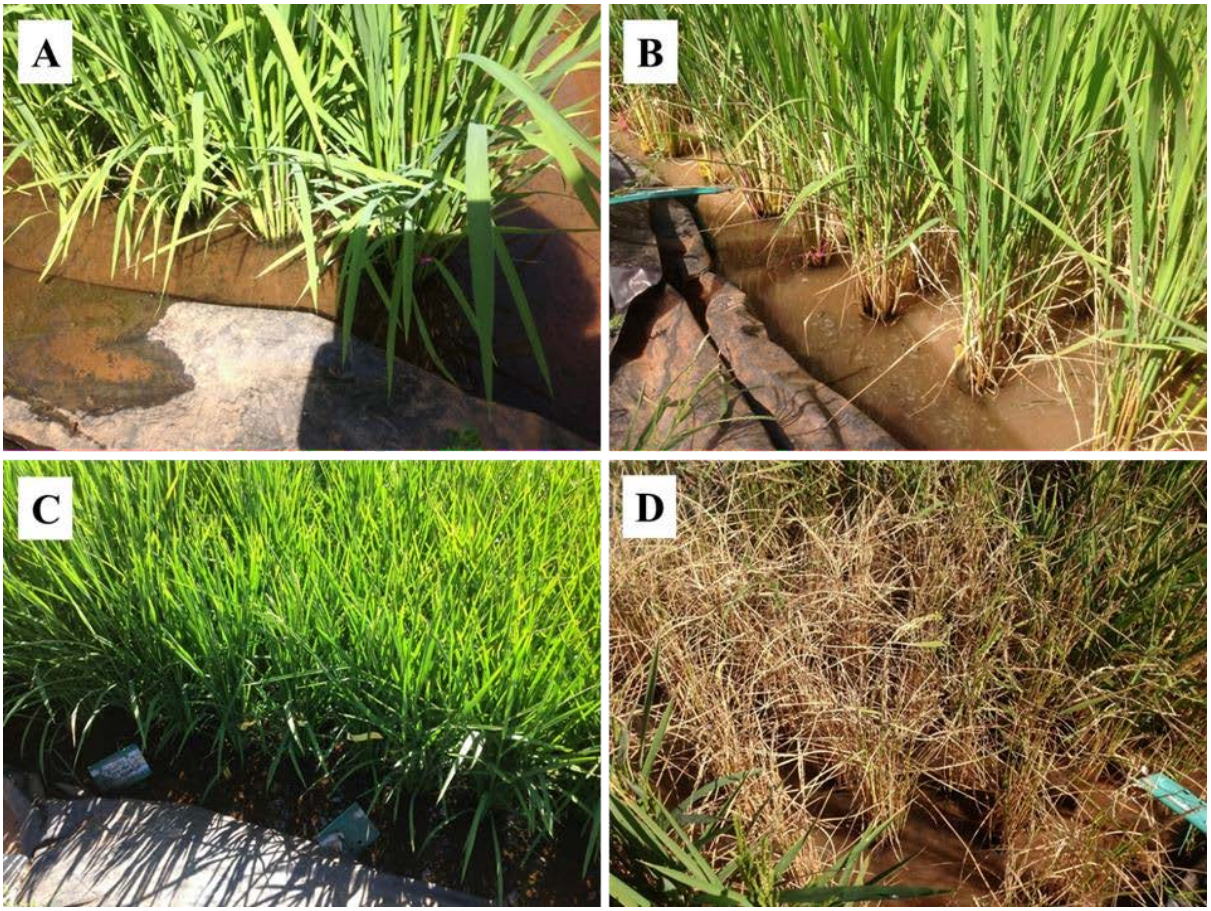

Figure 3.3: The effects of salinity stress. (A) represents control plot, note the vigour and colour. (B) represents the effects of salinity stress on the second Week of salinity exposure. (C) shows the control plot in Week 5, (D) shows the effects of salinity, note the extensive leaf damage caused by the salinity stress in Week five of the salinity exposure. The green leaves at the bottom of D is from the adjacent control plot.

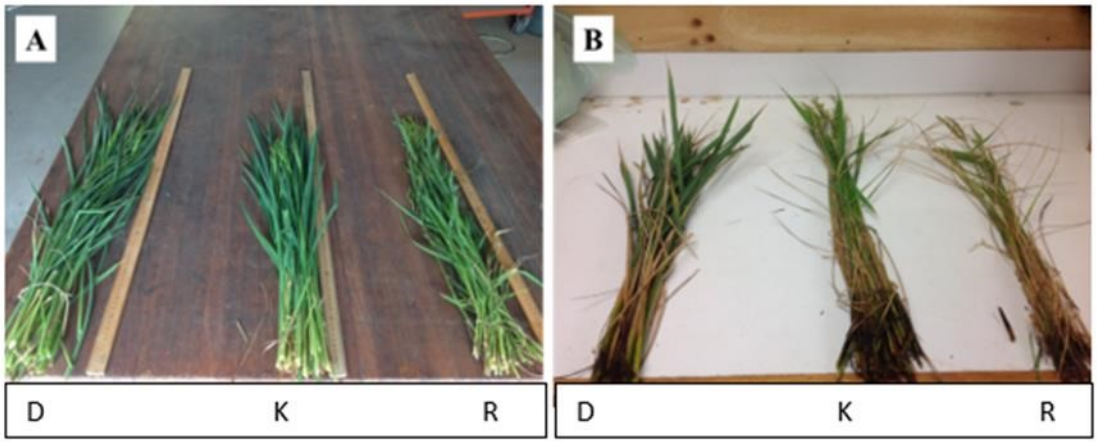

Figure 3.4: Comparison of salinity treated rice cultivars and their control. (A) represents the control group of the 3 genotypes grown in the field. (B) shows the effects of 100 mM NaCl on the 3 genotypes. D represents Doongara, K represents Koshihikari, while R represents Reiziq.

3.1.1: Fresh weight

Salinity treatment generally reduced the fresh weight among all cultivars (*p<0.0000*, Table 3.1, Figure 3.5 A, Figure 3.6 A) grown in both glasshouse and field conditions. Grown, Doongara and Koshi have significantly higher FW than Reiziq while Doongara has the highest FW during salinity stress and Reiziq consistently score the lowest (Figure 3.5 A). On the other hand, field grown control Doongara had the highest fresh weight followed by Koshi then Reiziq (Figure 3.6 A). At saline conditions Koshi's fresh weight was the lowest compared to the other two cultivars. Treatment: cultivar interaction is significant suggesting that FW response to salinity treatment is different among cultivars (*p=0.0366, p<0.0000*, Table 3.1). Scatterplot for FW grown at glasshouse and field conditions showed strong linear correlation (*r=0.76, p<0.0000*, Figure 3.7 A).

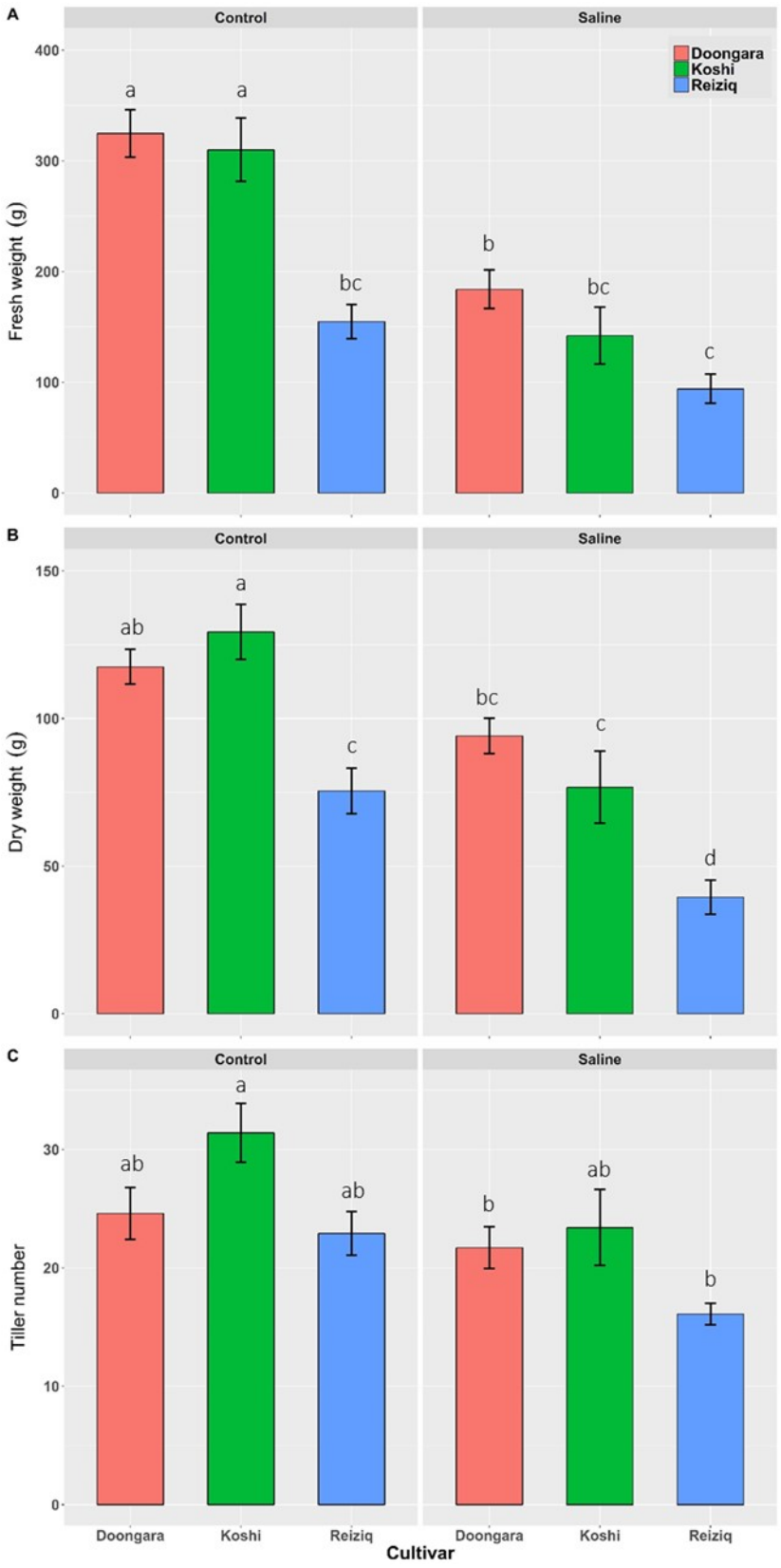

Figure 3.5: FW biomass in grams (A), DW in grams (B) and tiller numbers (C) of rice genotypes grown in the glasshouse. Letters above each bar represent ranking of means within each cultivar. Means are compared using Tukey's post-hoc at $\alpha = 0.05$

3.1.2: Dry weight (g)

Similar to FW, salinity treatment generally reduced the dry weight among all cultivars regardless whether grown in the filed or glass house (*p<0.0000*, Table 3.1, Figure 3.5 B, Figure 3.6 B). At control and saline conditions, Doongra and Koshi have significantly higher DW than Reiziq (Figure 3.5 B, Figure 3.6 B). Treatment: cultivar interaction is not significant among glass house grown cultivars (*p=0.2088*, Table 3.1, Figure 3.5 B) suggesting that DW response to salinity treatment is similar among cultivars. However, field trial shows lower dry weight for

Koshi more than Reiziq and Doongara demonstrating a cultivar-specific interaction (*p<0.0000*, Table 3. 1, Figure 3.6 B). Scatterplot for DW grown at glasshouse and field conditions showed moderately strong linear correlation (*r=0.59*, *p<0.0000*, Figure 3.7 B).

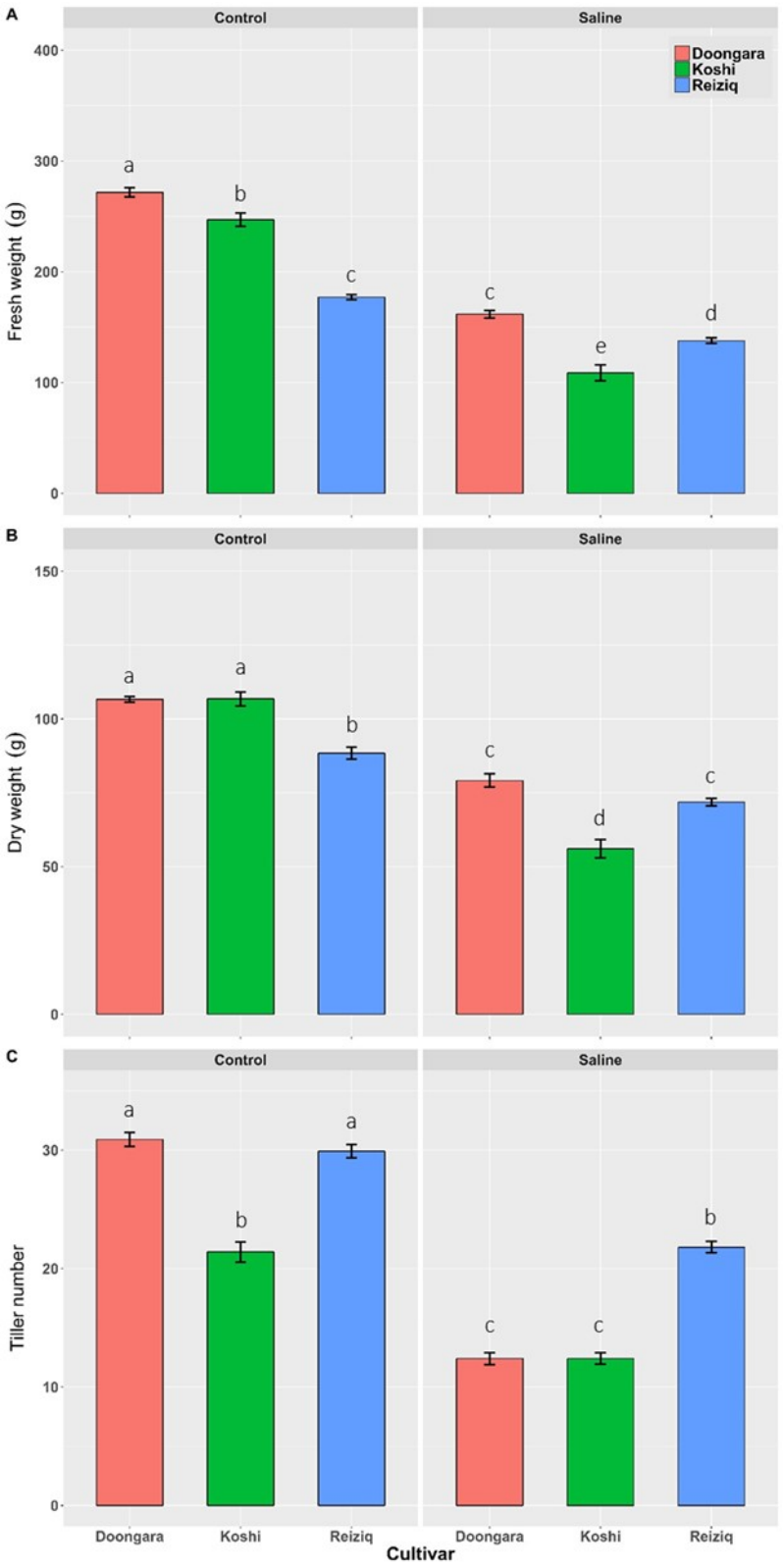

Figure 3.6: FW Biomass in grams (A), DW biomass in grams (B) and tiller numbers of the three rice genotypes grown in the field. Letters above each bar represent ranking of means within each cultivar. Means are compared using Tukey's post-hoc at $\alpha = 0.05$.

3.1.3: Tiller numbers

The response of tiller number (TN) grown in glass house and field showed stark variation (Figure 3.5 C, Figure 3.6 C). While all cultivars decreased TN due to salinity ($p<0.0000$, Table 3.1, Figure 3.5 C, Figure 3.6 C), field-grown cultivars show a treatment: cultivar interaction ($p<0.0000$, Table 3.1) while no significant interaction was observed in glasshouse grown cultivars ($p=0.4791$, Table 3.1) suggesting that cultivars respond differently to the niche they are grown to. The scatterplot for TN grown at glasshouse and field conditions showed no linear correlation ($r=0.044$, $p=0.74$, Figure 3.7 C) which further supports the observed response to growth conditions. Interestingly, Reiziq grown in the field during salinity stress had the highest TN at salt treatment and is similar to Koshi at control conditions (Figure 3.6 C).

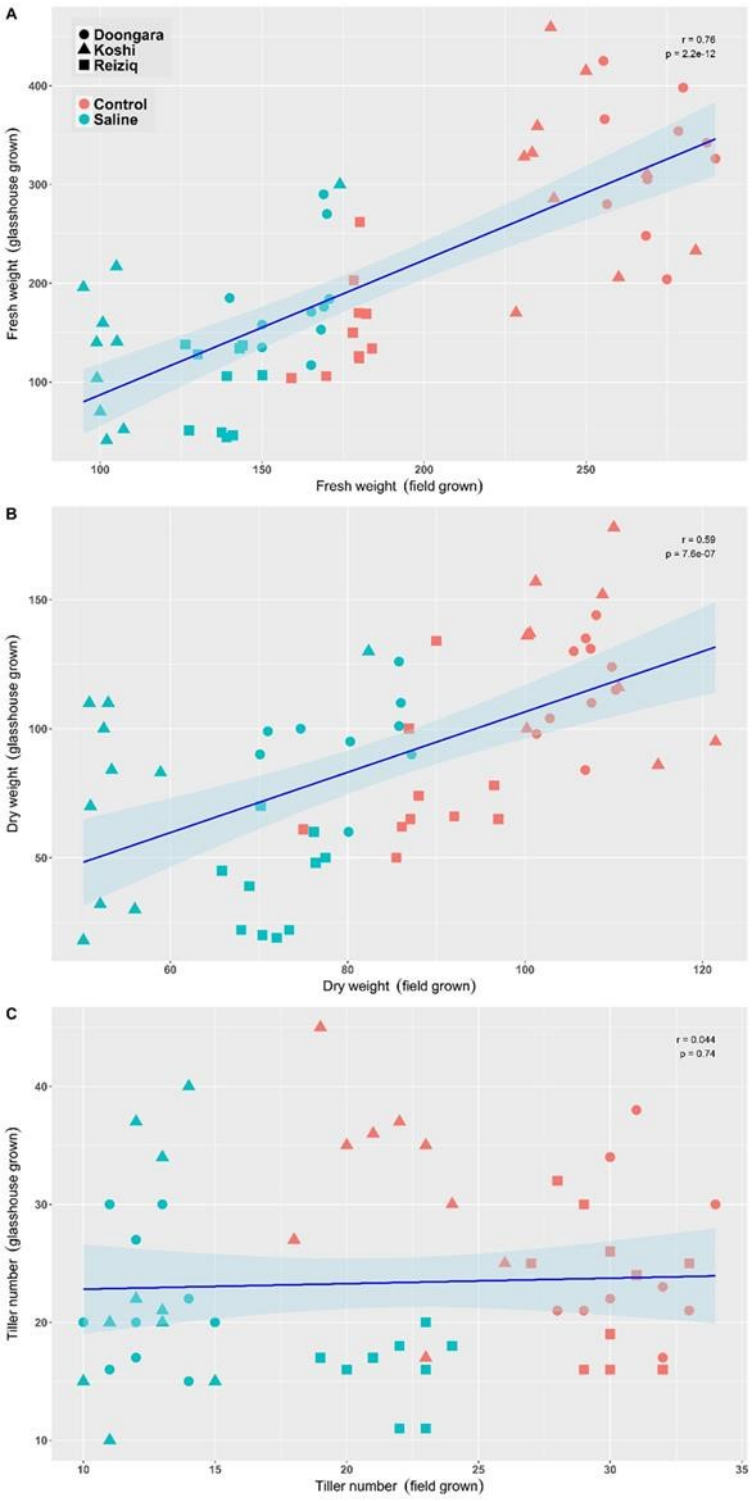

Figure 3.7: The relationship between FW (A), DW (B) and tiller numbers (C) of the three rice genotypes grown in glasshouse and in field conditions. The scatter plot with correlation of *r=0.044* is presented in (C). The scatter plot shows no linear correlation between tiller numbers grown in glasshouse and in the field (C).

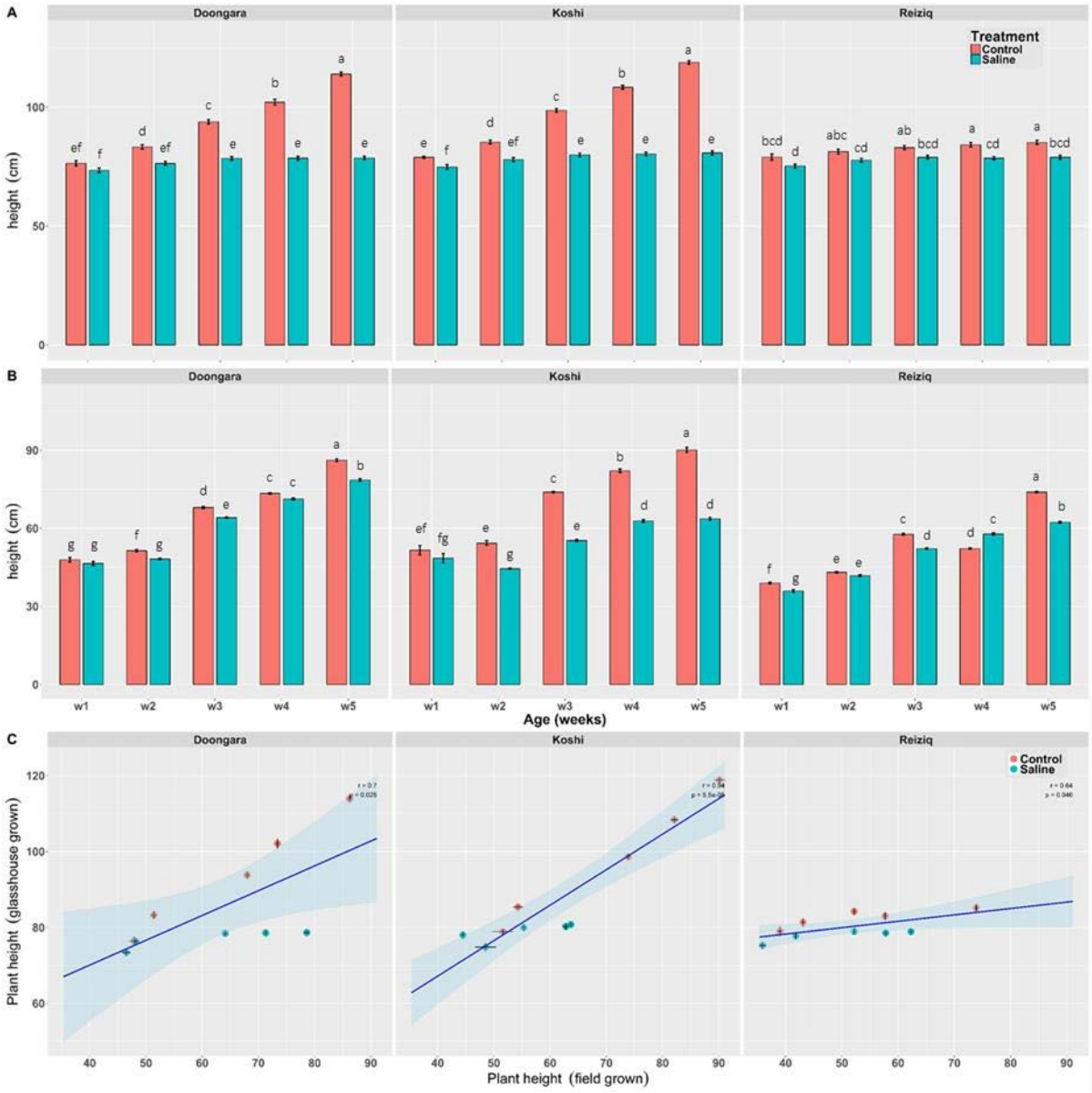

Figure 3.8: Plant height summary measured over five (5) weeks for three cultivars subjected to salinity stress and grown in glasshouse (A) and field (B). Scatter plot with correlation and p-values of (Doongara *r=0.73*, *p=0.06*, Koshi *r=0.15*, *p=0.75*, Reiziq *r=0.62*, *p=0.14*) between the glasshouse and field-grown plants are presented in C. Letters above bars represent mean comparison within Cultivars using Tukey's post hoc at $\alpha=0.05$

Plant height is generally taller at glass house conditions (Figure 3.8 A and 3.8 B) regardless of cultivar, age, and treatment. Plant height in Koshihikari and Doongara is progressively increasing with age at Control conditions whether glasshouse or field-grown. On the other hand, Reiziq control conditions in glasshouse did not significantly increased plant

height but the opposite was observed when grown in the field. Correlation analysis strongly predicts the height of glass house and field grown Koshihikari and Doongara but moderately linear in Doongara as supported by the variation response when grown in field and glass house conditions (Figure 3.8 C).

3.2: Effects of salinity on gas exchange in glasshouse experiment

3.2.1: Net CO_2 assimilation rate (A)

Net CO_2 assimilation rate (A, µmol $m^{-2}s^{-1}$) is not significantly different among cultivars (*p=0.8188*) at control conditions regardless of age (Figure 3.9 A, Table 3.1) but salinity treatment decreased *A* among cultivars (*p<0.0000*) regardless of age. The effect of salinity to *A* is further exacerbated as age progresses evident in the Treatment: Age interaction (*p=0.0367*, Table 3.1). Among cultivars, Reiziq seems to tolerate salinity stress on the early onset of saline conditions as *A* net is not affected after week 1 (Figure 3.9 A) while *A* net of the two other cultivars decreased significantly after one week of salt treatment.

Table 3.1. Statistical summary of the two-way and three-way ANOVA for the effects of salinity treatment on, cultivar, and age on various parameters

Parameter	Main Effects (p)			Interactions (p)			
	Treatment	Cultivar	Age	Treatment X Cultivar	Treatment X Age	Cultivar X Age	Treatment X Cultivar X Age
CO_2 Assimilation Rate, A_{net} (µmol m^{-2} s^{-1})	**0.0000**	0.81882	0.3149	0.0779	**0.0367**	0.4481	0.5232
Stomatal conductance, g_s (mol m^{-2} s^{-1})	**0.0000**	**0.0518**	0.8612	0.0975	0.5076	0.9158	0.9841
Leaf intrinsic water-use efficiency, WUE_i [µmol $[CO_2]$ (mol $H_2O)^{-1}$]	**0.0000**	0.7960	**0.0000**	0.6338	**0.0000**	0.7075	0.4239
Transpiration rate, E (mol m^{-2} s^{-1})	**0.0000**	**0.0423**	0.8556	0.0670	**0.0108**	0.8329	0.9772
CO_2 Assimilation Rate (field grown), A_{net} (µmol m^{-2} s^{-1})	0.0802	**0.0203**	**0.0118**	0.4255	0.4210	0.4995	**0.0000**
H^+ flux, H^+ (nmol m^{-2} s^{-1})	**0.0220**	0.1483	**0.0000**	0.8557	**0.0000**	0.1332	**0.0005**
Na^+ flux, Na^+ (nmol m^{-2} s^{-1})	**0.0000**	0.5140	**0.0005**	0.7826	**0.0005**	0.6625	**0.0000**
Cl^- flux, Cl^- (nmol m^{-2} s^{-1})	**0.0000**	0.4313	**0.0115**	0.4700	**0.0117**	0.7387	**0.0000**
K^+ flux, K^+ (nmol m^{-2} s^{-1})	**0.0000**	0.3402	**0.0028**	0.1677	**0.0018**	0.7301	**0.0000**
Plant height (glasshouse), height (cm)	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Plant height (field), height (cm)	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Fresh weight (glasshouse), FW (grams)	**0.0000**	**0.0000**	*NA*	**0.0366**	*NA*	*NA*	*NA*
Dry weight (glasshouse), DW (grams)	**0.0000**	**0.0000**	*NA*	0.2088	*NA*	*NA*	*NA*
Tiller number (glasshouse), TN (tillers)	**0.0028**	**0.0017**	*NA*	0.4791	*NA*	*NA*	*NA*
Fresh weight (field), FW2 (grams)	**0.0000**	**0.0000**	*NA*	**0.0000**	*NA*	*NA*	*NA*
Dry weight (field), DW2 (grams)	**0.0000**	**0.0000**	*NA*	**0.0000**	*NA*	*NA*	*NA*
Tiller number (field), TN2 (tillers)	**0.0000**	**0.0000**	*NA*	**0.0000**	*NA*	*NA*	*NA*

*bold values indicate significant effects at α=0.05. NA indicate parameters where three-way ANOVA is not applicable.

3.2.2: Stomatal conductance in glasshouse

Stomatal conductance (g_s) is generally higher in control conditions than the salinity exposed conditions (*p<0.0000*, Table 3.1, Figure 3.9 B) suggesting stomatal closure during saline conditions. Stomatal conductance immediately dropped after one week of salinity treatment. There was no significant variation among cultivars when compared in control and treatment conditions (Figure 3.9 B) but there is a marginal cultivar variation regardless of treatment and age (*p=0.0518*, Table 3.1). g_s did not vary as age progresses whether in control or saline conditions (*p=0.8612,* Table 3.1).

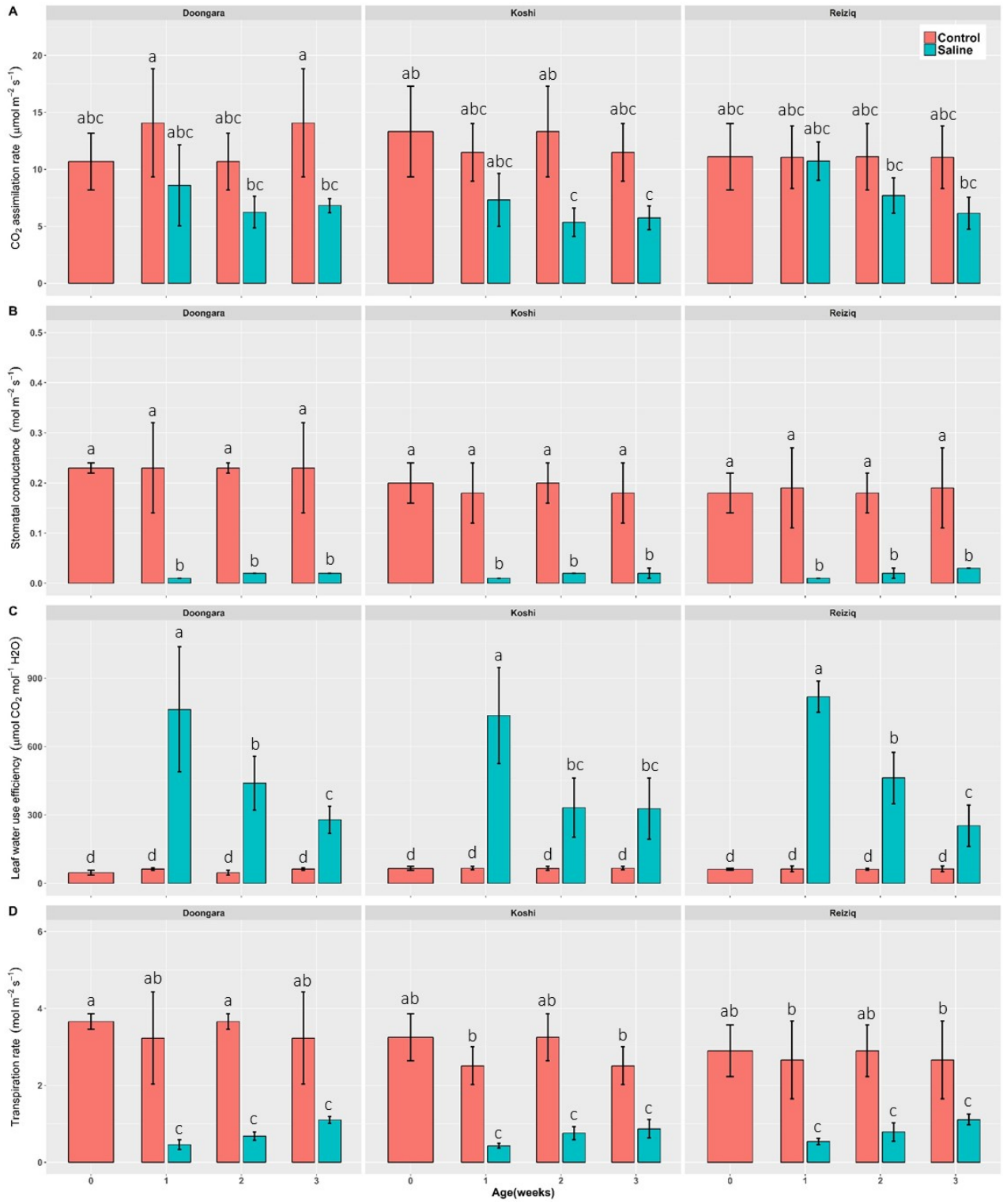

Figure 3.9: Gas exchange parameters of the three cultivars grown under 100 mM NaCl and 0 mM NaCl in glasshouse conditions. (A) represents CO_2 assimilation rate (*A*), (B) represents stomatal conductance (g_s), (C) represents leaf water-use efficiency (*WUEi*), (D) represents transpiration rate (*Tr*). Values are means ±SE. Means were compared using Tukey's post-hoc at $\alpha = 0.05$.

3.2.3: Leaf intrinsic water-use efficiency (WUEi) in glasshouse

Leaf intrinsic water-use efficiency (WUE_i) was measured as unit CO_2 going inside the leaf intercellular spaces through the stomata per unit H_2O moving out of the leaf via transpiration (A/g_s). There were no significant differences among cultivars either at control and saline conditions (Figure 3.9 C, Table 3.1, *p=0.7960*) but significant variation exists due to salinity stress (*p<0.0000,* Table 3.1, Figure 3.1 C). WUE_i is enormously high during salinity stress due to very low steady-state conductance g_s (Figure 3.9 B, Figure 3.9 C). WUE_i decreases as age progresses for control variables *(p<0.0000,* Table 3.1) and the response of WUE_i to salinity stress is age-dependent *(p<0.0000,* Treatment: Age interaction, Table 3.1). A scatter plot (Figure 3.10) revealed high dependency of WUE_i to g_s (*r= -0.85*, *p<0.0000,* Figure 3.10 A) over (*A*) (*r= -0.55*, *p=0.01,* Figure 3.10 B).

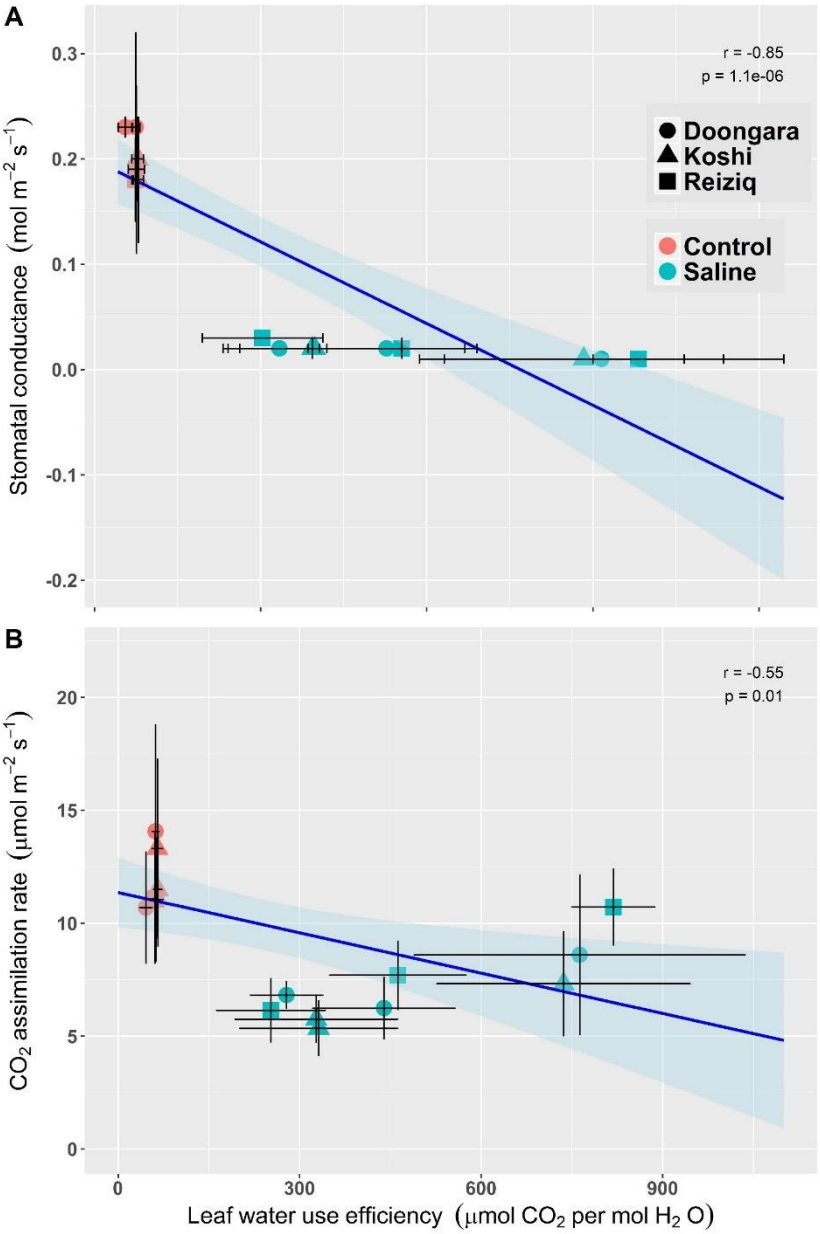

Figure 3.10: Scatter plot shows controls of leaf WUEi (g_s and A). (A) represents stomatal conductance (g_s), (B) represents CO_2 assimilation rate (A). p-values and correlation coefficient (*r*) are also presented.

3.2.4: Transpiration rate (Tr) in glasshouse

Transpiration rate (Tr) is lower at saline conditions (*p<0.0000,* Table 3.1) and varies among cultivars regardless of age and treatment (*p=0.0423,* Table 3.1, Figure 3.9 D). Transpiration rate tends to vary as age progresses (*p=0.0108,* Table 3.1, Figure 3.9 D) evident in the Treatment: Age interaction. Transpiration rate highly reflects the patterns in stomatal

conductance (Figure 3.9 B) as they both rely on the unit of H_2O vapor moving out of the stomata.

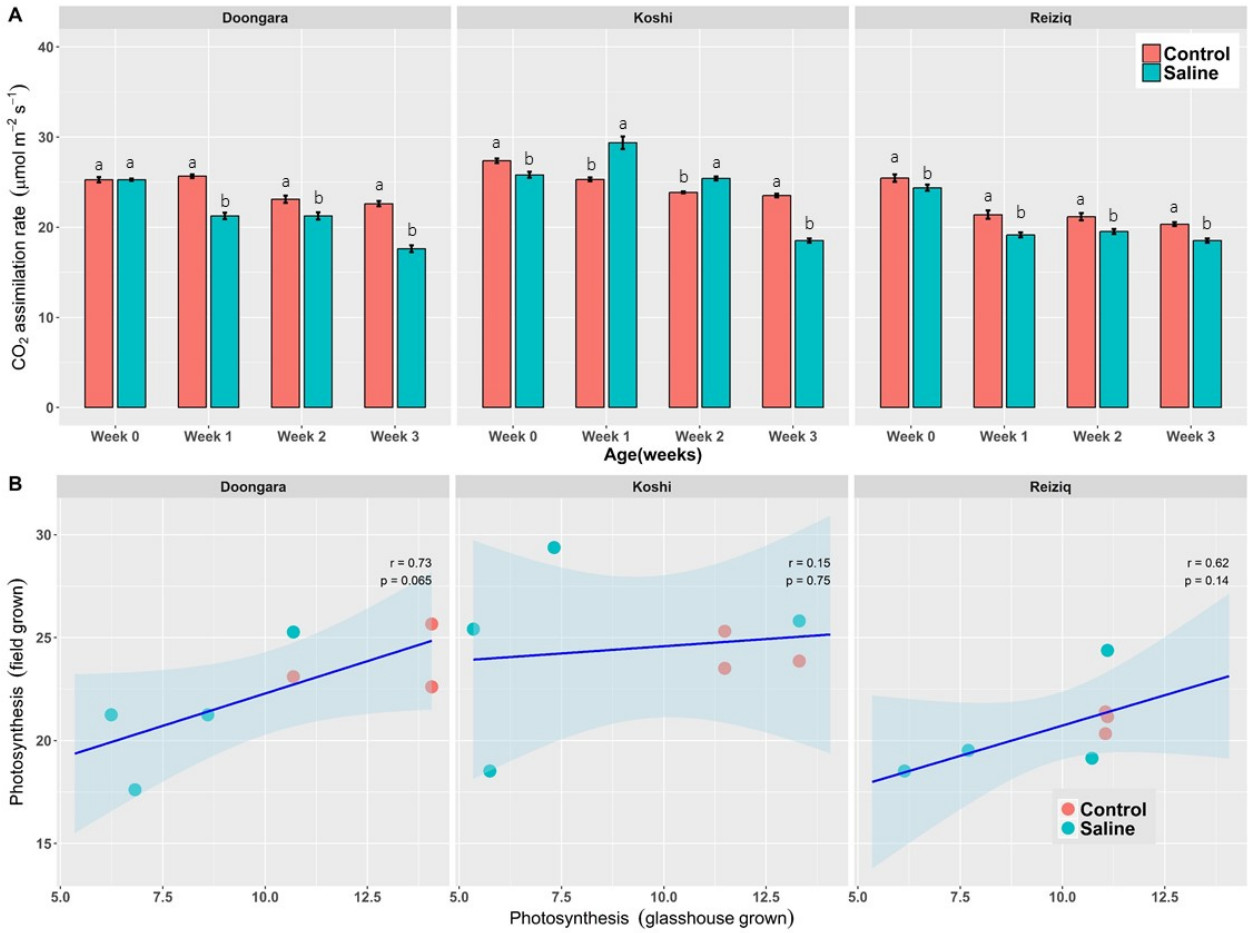

Figure 3.11: Photosynthetic measurements of both the control and treatment, (A) shows the correlation between (A) measurements in glasshouse and the field. (B) represents p-values as well as (r) values between the three cultivars.

3.3: Effects of salinity on gas exchange

3.3.1: Correlation of gas exchange in glasshouse and field

Regardless of treatment and age, there was cultivar variation (*p=0.0203*, Table 3.1) evident in Koshihikari's response to salinity weeks 1 and 2 whereby *A* increased relative to the control while Doongara and Reiziq performed the opposite (Figure 3.11 A). Age effect is evident regardless of salinity treatment (*p=0.0118*, Table 3.1, Figure 3.11 A). Scatterplot of glasshouse and field measurements (Figure 3.11 B) showed no evidence of linear relationship suggesting a variation response to growth condition.

3.4: Effect of salinity on fluxes in the field

3.4.1: H^+ fluxes

H^+ flux showed decreased flux among salinity treated cultivars during the first two weeks of treatment and began to elevate at week 3 (Figure 3.12 A). There are significant differences between Treatment (*p=0.0220*, Table 3.1) and Age (*p=0.0000*, Table 3.1), but no significant difference between cultivars (p= 0.1483, Table 3.1). There are also significant interactions between (Treatment × Age *p=0.0000* and Treatment × Cultivar ×Age *p=0.0005*, Table 3.1), but not significant differences between (Treatment × cultivar *p=0.8557* and Cultivar × Age *p=0.1332*, Table 3.1).

3.4.2: Na^+ fluxes

Na^+ flux profile shows progressive efflux of Na^+ ions peaking at week 4 among all cultivars (Figure 3.12 B, Treatment: Age interaction *p=0.0005*, Table 3.1). Significant differences exist between fluxes of the treatment and age (*p=0.0000*, *p=0.0005*, Table 1.3) but no significant differences were found between cultivars (*p=0.5140*, Table 1.3). There are also significant differences in the interactions between (Treatment × Age: *p=0.0005* and Treatment × Cultivar × Age: *p=0.0000*, Table 1.3). In contrast, no significant differences were observed in the interactions between (Treatment × Cultivar: *p=0.7826* and Cultivar × Age: *p=0.6625*, Table 1.3).

3.4.3: Cl^- fluxes

Chloride efflux also progressively increased along with age and salinity treatment (Figure 3.12 C, treatment: age interaction *p=0.0117*, Table 3.1). Doongara and Koshihikari decreased efflux of chloride ions after week 4 while Reiziq continued to efflux Cl^- out of the mesophyll tissues (Figure 3.12 C). Significant differences were found between treatment and age (*p=0.0000*, *p=0.0115* respectively, Table 1.3). However, no significant differences were found between cultivars (*p=0.4313*, Table 1.3). Similarly, the interactions between (Treatment × Cultivar × Age *p=0.0000* and Treatment × Age *p=0.0117*) were significant, while no significant variations were found in the interactions between treatment and cultivar (*p=0.4700*,

Table 1.3) and between cultivar and age (*p=0.7387*, Table 1.3).

3.4.4: K^+ fluxes

K^+ flux profile elevated efflux among salinity treated plants but only at a margin below -250 nmol m-2 s-1 until week 4. Week 5 showed a greater efflux rate among all cultivars (Figure 3.12 D). This study found variations in correlation of fluxes between the three cultivars grown in the glasshouse and field (Figure 3.14). There are significant differences between treatment and age (*p=0.0000* and *p=0.0028*, respectively, Table 3.1) but no differences were found between cultivars (*p=0.3402*, Table 3.1).

3.5: Effect of salinity on fluxes in glasshouse

3.5.1: H^+ fluxes

H^+ flux showed similar patterns in Koshihikari and Reiziq compared to their control conditions while greater influx (and consequently mesophyll acidification) was observed in Doongara control at weeks 1 and 2 (Figure 3.13 A).

3.5.2: Na+ fluxes

Na^+ flux showed no variation during control conditions among the three cultivars regardless of age while Na^+ efflux showed greater rates at week 1 and continued to decline at week 2 and finally Na^+ influx at week 3 among the three cultivars (Figure 3.13 B).

3.5.3: Cl^- fluxes

Very minimal Cl^- efflux was evident among three cultivars at control conditions while progressive Cl^- efflux were observed among salinity treated cultivars across age (Figure 3.13 C).

3.5.4: K^+ fluxes

K^+ pattern reflects Cl^- pattern such that efflux is progressively increasing with age with respect to salinity treatment but not with the control-grown cultivars (Figure 3.13 D).

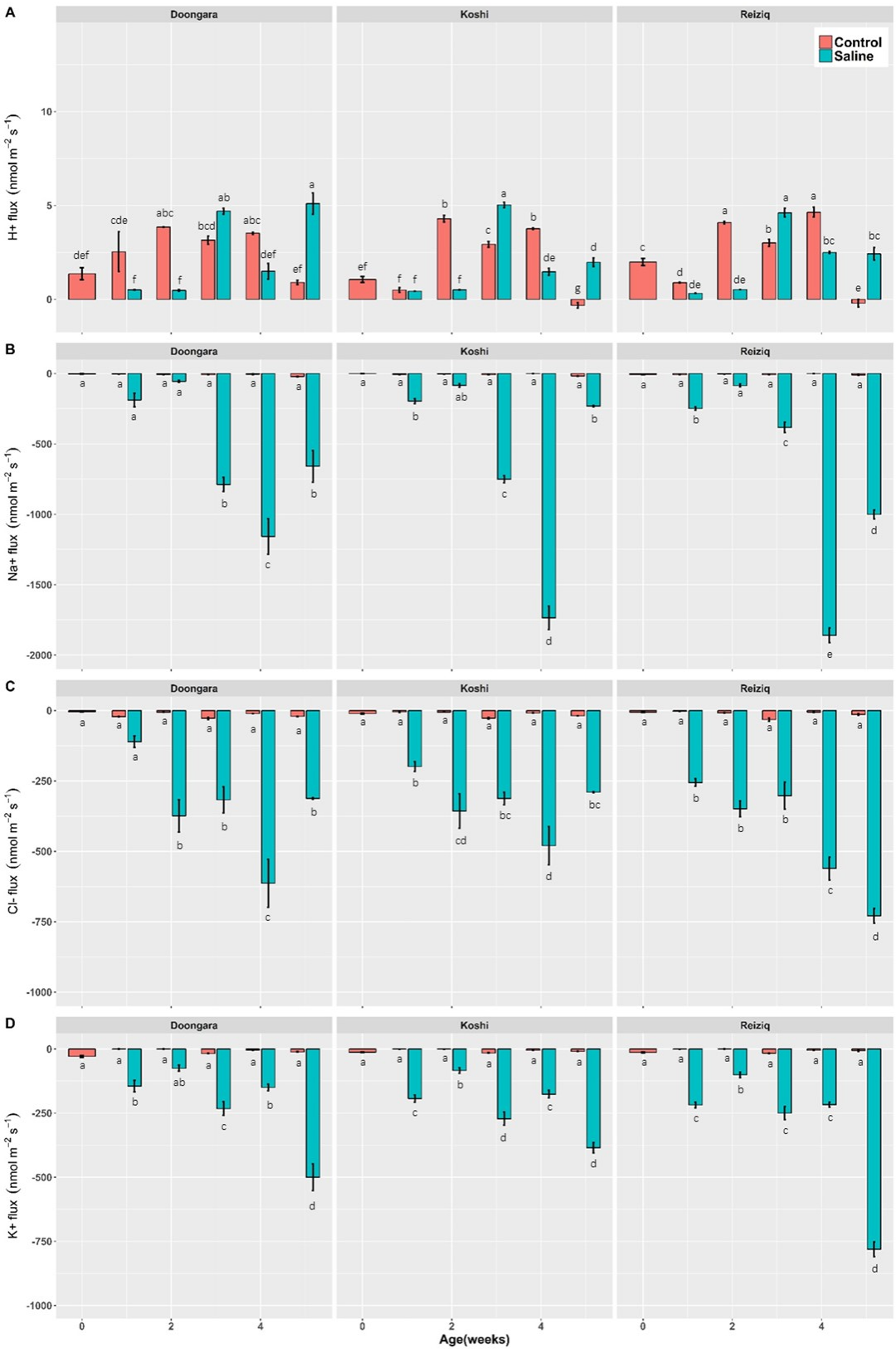

Figure 3.12: MIFE measurements of H^+(A), Na^+ (B), Cl^- (C), and K^+ (D) of three rice cultivars subjected to salinity stress over a period of four weeks grown in field conditions. Letters above each bar represent ranking of means within each cultivar using Tukey's post hoc at $\alpha=0.05$.

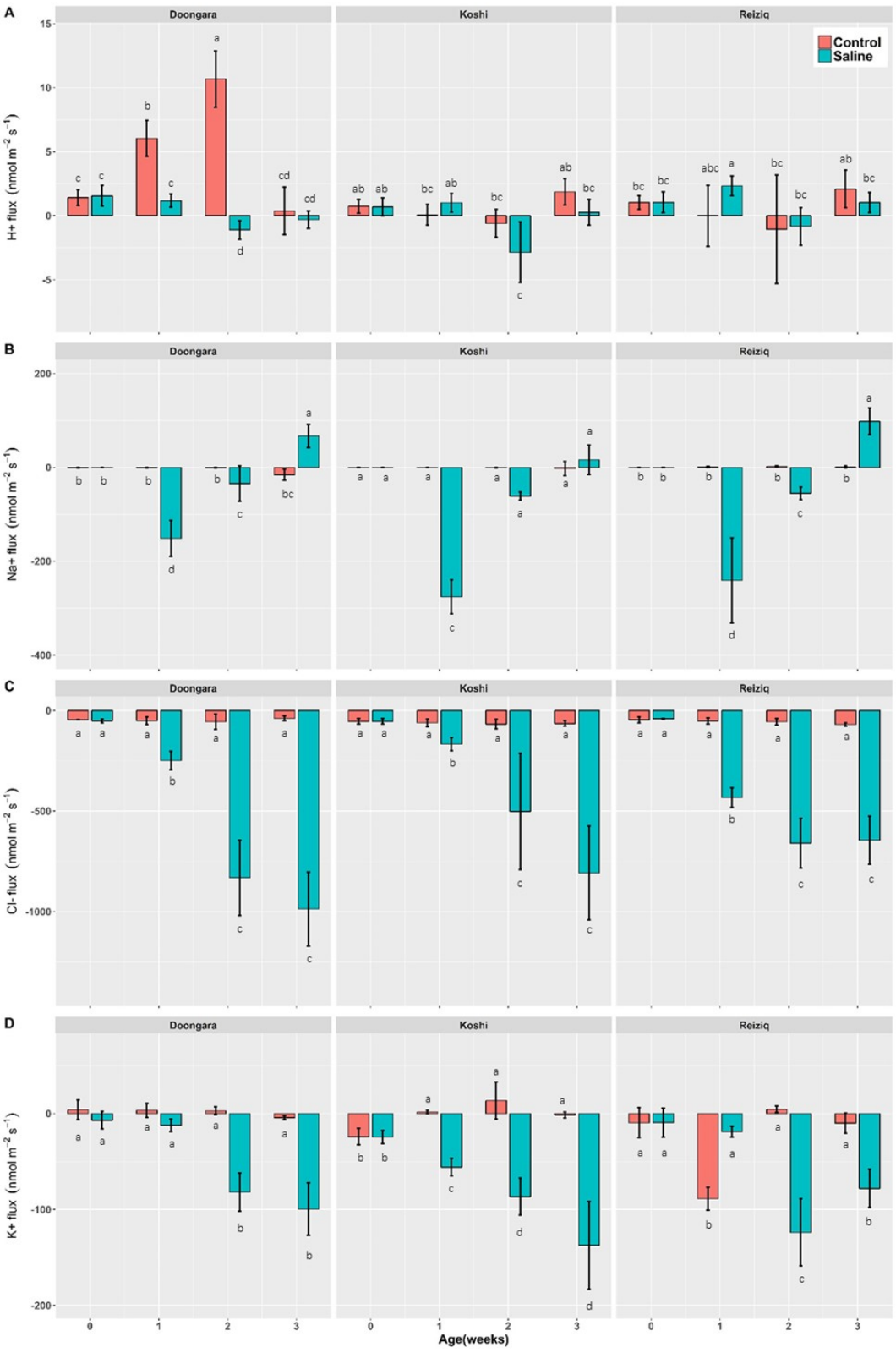

Figure 3.13: MIFE measurements of H^+(A), Na^+ (B), Cl- (C), and K^+ (D) of three rice cultivars subjected to salinity stress over a period of four weeks grown in glasshouse conditions. Letters above each bar represent ranking of means within each cultivar using Tukey's post hoc at $\alpha=0.05$

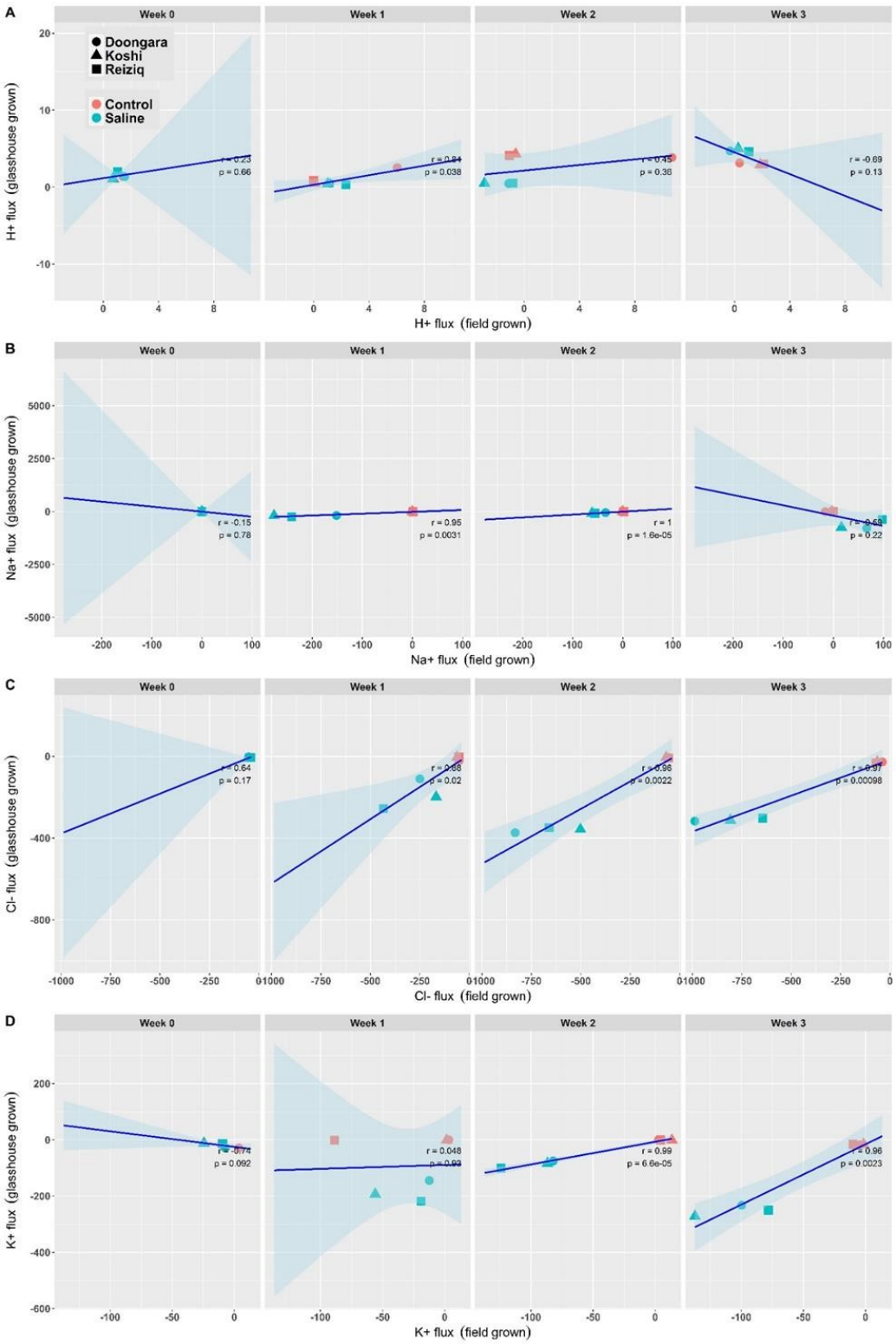

Figure 3.14: MIFE correlation scatter plot of H^+(**A**), Na^+ (**B**), Cl^- (**C**), and K^+ (**D**) of three rice cultivars grown in glasshouse and field conditions and grouped by Age. Coefficient of correlation(r) and respective p-value are presented.

4: Discussion

4.1: Agronomic traits need to be evaluated in the breeding and development of salinity tolerance in rice

Plant height is generally taller at glasshouse conditions (Figure 3.8 A and 3.8 B) regardless of cultivar, age, and treatment. Plant height in Koshihikari and Doongara has progressively increased with age at Control conditions whether glasshouse or field-grown. On the other hand, Reiziq control grown in the glasshouse did not significantly increased plant height but the opposite was observed when grown in the field. Correlation analysis strongly predicts the height of glass house and field grown Koshihikari and Doongara but moderately linear in Doongara as supported by the variation response when grown in field and glass house conditions (Figure 3.8 C). The difference in plant height between the glasshouse and field could be attributed the controlled conditions in the glasshouse, which allows the precise control of all aspects of growing conditions. In contrast, growing conditions in the field are subject to the prevailing abiotic and biotic stresses in open fields. This allows glasshouse crops to reach their potential when compared to field grown crops. Overall results show that salinity stress attenuated the height of all three cultivars (Figure 3.8), On average, control plants were 20 to 30 cm taller that treatments variables. However, initial onset of salinity exposure did not attenuate plant height in Doongara genotype, however, Koshihikari and Reiziq were affected.

Breeding for salinity tolerance has been an extremely difficult task, which is mostly linked to unfavourable agronomical traits from salinity tolerance donor parents (Ashraf and Akram 2009, Gregorio et al. 2013). On the other hand, there are many candidate genes and genotypes that can be useful in breeding programme. It is vital that the superior agronomic traits (e.g. high yield, top quality, disease resistance, wide adaptation to different environments) of the elite commercial cultivars should be retained in new breeding lines for salinity tolerance. For example, as shown in this study Reiziq showed efficient WUEi under salinity tolerance and has the highest yield among Australian grown rice varieties. This makes

Reiziq a good candidate for breeding programs focused on WUEi, salinity tolerance, and high yield in rice.

In this experiment, Doongara and Koshihikari exhibited the greatest disparity of growth in both control and treatment in Week two, but not with Reiziq. The growth of Doongara in the early stages of salinity exposure could be linked to the difference in osmotic potential between the root and soil (Marcelis and van Hooijdonk 1999, Horie et al. 2012, Schroder et al. 2014). Overall, reductions in plant height observed in all three genotypes subjected to salinity stress are in agreement with previous studies that reported high salt concentrations decreased plant height (Munns and Tester 2008), altering root and shoot relationship (Tattini et al. 1995) and restricting leaf expansion (Cramer 2002).

Rice tillering is a desired agronomic trait and a crucial parameter that affects panicle number per unit and hence yield (Gallagher and Biscoe 1978. Yoshida 1981, Miller et al. 1991, Peng et al. 1994, Moldenhauer and Gibbs 2003, Wang et al. 2007, Badshah et al. 2013). Panicle initiation rate is correlated with grain yields in rice (Wang et al. 2007). Conversely, high tiller numbers have been associated with poor grain filling, reduced panicle size and high tiller abortion rate (Peng et al. 1994, Ahmad et al. 2005, Badshah et al. 2013).

The response of tiller number (TN) for genotypes grown in the glasshouse and field showed stark variation (Figure 3.5 C, Figure 3.6 C). Although all the 3 genotypes displayed decreased TN due to salinity exposure ($p < 0.0000$, Table 3.1, Figure 3.5 C, Figure 3.6 C) field grown cultivars show a treatment: cultivar interaction ($p < 0.0000$, Table 3.1), however, no significant interaction was observed in glasshouse grown genotypes ($p = 0.4791$, Table 3.1) suggesting that cultivars respond differently to the niche they are grown. This agrees with an earlier study conducted by Yoshida et al. (1981) on rice tillering dynamics, who found that substantial variation in rice tiller numbers were closely related to plasticity with respect to growth conditions. The results in this experiment seem to be consistent with other similar

studies which found that the exposure of NaCl decreased tiller numbers (Gridhar 1988, Grattan et al. 2002). Similar results were also reported on rice plants subjected to salinity stress (Castillo et al. 2004, Moradi and Ismail 2007).

The tiller numbers grown in the glasshouse and field showed no linear correlation as evident in the scatterplot (*r= 0.044*, *p= 0.74*, Figure 3.7 C) which supports the observed response to the growth conditions. Ironically, Reiziq genotypes grown in the field during salinity treatment had the highest TN which is closer to Koshihikari's TN in the control variables. Previous studies have reported that rice tillering depends on genotype and nutrients available for growth and development (Dingkuhn and Kropff 1996). In rice, tiller numbers exceeding 40 per plant with a senescence rate of >50 % have been observed (Peng et al. 1994). This is corroborated by similar studies which found that tillering beyond a sustainable threshold is aborted or corrected by senescence (Dingkuhn and Kropff 1996).

This reduction in tiller count could be attributed to the water deficit associated with the severe osmotic stress and ion toxicity (Munns and Tester 2008). Upon salinity exposure, the growth and development of rice plants are affected resulting in plants exhibiting smaller tiller size and reduced tiller numbers (Munns and Tester 2008, Rajendran et al. 2009, Horie et al. 2012). Therefore, the study of tillering behaviour with respect to salinity exposure in rice is a crucial step towards the understanding of morpho-physiological effects of salinity stress on rice.

4.1.1: Mesophyll tissue tolerance is a key determinant to improve salinity tolerance

Salinity stress inhibits CO_2 assimilation rate *A* due to the accumulation of NaCl in mesophyll tissues which is also referred to as the 'ionic effects' (Yamane et al. 2012, Wang et al. 2018) and 'osmotic effects' resulting from low leaf water potential induced by low osmotic potential (Delfine et al. 1999, Centritto et al. 2003, Moradi and Ismail 2007, Chaves et al. 2011, Chen et al. 2015). Recent studies have shown that mesophyll shape is extensively altered by

dehydration resulting from the osmotic stress (Scoffoni et al. 2016, Scoffoni et al. 2017). Therefore, changes in mesophyll shape could be one of the potential reasons for the reduced FW and DW observed in this experiment. In addition, salinity is deleterious to all tissues of the plant; however, the most noticeable damages are seen in the aerial parts of the plants under salinity stress. Results in this experiment shows that salinity exposure significantly reduced the fresh weight of all cultivars ($p < 0.0000$, Table 3.1, Figure 3.5 A, Figure 3.6) grown in both the glasshouse and field conditions. In the glasshouse plants, Doongara and Koshihikari exhibited significantly higher FW in the control compared to Reiziq, while Doongara was found to have the highest FW in the treatment genotypes, giving Reiziq the lowest score among the treatment plants (Figure 3.5 A). On the other hand, field-grown Doongara had the highest FW followed by Koshihikari and Reiziq in the control group (Figure 3.6 A). In contrast, Koshihikari scored the lowest FW in the treatment group when compared to Doongara and Reiziq. The observed decrease in FW in the treatment group is comparable to the findings of previous studies which indicated that the reductions in fresh weight (FW) and dry weight (DW) caused by salinity exposure are closely associated with a decrease in leaf numbers or leaf abscissions (Hernandez et al. 1995, Alarcon et al. 1999, Chartzoulakis et al. 2002, Torrecillas 2003, Rodriguez et al. 2005, Alarcon et al. 2006, Navarro et al. 2008). This experiment showed that the treatment: genotype interaction is significant suggesting that FW response to salinity stress is different among cultivars ($p= 0.0366$, $p= <0.0000$, Table 3.1). Likewise, the scatterplot for the three genotypes grown in glasshouse and field conditions showed strong linear correlation ($r= 0.76$, $p <0.0000$, Figure 3.7 A).

Similarly, salinity interferes with the process of cell expansion and division causing significant decrease in leaf area index and ultimately hampering photosynthetic processes (Netondo et al. 2004). In addition to that, in the glasshouse experiment, Doongara produced less biomass, compared to other cultivars grown in the field. This could be related to the onset

of low night temperature during the last 4 weeks before harvest. Previous studies have reported that low temperature affect rice biomass and yield in two ways. First, chilling affects the development of the shoot apex, which controls panicle differentiation, leading to spikelet infertility and potential yield loss (Takeoka et al. 1992). This damage usually occurs during the formation of pollen sacs, causing male sterility (Heena et al. 1984). Secondly, low temperature hampers photosynthesis activities, which decreases metabolites required for growth, development and yield (Smillie et al. 1988). Lewin and McCaffery (1985) reported that lowering day or night temperature by just 5º C resulted a reduction in biomass by two-thirds. Moreover, plants grown in glasshouse conditions will behave differently than those grown in the field. Different abiotic stresses including low and high temperatures, wind, humidity and other environmental factor could impact the growth parameters in field grown crops. However, plants grown in glasshouse environments are considered to be an artefact that may not be replicated in open fields.

Similar to FW, salinity treatment generally reduced the DW among the 3 genotypes, irrespective of where grown in the glasshouse or the in the field (*p <0.0000,* Table 3.1, Figure 3.5 B, Figure 3.6 B). At both the control and treatment groups, Doongara and Koshihikari have significantly higher DW in comparison to Reiziq (Figure 3.5 B, Figure 3.6 B). This reduction of FW and DW could be attributed to accumulation of toxic NaCl in rice cells which manifested in the form chlorosis, senescence, necrosis and death in older leaves observed in the treatment groups (Figures 3.1, 3.2, 3.3 and 3.4). Earlier studies have shown that plants sequester salt in the old leaves, leaf sheaths, and stems to protect the young leaves and reproductive tissues (Singh and Flowers 2010, Sarhadi et al. 2012). Moreover, it has been suggested that the accumulation Cl^- in salt-stressed leaves activate 1-aminocyclopropane-1carboxilic acid (ACC) synthesis and its transformation into ethylene, delivering enough hormones to cause leaf abscission in plants (Tudela and Primo-Millo 1992, Dodd 2005).

Similar studies have reported that osmotic and salt stress also facilitated the conversion of ACC into ethylene in rice (Kao and Yang 1983, Khan et al. 1987, Basu and Ghosh 1991, Lutts et al. 1996), in *Allenrolfea occidentalis* (Chrominski et al. 1988) and in tomato plants (Albacete et al. 2008, Ghanem et al. 2008). A reduction in total leaf area has been linked to oxidative damage resulting from massive build-up of Na^+ (Albacete et al. 2008, Ghanem et al. 2008). Other studies have asserted that leaf area reduction may be an evolutionary response to minimise water loss when stomata pores are closed (Save et al.1994, Ruiz-Sanchez et al. 2000). This strategy could also be interpreted as a mechanism favouring the sequestration of toxic ions in the roots, instead of the shoots (Colmer et al. 2005, Munns and Tester 2008).

4.1.2: Genotypic difference in photosynthetic performance is consistent in both glasshouse and field

The net CO_2 assimilation rate (*A*) is not significantly different among cultivars ($p = 0.8188$) in the control group regardless of age (Figure 3.9 A, Table 3.1). In contrast, salinity treatment decreased *A* among the three cultivars ($p < 0.0000$) regardless of age. The effect of salinity to *A* is further exacerbated as age progresses which is evident in the Treatment: Age interaction ($p = 0.0367$, Table 3.1). This reduction in *A* may be related to the direct effects of NaCl on stomatal resistance via loss of guard cell turgor, atmospheric vapour pressure and root-activated chemical signals (Dionisio-Sese and Tobita 2000, Chaves et al. 2009). Another limiting factor in *A* rate could be attributed to the accumulation of Na^+ in the mesophyll leading to stomatal closure (Flexas et al. 2004, Chaves et al. 2009). Moreover, research has shown that increased salinity in the rhizosphere tends to limit transpiration rate, because of the lower water potential and the movement of abscisic acid (ABA), from root to shoot to trigger stomatal closure (Zheng et al. 2001). High concentrations of ABA have been shown to play a major role in the activation of plasma membrane-localised anion channels and raising cytosolic Ca^+ (Hamilton et al. 2000, Kohler and Blatt 2002). Consequently, causing H_2O_2 generation, loss of guard cell volume, potassium efflux and ultimately stomatal closure

(Zhang et al. 2006, Wang et al. 2012). Other major antioxidants such as Ascorbate (AsA) redox state has also been reported to influence transpiration rate and stomatal movement (Chen and Gallie 2004). Recently, several studies have reported that salinity stress can break down chlorophyll pigmentation in plants (Li et al. 2010, Yang et al. 2011) and carotenoid contents (Li et al. 2008, Gomathi and Rakkiyapan 2011), thus, causing reduction in photosynthesis and decreased photoprotection, respectively. Therefore, salinity stress may have direct effect on stomatal conductance and indirect effects on photosynthetic parameters in rice plants. The effects of Na^+ on enzymatic factors of photosynthesis could also be a factor as proposed by Seemann and Sharkey (1986) who found that the capacity of Rubisco significantly decreased under 100 mM of NaCl in *Phaseolus Vulgaris* L. Previous reports show that salinity stress adversely impacted on plant water relations and decreased stomatal conductance (Flowers and Colmer 2008, Bazihizina et al. 2012). It is well documented that the build-up of Na^+ ions in the root zone leads to a decrease in the water potential via decrease in osmotic potential (Sanchez-Blanco et al. 2004, Franco et al. 2011). Among the three cultivars tested in this study, Reiziq seems to tolerate salinity stress on the early onset of saline exposure, as *A* is not affected after Week 1 (Figure 3.9 A) whereas the *A* of Doongara and Reiziq have significantly declined after one week of salt treatment.

Stomatal conductance was found to be generally higher in the control group compared to the treatment ($p< 0.0000$, Table 3.1, Figure 3.9 B) suggesting stomatal closure during salinity exposure. There was no significant variation between the three cultivars in both the control and treatment (Figure 3.9 B). Although only marginal variation was observed regardless of treatment and age ($p= 0.0518$, Table 3.1). However, in the field the reduction in stomatal conductance may be attributed to the loss of leaf turgor which in turn reduces net CO_2 assimilation rate and ultimately hampering photosynthetic processes. Similar findings to this experiment was reported in several studies (Farquhar and Sharkey 1982, Kramer and Boyer

1995, Sibole et al. 1998, Sultana et al. 1999, Megdiche et al. 2008, Stoeva and Kaymakanova 2008). However, stomatal closure is also a protective mechanism employed by plants to save water which also enhances their water use efficiency (Chaves et al. 2009). Similarly, Koyro (2006) suggested decreased stomatal conductance is a form of adaptive mechanism designed to reduce the concentration of salt in the leaves, therefore maintaining subtoxic levels while reducing the transpiration rate. In this study, stomatal conductance immediately dropped after one Week of salinity treatment (Figure 3.9 B). A recent study comparing responses of some Barley cultivars with varying salinity tolerance levels, found that lower stomatal conductance during the initial exposure indicated salinity tolerance (Vysotskaya et al. 2010). Similar findings were reported in other plants (Banon et al. 2012, Gomez-Bellot et al. 2013). In this study there was only some marginal differences between the three cultivars tested regardless of age ($p= 0.0518$, Table 3.1). Stomatal closure during salinity exposure may be triggered by the accumulation of abscisic acid (ABA) (Mulholland et al. 2003, Fricke et al. 2004, Vysotskaya et al. 2010), however, Tardieu and Simonneau (1998) pointed out that stomatal closure is more of maintaining water relations which may be associated to an interaction between hydraulic and chemical signalling.

Salinity tolerance in plant is a cumulative process which depends on maintaining a multitude of physiological traits such as ion homeostasis, low Na^+ and K^+ ratio in root and shoot, vacuolar sequestration, Na^+ extrusion from shoot, and Na^+ exclusion (Munns and Tester 2008, Ward et al. 2009, Horie et al. 2012). This study shows that Na^+ flux profiles exhibited by all three genotypes were progressive along with duration and treatment (Figure 3.12 B, treatment: age interaction, $p = 0.0005$, Table 3.1).

4.1.3: Effects of salinity on mesophyll cell ion flux

Considering the deleterious effects of Cl^- in many woody species such as grapevine and *Citrus*, it is surprising that the bulk of research on salinity tolerance focused on Na^+ rather than Cl^-, given that both are toxic when excessive amounts accumulate in the cytoplasm (Munns and Tester 2008, Teakle and Tyerman 2010). However, studies have shown that in some species including rice (Lin and Kao 2001) and Wheat (Kinraide 1999, Husain et al. 2004, Munns 2004, Plett and Moler 2009), Na^+ concentrations in their shoot are negatively correlated with salinity tolerance (Plett and Moler 2009). In this experiment, Cl^- efflux progressively increased with age and salinity treatment in the 3 genotypes (Figure 3.12 C, treatment: age interaction $p = 0.0117$, Table 3.1). The present findings seem to be consistent with earlier studies that found NaCl induced progressive K^+ efflux in several species such as *Arabidopsis* (Lorenzen et al. 2004), in *Hordeum Vulgare* (Yamashita and Matsumoto 1996, Britto et al. 2004), *Diplachne fusca* (Bhatti and Wieneke 1984), and *sorghum bicolor* (Boursier and Lauchli 1989). Results in this study also shows that Doongara and Koshihikari decreased efflux of Cl^- after 4 Weeks while Reiziq continued to efflux Cl^- out of the mesophyll tissues (Figure 3.12 C). This result agrees with similar studies that found genotypic variations in Cl^- efflux, for example, Sun et al. (2009) observed no Cl^- efflux in the salt-sensitive *Populus popularis* treated with 100 mM NaCl, while salt-tolerant *Populus euphratica* subjected to the same condition displayed significant Cl^- efflux. Furthermore, a recent similar study using microelectrode ion flux estimation (MIFE) has detected Cl^- efflux in bean mesophyll exposed to salinity stress (Shabala 2000). In another study, using recombinant fluorescence probes, Lorenzen et al. (2004) also observed similar Cl^- efflux in salt stressed transgenic *Arabidopsis*. With this limited data, it is difficult to draw conclusions, therefore, further studies are needed to explore whether increased Cl^- efflux from roots may play a role in the response variations between different

genotypes. It is also worth noting that measurements of Cl^- could be negatively affected by the low signal-to-noise ratio for liquid ion exchanger (LIX) when used in high concentration.

In plant cells, K^+ is an important nutrient involved in the regulation of protein synthesis, ribosome functions, adjustment of plasma membrane electrical potential, cytosolic pH homeostasis and neutralisation of anionic groups (Shabala and Cuin 2008, Marschner 2011). K^+ is also vital for plant survival of plants under salinity stress given that K^+ is involved in the activation of more than 50 enzymes in the cytoplasm (Shabala and Cuin 2008, Marschner 2011, Wang et al. 2012). In this experiment, there is a progressive increase in K^+ effluxes among the 3 genotypes tested in Week 4, but only with a margin of < 250 m^{-2} s^{-1}, however, in Week 5 greater efflux rate was observed on all 3 genotypes (Figure 3.12 D). This finding support previous studies on K^+ effluxes induced by salinity stress, for instance, Shabala and Pottosin (2014) reported that upon salinity exposure, K^+ concentrations declined significantly, due to K^+ efflux through the leaf and root cells. Studies in *Arabidopsis* have shown a strong correlation between K^+ accumulation and salt tolerance (Shabala et al. 2005). Similar results were also reported in barley (Chen et al. 2005, Britto et al 2010), wheat (Cuin et al. 2008), cotton (Cramer et al. 1985), bean (Nassery et al. 1975), pea (Shabala et al. 2007), alfa-alfa (Smethurst et al. 2008) and quinoa (Bonales-Alatorre et al. 2013). The decline in K^+ concentrations induced by salinity exposure has been associated with depolarisation of the membranes resulting in K^+ efflux via K^+ outward rectifying channels (KORCs) (Pottosin and Dobrovinskaya 2014). In addition to that, K^+ retention in leaf mesophyll cells has been reported to indicate salinity tolerance and has been used to discriminate between salt-sensitive and salt-tolerant barley and wheat genotypes (Wu et al. 2013, Wu et al. 2015, Wu et al. 2016). Likewise, the capacity of the root in retaining K^+ in wheat cultivars has been suggested to be used as a marker for breeding salt-tolerant crops (Cuin et al. 2008). In a recent study (Coskun et al. 2013) investigated mechanisms involved in the stimulation of K^+ efflux in barley cultivars subjected

to salinity stress. The same study concluded that loss of membrane cohesion caused by osmotic and ionic effects were the underlying factor involved in activating K^+ efflux (Coskun et al. 2013). This is supported by the findings of earlier studies (Nassery 1979, Cramer et al. 1985, Britto et al. 2010). However, Shabala et al. (2006) and Pottosin and Dobrovinskaya (2014) differed in that view and suggested that depolarisation of membranes is the root cause of K^+ efflux in plant cells subjected to salinity stress. Although this study did not identify significant difference between the 3 genotypes tested, however, it reinforces previous studies that concluded that K^+ is a valuable screening tool for salinity tolerance traits in crops (Chen et a. 2005, Chen et al. 2008, Cuin et al. 2008).

4.2: Statement of Potential Impact

The impact of rice research on world economy is well documented since the release of IR8 rice cultivar by the International Rice Research Institute in the Philippines (Renkow and Byerlee 2010, Yamano et al. 2016). IR8 is a cross between Dee-Geo-Woo-gen (DGWG) and Peta rice (Khush 2005). Dubbed as the "Miracle Rice", IR8 became the single most successful cultivar that lifted millions out of poverty (Thirtle et al. 2003, Renkow and Byerlee 2010, Evenson and Gollin 2013). Consequently, rice production in the Philippines has increased from 3.7 to 7.7 million tonnes in less than twenty years, making Philippines a rice exporter for the first time (Evenson and Gollin 2013). In 1967 India also adopted IR8 and is currently producing 104 million tonnes annually (Evenson and Gollin 2013). Prior to the introduction of IR8, maximum rice yields in India stood at < 2 t/ha however, in less than two decades that figure rose to > 6 t/ha. This increase in yield has reduced the cost of rice from $ 550 a ton in 1976 to $ 200 in 2001. According to a report prepared by Brennan and Malabayabas (2011) for the Australian Centre for International Agricultural Research (ACIAR), the improved rice varieties subsequently released by IRRI from 1985 to 2009 has boosted harvest yield by up to 13 % in

the Vietnam, Philippines and Thailand. Consequently, rice farmers in these three countries were able to harvest an additional $ 1.46 billion worth of rice annually (Brennan and Malabayabas 2011). In Vietnam, the IR8 rice has raised farmer income by $ 127 per hectare (Maredia & Riatzer 2012). This increase in disposable income can have positive implications on other areas such as food security, environmental protection, empowering women, tackling climate change and reducing poverty. A study from India and China by Fan et al. (2005) reported that, in the period between 1981 to 1999, IRRI's genetically improved modern varieties (MVs) has helped more than 6.8 million Chinese citizens move out of poverty. Likewise, more than 14 million Indian nationals were also lifted out of poverty between 1991 to 1999. Furthermore, the cost of salinity to the general economy stems from the reduced yield and hence income to the small farmer. This is particularly more pronounced in arid and semiarid regions with irregular rainfall patterns and limited alternative water source for irrigation. The cost of salinity may differ from region to region depending on farm inputs, and profits generated in a growing season. For example, assuming that a farmer in a broad-acre dry land farming in Australia spends Aus$300 h^{-1} and harvests three tonnes per hectare, with a net value of Aus$200 ha^{-1} per tonne. This gives the farmer a gross profit of Aus$600 ha^{-1} and a net profit of Aus$300 ha^{-1}. However, if that yield is reduced by salinity stress to two tonnes per hectare then the gross-income falls to Aus$400 $ha^{-1}$and the net profits falls to just Aus$100 ha^{-1}. Recurring losses caused by rising water table salinity and other forms of salinity means huge loss to the farmer and to the national income. Therefore, even a one tonne deficit in production may cause farmers to abandon farming and revert to pastoral practices which could result in negative economic consequences and a further threat to food security.

Thus, the significance and potential impacts of screening salinity tolerance in rice are at least five-fold:

- The combination of novel morpho-physiological tools for screening rice populations will allow us to identify salt tolerant cultivars that can be used for breeding purposes
- The findings in this experiment may contribute to the breeding of salt-resistant rice in the future that could help farmers around the world in exploiting 960 million hectares of salinized lands to increase rice production for global food security (Rengasamy 2006).
- The results provide an essential conceptual advance in our knowledge on how different rice genotypes response to salinity stress.
- Growing rice and other crops in salinized soils will act as a carbon sink, thus mitigating effects of climate change. In addition to that, brackish and saline waters could be used to irrigate these fields, thus saving precious water for human consumption.
- In terms of social benefits, results from this project may help create jobs for people living in salinity affected regions of the world which will increase economic development while reducing poverty. Moreover, the findings in this study may contribute in the efforts to minimise or save Australia approximately AU$305 per year in the Murray Darling Basin alone (Wilson 2003). On a global scale, the savings could be as high as AU$27 billion per year (Qadir et al. 2014).

Thus, the results of this experiment align with the environmental, water and food security, educational research advancement strategies designed to increase food and fibre production. Therefore, improving salinity tolerance in rice will be a great boost to the world's rice production. In addition to that, growing rice and other crops in marginal wastelands of the world will be a significant boost to the fight against hunger and poverty.

4.3: Limitations and future research directions

Time and resource constraints have limited the extent of this experiments. The time allocated for this type of research was not sufficient because growing crops takes a long time. There have been two crop failures in the first six months due to wind damage to the roof of the glasshouse and pest problems. During the experiment period, many challenges have been met. For example, the onset of low night temperature has cofounded the effects of salinity on the field experiment and also affected yield components compared glasshouse grown crops. Therefore, sowing rice in late October or early November could have prevented chill exposure, particularly to the tropically grown Indica varieties, which is less-tolerant to low temperature when compared to the temperate growing Japonica varieties. This type of experiment requires a longer time than the current allocation of one year for Master of Research. An additional six month could have positive implications for students undertaking agricultural experiments. The course work could have been limited to six months to give students time to conduct quality research.

In the physiology study, there exists considerable genetic differences for salinity tolerance with rice, therefore, the inclusion of more genotypes for both the Indica and Japonica genotypes could be used for screening purposes. Wider range of genotypes with multivariate analysis could have been more suitable for screening rice germplasm because different mechanisms exist in different genotypes of rice. Further physiological experiments such as proline and chlorophyll content, total soluble sugars and concentration of Na^+ in the xylem sap could have added more depth to this study. In addition to that, it would have been interesting to investigate genotypes with varying tolerances to salinity and to compare their physiological responses to a single reference tolerant or susceptible genotype. Morpho-physiological results showed that Reiziq performed better in water-use efficiency compared to other cultivars, however further studies are needed to confirm.

4.4: Conclusions

Salinity intrusion into the fertile regions of rice-producing countries around the world is a major setback for meeting the demands increasing world population. Although salinity is a global phenomenon, it is more prevalent in arid and semi-arid regions in Asia, Africa, and Australia. Currently, large areas of prime agricultural land are degraded by salinity and is expected to grow in the future. This is compounded by the projected increase of World population in the coming decades. Therefore, to meet the demands of the growing population rice production must also increase. Rice is the second most agronomically important crop in the world after wheat, feeding more than half of the world's population. Moreover, rice consumption per capita has increased in many parts of the world including Africa and South America.

The experiments presented in this thesis were set to address the above-mentioned issues and was focused on the enhancement of salinity tolerance in rice by examining the effects of salinity stress on morphophysiological traits such as gas exchange, agronomical growth parameters, and net fluxes of basic ions. Currently, many techniques have been implicated for manipulating rice plants to grow in saline environment without incurring yield penalty, however, these studies failed to produce a rice variety that can tolerate more than 10 dSm-1 of salinity to date. Rice response to salinity stress is multifaceted and complex which makes the task of developing salt tolerant a difficult. Although, the mechanisms of salinity tolerance in rice are yet to be determined, due their complexity, the traits displayed by the 3 rice genotypes may contribute to the endeavour in enhancing salinity tolerance in cultivated rice and other important crops. This study found variations in the response of the three cultivars to salinity stress thus providing evidence that morpho-physiological studies can be used to screen plants for salinity tolerance. However, further studies are needed to confirm these findings.

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6: Appendices

Appendix 1

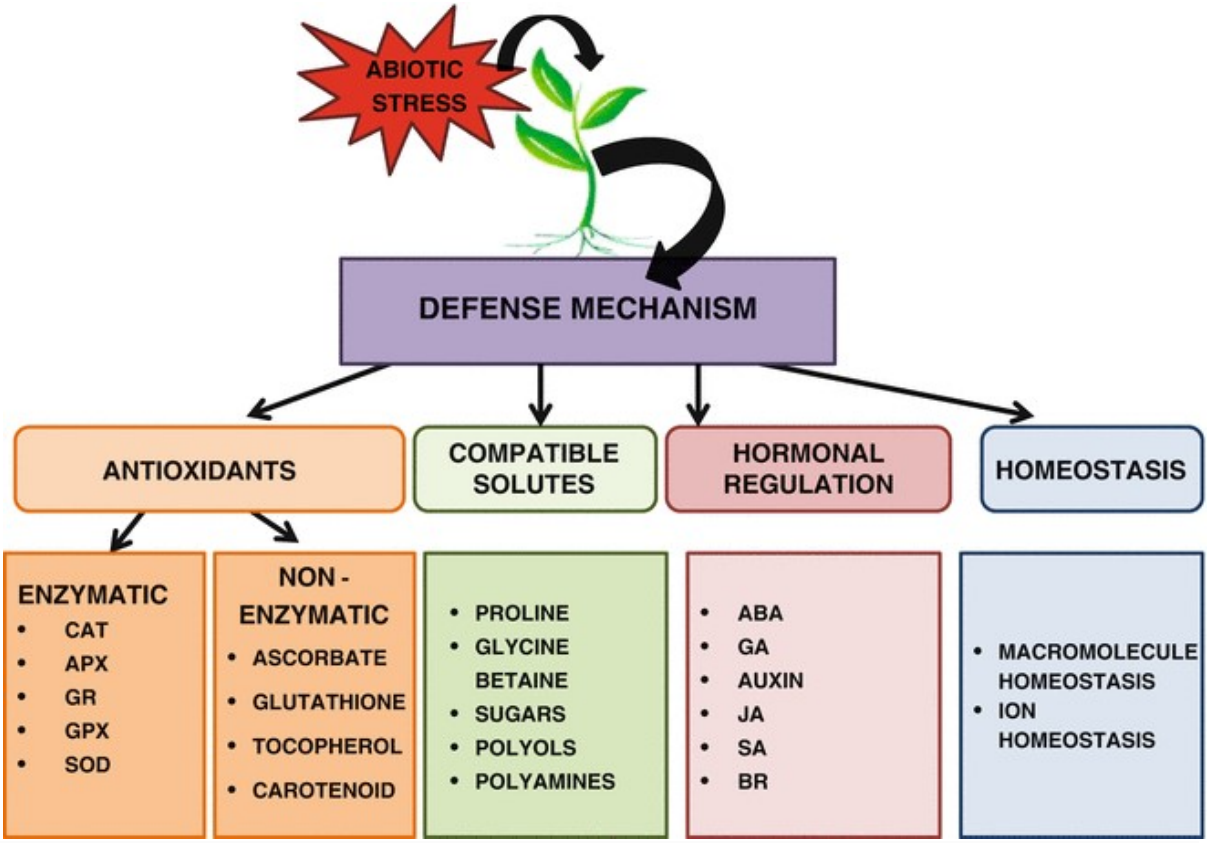

Figure A.1: Abiotic stress defence mechanism of plants including antioxidants, compatible solutes, hormonal regulation and homeostasis (Sengupta et al. 2016).

Appendix 2

Figure A.2: Backhoe loader used for digging the two adjacent trenches for the field experiment.

Figure A.3: Trench preparations for the field experiment

Figure A.4: Heavy duty polythene sheets (100 microns) used to prevent salt leaching to the soil.

Appendix 3

Figure A.5: Comparison between Doongara treatment and control after eight Weeks of salinity exposure in the glasshouse

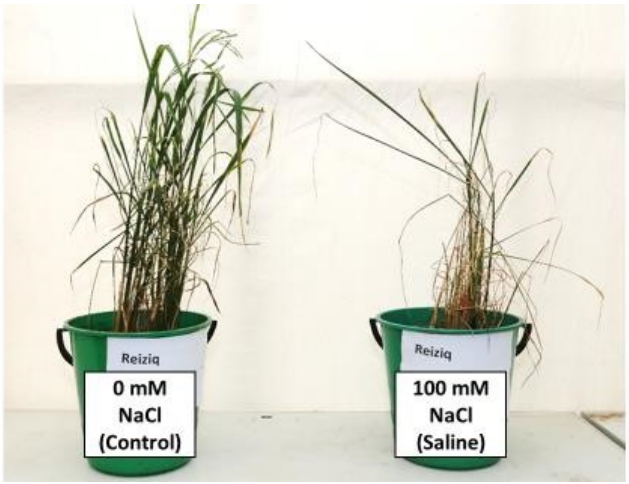

Figure A.6: Comparison between Reiziq control and treatment after eight Weeks of salinity treatment in the glasshouse.

Figure A.7: Comparison of fresh biomass of the three cultivars from the field test.

Figure A.8: Comparison of the controls between the three cultivars grown in the glasshouse.

Figure A.9: Rice plants 21 days after transplant before salinity application

Appendix 4

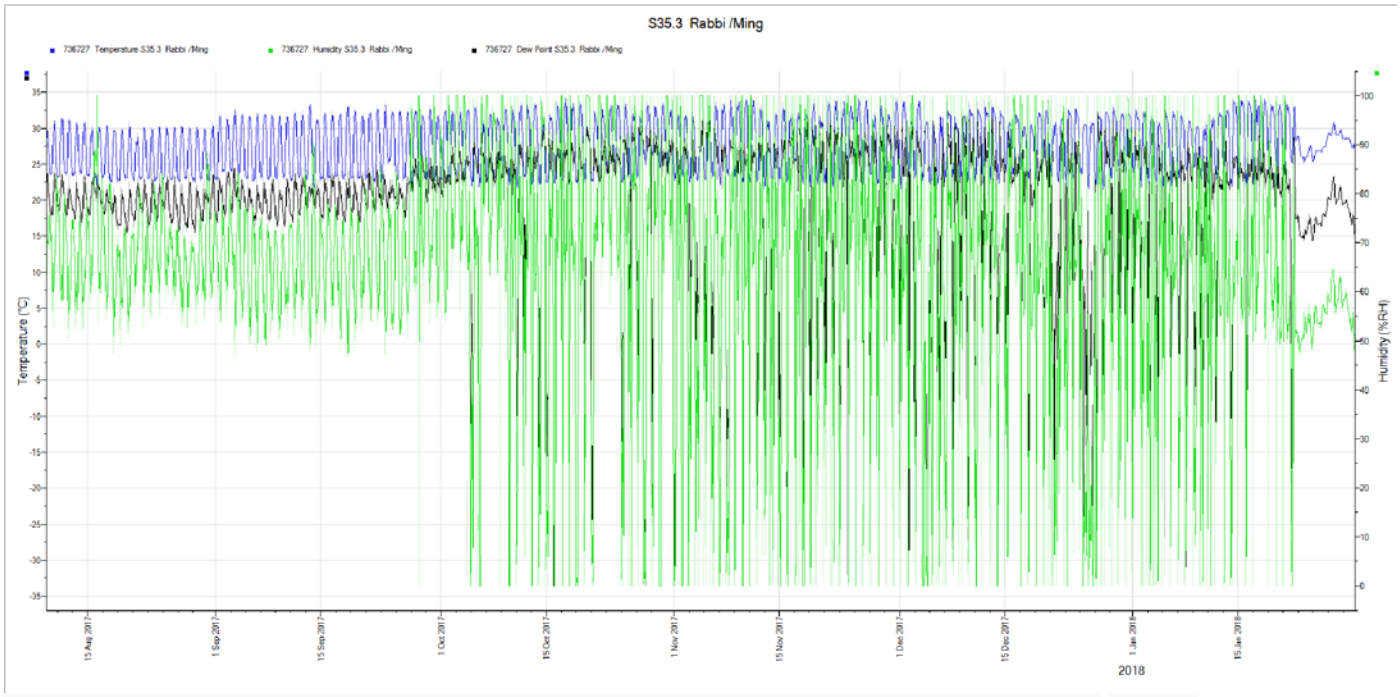

Figure A.10: Graph of the temperature and humidity obtained from TinyTag TG data logger.