Genome Wide Association and Forward Genetic Studies to Identify Genes Involved in Salt Tolerance

Juan Patishtan Perez

PhD

University of York

Biology

September 2016

Abstract

Salinity tolerance varies considerably between natural accessions of rice. In this project, the main aims were to identify well-known genes and novel determinant candidate genes for salinity tolerance using genome wide association study (GWAS) and forward genetic screening. To assess salinity tolerance in rice and Arabidopsis, relative growth rate of plants and cations in roots and shoots were analysed. This research has found that relative growth rate, root K^+, and shoot Na^+ traits are parameters for salinity tolerance in both species. Moreover, the growth parameter is correlated with the root K^+ and shoot Na^+. Regarding the identification of determinant genes for saline conditions, the GWAS approach has revealed 120 association signals and several of them were not revealed before using genomic mapping. The association signals contained ~1500 novel candidate genes across the rice genome and 32 well-known published candidate genes that play roles in saline conditions. One of the relevant outcomes was that the genomic region of the well-known *qSaltol* QTL correlated with a SNP position identified by this approach. The *qSaltol* has already been introgressed into elite rice cultivars and assessed for its effectiveness in saline field conditions. Concerning forward genetic screening, this approach has identified two candidate genes that have molecular functions which correlate with the candidate genes identified by GWAS. These findings may contribute to our understanding of the complex and dynamic mechanisms of plants and the roles of genes to achieve salinity tolerance in plants.

Table of Contents

List of Tables

List of Figures

List of Appendices

Preface

Plants encounter a wide range of biotic and abiotic stresses during their life cycle. Regarding abiotic stress, salinity is one of the main stresses which affect nearly one billion hectares of arid and semi-arid areas of the world. Salinity occurs when the concentrations of ions such as Na^+ and other soluble salts in a medium exceed the electrical conductivity of 4 dS/m which is equivalent to 50 mM NaCl. Under such conditions, most glycophytic plants such as rice and Arabidopsis are unable to complete their life cycle. Furthermore, salinity affects plants in osmotic and ionic ways. Whereas the former takes place after the onset of salt stress, the latter may take place after days or even weeks depending on the plants species, weather conditions and strength of the saline conditions.

To improve salinity tolerance in plants, it is crucial to understand the complex and dynamic mechanisms of plants under saline conditions. For many years, scientists have been using different techniques to identify genes that are involved in saline conditions by using forward genetic screening and genomic mapping i.e. quantitative trait locus (QTL) studies. Recently, genome wide association study (GWAS) has emerged as a new innovative method for studying associations between genotypes (SNPs) and phenotypes. GWAS identifies single nucleotide polymorphism markers that are significantly associated with a trait of interest across a diverse population.

The main aims of this project are (a) to identify well-known genes and (b) to identify novel determinant candidate genes for salinity tolerance using GWAS and forward genetic screening. The current project divides across three research chapters using rice and Arabidopsis species as studies, to fulfil specific objectives in each section. For the first research chapter, the objectives are (a) to assess a correlation between osmotic and salinity tolerance in rice cultivars and (b) to identify common physiological responses of rice cultivars to osmotic and saline conditions. For the second research chapter, the objectives are to identify relatively well-known and novel determinant genes for osmotic and ionic components of salinity through GWAS. This study also assesses the efficiency of GWAS to get insights of amino acid changes of SNP positions, and evaluates correlations between genetic and physical mapping approaches. For the third research chapter, the objective is to identify novel determinant genes for salt tolerance using a forward genetic screen.

The current study identified 120 association signals and ~1500 candidate genes for rice accessions exposed to saline conditions. Interestingly, the GWAS identified association signals for rice accessions exposed to saline conditions that had been not revealed before using genetic mapping. Moreover, this approach also identified specific and relatively well characterised genes that mediated K^+ and Na^+ transport. Likewise, forward genetic screening identified 2 novel genes that might have important roles in saline conditions. The research has also shown that there was a strong correlation between osmotic and salinity tolerance in rice cultivars. Furthermore, rice cultivars exhibited multiple common mechanisms such as changes in K^+, WL, increase of ROS production, when they were exposed to short and long term osmotic and saline conditions. This research therefore provides useful information for future studies to enhance our understanding of the multiple, dynamic and complicated mechanism of plants under saline conditions.

Acknowledgements

Special thanks to my supervisor Dr. Frans Maathuis for his enormous pacience, knowledge, great guidance and support throughout my PhD. Likewise, thanks to the training advisory panel (TAP), Dr. Michael Schultze and Dr. Ian Graham who contributed and enriched this project.

Thanks to my fellow workers and lab members for their support: Tom Hartley, Izhar Ahmad, Nahed Mohammed, Radwa Mohamed, Emma Lindsay, Joseph Vaughan, Thomas Irving. As well as, thanks to those friends who supported me on special occasions: Kyla Mccarthy, Alison Fellgett, Kuntal Singh, Dinesh Manandhar, Mohamed Mohamed, Laziana Ahmad, San Juana Castillo, Rachael Oakenfull and Angela Roman.

Thanks to my †father and mother who guided me during childhood. Likewise, thanks to Claudia Acosta, Juan Raphael Patishtan and my siblings for their company.

Thanks to Raul Rodríguez, Elsa Edith Rivera and Harmony Dawn for their greatest friendship and guidance.

Also special thanks to Mexican institutions that supported me throughout the PhD.

Author's Declaration

Unless otherwise acknowledged, I declare that this thesis is a presentation of original work and I am the sole author, except where otherwise stated.

For chapter 3, the 700,000 single-nucleotide polymorphisms (SNPs) was obtained from Department of Biological Statistics and Computational Biology, Cornell University.

This work has not previously been presented for an award at this, or any other, University.

All sources are acknowledged as References.

Chapter 1

Rice background, diversity and approaches to identify determinant genes for salt tolerance

1.1 Introduction

Plants encounter a wide range of biotic and abiotic stresses during their life cycle. These stresses may have stronger effect on plants in the coming years due to global warming and climate change. Global warming will alter global temperature and precipitation patterns which already highly impact the productivity of crops (Mickelbart *et al.*, 2015; Oppenheimer and Anttila-Hughes, 2016). It is expected that weather and climate patterns will intensify, including a rise in the frequency of natural disasters.

In biology, stress refers to biotic and abiotic stimuli or conditions out of the range that supports optimal growth and development of an organism. Plant growth is negatively affected by stress. Regarding biotic stresses, plants face challenges from pathogen and pest attacks. In the future, these may be more devastating and include new pathogens and pests. There is the concern that many pests could alter their ecology, increase in genetic variation and increase their resistance to chemical controls (Strange and Scott, 2005; Fitt *et al.*, 2016).

The main abiotic stresses that cause crop yield reduction are drought, flooding, extreme temperatures and salinity (Bray *et al.*, 2000). In particular salinity affects nearly one billion hectares of arid and semi-arid areas of the world (Dagar and Minhas, 2016) and this will expand in the coming years. In most cases, salinity has a high impact on growth when the Na^+ concentration in the growth substrate is 4 deciSiemens per meter (dS/m) or more. This is equivalent to approximately 40 mM NaCl with an osmotic pressure ~ 0.2 MPa (Bernier *et al.*, 2008). Most land plants are glycophytic and unable to complete their life cycle above this salt concentration, however, halophytes can survive at much higher concentrations of Na^+ (Pantoja *et al.*, 1989; Albert, 1975; Hasanuzzaman *et al.*, 2014).

Soil salinity occurs naturally in many places while also being accelerated by human activities. Natural salinisation occurs in coastal regions where salt water inundates fields, but also phenomena such as rain and wind may carry and deposit high amounts of salt on the soil surface. To illustrate, rain containing 10 mg k^{-1} of salt would deposit 10 kg ha^{-1} for each 100 mm of rainfall per year (Munns and Tester, 2008). Agricultural activities can increase soil salinisation due to land clearing for agriculture, incorporation of high amounts of fertilisers and, most of all, improper irrigation

management (Gustafsson and Westman, 2002; Mahmuduzzaman *et al.*, 2014; Smedema and Shiati, 2002).

There are agricultural practices to mitigate soil salinity. Improving drainage and applying high water table irrigation techniques can temporary displace the excess of Na^+ in soils (Soda *et al.*, 2016; Oliveira *et al.*, 2016). In addition, increasing levels of Ca^{2+} and incorporation of manure may counteract the negative effect of Na^+ (Andreola *et al.*, 2016; Jabeen and Ahmad, 2016).

1.2 Salinity Tolerance

1.2.1 Tolerance and sensitivity to salinity

Tolerance refers to a plant's capacity to respond to external stimuli without significant changes in phenotype. The phenotype of a plant is the expression of the plant's genetic constitution, or genotype, modified by the environment. Susceptibility or sensitivity refers to an organism's negative response to external stimuli, accompanied by significant changes in phenotype. Typically, a plant's tolerance will depend on the genes that are functioning at the stage of development during which the stress occurs. For most stress conditions, tolerance to salinity can be defined in absolute and relative values. For example, growth rates per day (g per day) or relative growth rates (RGRs) can be used to compare levels of tolerance (Caton *et al.*, 2003). Alternatively, changes in growth rates, relative to the non-stressed condition, can be applied.

1.2.2 Agronomical phenotypes: biomass and grain-yield

Salinity affects plants at all stages of development and affects all tissues. In an agronomic context, plant growth is not necessarily the most convenient or appropriate parameter to measure. In the case of cereals such as rice a reduction in grain yield may be the most important trait. In general, plant biomass (composed of roots and shoot tissues) correlates well with other, easily accessible traits such as plant height or number of tillers (Gain *et al.*, 2004). However, recent study showed that grain yield in rice is only associated with flag leaf biomass but not with the whole plant biomass (Ahmadizadeh *et al.*, 2016).

1.3 Plant Responses to Salinity

1.3.1 Halophytes and glycophytes

At low concentrations, Na^+ is often beneficial to cereals. For example, grass and wheat grown under low concentrations of Na^+ have shown positive growth responses, particularly in conditions with limited K^+ supply (Elzam and Epstein, 1969; Box and Schachtman, 2000). Recent studies have also shown that mild salinity improves strawberry and citrus fruit quality (Galli *et al.*, 2016; Yamada *et al.*, 2015). However, in high saline conditions (> 4 dS), most plants are unable to complete their life cycle. Wheat, barley and rice are classified as glycophytes but their tolerance to salinity is widely different. Wheat and barley are the most tolerant cereal crops while rice is the most salt sensitive (Bado *et al.*, 2016). Furthermore, within the same species, there is also a wide variety of salt tolerance.

Halophytes can grow and complete their life cycle at levels of salinity that may reach 500 mM NaCl or more. Halophytes are capable of this feat through multiple mechanisms which include efficient compartmentalisation of Na^+ in vacuoles for osmotic regulation and removal of excess Na^+ through specialised organs such as bladders and glands (Grigore *et al.*, 2014; Flowers *et al.*, 2015). For example, the halophytic wild rice, *Porteresia coartat* Tateoka has been shown to grow in high saline conditions and exclude Na^+ through root hairs (Garg *et al.*, 2013; Flowers *et al.*, 1990).

1.3.2 Plant responses to salt stress: two distinct phases through time

Salinity affects in osmotic and ionic ways and plants may respond to these conditions by using different mechanisms and genes. The osmotic component of salinity occurs after the onset of salt, which leads to the reduction of water transport and partial stomatal closure. Interestingly, plants temporary adapt to saline conditions using K^+, Na^+ and osmoprotectants for osmoregulation (Le Rudulier *et al.*, 1984; Chen *et al.*, 2005). However, in long term salt exposure, plants are affected by the continuing osmotic damage plus the excessive accumulation of Na^+ in plant cells, i.e. the ionic component of salinity. High concentrations of Na^+ inhibit enzyme activities and compete against K^+ which is particularly important for enzyme functions (Bertorello *et al.*, 1991; Tester and Davenport, 2003). To counter ionic toxicity, plants have mechanisms to compartmentalise Na^+ in vacuoles and to remove the excess of Na^+ from the cytosol to the apoplast where it is less toxic (Davenport *et al.*, 2005; Mühling and Läuchli, 2002; Maathuis and Amtmann, 1999).

1.4 Rice Background and Domestication

Rice has been farmed for thousands of years and nowadays is a staple food in over a hundred countries. The genus *Oryza* belongs to the tribe *Oryzeae* of the family *Poaceae*. There are 12 genera within the *Oryzeae* tribe (Vaughan *et al.*, 2003). The genus *Oryza* contains approximately 22 species of which 20 are wild species and two, African *O. glaberrima* and Asian *O. sativa*, are cultivated species (Vaughan *et al.*, 2003). The *O. glaberrima* is grown in West African countries while *O. sativa* is the most widely grown throughout the world.

African rice, *O. glaberrima,* is a species closely related to *O. sativa*. The global interest of *O. glaberrima* is its tolerance to abiotic stress conditions and yields (Sikirou *et al.*, 2016; Kikuta *et al.*, 2016). The *O. glaberrima* has five groups and its domestication occurred between 4000 and 5000 years ago along the Niger River western Africa (Manning and Timpson, 2014; Meyer *et al.*, 2016; Semon *et al.*, 2005).

The *O. sativa* derives from several groups of the *Oryza rufipogon* and their domestication occurred through multiple events. The domestication of *O. sativa* may have occurred between 12,000 and 7,000 years ago. Based on chloroplast, low-copy nuclear markers and 108 accessions, Huang *et al.* (2012a) identified two genetically distinct *O. rufipogon* groups. Using a higher number (~280) of accessions, Kim *et al.* (2016) identified six *O. rufipogon* groups. These groups of accessions showed a wide geographical distribution. The north-eastern group (Ruf-I) and the group from South Asia and Indochinese peninsula (Ruf-II) were identified by Huang *et al.* (2012a), while Kim *et al.* (2016) found that *O. rufipogon* was distributed in Nepal, Papua New Guinea, Himalayan mountains, South and South East Asia. Three of six groups might have derived from *Oryza nivara* and the other three groups might have evolved from *O. rufipogon* lineages. Therefore, these groups of the *O. rufipogon* were broadly adapted to diverse climates and would have provided wide genetic diversity to the *O. sativa* domestication.

Rice *indica* and *japonica* populations derived from *O. sativa* species. Over a thousand years ago, people recognised that *indica* (Hsien) and *japonica* (Keng) are the two main domesticated varieties (Callaway, 2014). This ancient domestication of *indica* and *japonica* might have occurred based on phenotypic traits such as plant architecture and grain yield (Jin *et al.*, 2008; Tan *et al.*, 2008). Thousands of years later, many researchers have taken the task of identifying the genetic diversity of these varieties and their possible lineages, by using different genetic markers and approaches.

There are several hypotheses for the origin and domestication events of *indica* and *japonica* populations. Findings of Huang *et al.* (2012a) showed that the *indica* population might have derived from a north-eastern Asian *O. rufipogon* group. However, Kim *et al.* (2016) demonstrated that two of six groups from Himalayan mountains and south-east Asia showed relationships with the *indica* and *japonica* populations. *Indica* rice is predominant in tropical and subtropical regions while *japonica* rice is more adapted to temperate regions (Zhang *et al.*, 1992). The domestication of either *indica* or *japonica* might have occurred through local, introduced and improved ancient rice varieties.

Nowadays, rice is grown in either lowland or highland conditions. The former refers to paddy fields while the latter refers to the direct sowing and growth of seeds in non-flooded aerobic conditions (Bernier et al., 2008). The cultivation of rice in paddy fields consists of growing rice in lands with ridges and irrigation which is achieved through canals. In highland conditions, rice is grown in rain-fed, naturally well-drained soils without surface water accumulation, usually without irrigation system (Ahmadi *et al.*, 2004). The rice grain yield in low and highland conditions is variable, typically ranging from 2-7 tons per hectare (Weerakoon *et al.*, 2011; Bernier *et al.*, 2007; Koutroubas and Ntanos, 2003).

1.4.1 Traits for rice domestication

Plant architecture and grain yield traits were critical traits for rice domestication. Research findings have shown that *O. rufipogon* has a wide tiller angle and short stature with many tillers, whereas domesticated rice shows relatively erect growth and fewer tillers which allows for effective high-yield cultivation (Jin *et al.*, 2008; Tan *et al.*, 2008). Ancient people might have considered the amount of grains that early domesticated plants produced and nowadays this is one of the most important traits. Much study has shown that there is a relationship between erect plant architecture and high grain yield in cultivated rice (Tan *et al.*, 2008; Li *et al.*, 2009). With grain yield having been the main breeding objective, many scientists are now working on finding and characterising alleles that were lost during rice domestication, especially genes that may be related to tolerance of biotic and abiotic conditions.

1.4.2 Rice genetic diversity

The genetic diversity of *O. sativa* is summarised into *indica* and *japonica* populations. Both of the main populations contain thousands of varieties with a wide range of resilience phenotypes for different conditions (IRRI, 2016). Furthermore, the *indica* population is divided into *indica, australis* and *admixed-indica* subpopulations, whereas *japonica* is divided into *tropical-japonica*, *temperate-japonica*, *aromatic* and *admixed-japonica* (McCouch *et al.*, 2016; Huang *et al.*, 2010). Much study

has been done to find differences between *indica* and *japonica* populations for grain yield, morphological and physiological responses to biotic and abiotic conditions. According to research, the *indica* population is genetically more diverse than the *japonica* population when using different markers and either using a small or medium group of rice accessions (Zhang *et al.*, 1992; Ni *et al.*, 2002). Regarding morphological traits, Han et al. (2016) found that heading date is similar in both of the populations but there was no common allele. Moreover, Bai *et al.* (2016) identified common and different alleles between *indica* and *japonica* populations for panicle architecture. Furthermore, Ntanos and Koutroubas (2002) showed significant difference between *indica* and *japonica* populations for biomass and grain yield. Regarding biotic and abiotic stresses, there was no significant difference between *indica* and *japonica* tolerance to rice blast pathogen (Zhu *et al.*, 2016a). The temperature, drought and salinity effects on growth of plants seem to be different between *indica* and *japonica* populations (Yoshida and Hara, 1977; Lee *et al.*, 2003; Xuan *et al.*, 2016). Furthermore, within populations, there are also differences among varieties in regard to drought and salinity tolerance along rice plant life (Munasinghe and Price, 2016; Kumar *et al.*, 2015).

1.5 Impact of Salinity on Agriculture and Rice

Salinity is one of the major limiting factors that reduce crop yields. Globally, salt affected areas account for about ~ 400 million ha saline soils and ~ 430 million ha sodic soils (Land, 2008). Salinisation of agricultural lands is particularly widespread in arid and semiarid environments where crop production requires designed irrigation schemes. The concentration of salt in agricultural lands varies between 0.5 and 32 dS/m (Rasool *et al.*, 2013). This wide variation depends on soil agricultural history, season, topography, soil physical and biological properties, water and fertiliser sources, irrigation and drainage systems (Acosta *et al.*, 2011; Kaur and Singh, 2011). On the other hand, the salinity tolerance in cereal and vegetable crops is also widely variable (Hanson *et al.*, 1999). For example, wheat, sugar beet, canola, cotton, and barley are relatively high salt tolerant crops. However, crops such as rice, sugar cane, carrot, onion, lettuce, and strawberry are salt sensitive crops (Patade *et al.*, 2009; Shannon and Grieve, 1998).

1.5.1 Impact of salinity on rice production: lowland and highland conditions

Rice is considered as a major food crop across major countries worldwide. Rice is grown in over 100 countries covering an area of more than 150 million hectares of land. Most of these agricultural areas are salinised to some degree. Nevertheless, the production of rice has increased from 257 million tons to 600 million tons between 1966 and 2000. Nowadays, the average rice yield fluctuates between 5 and 7 tons per hectare (Khush, 2005; Weerakoon *et al.*, 2011). In the near future,

the demand of this cereal will increase due to the rising human population. To meet this challenge, scientists work on developing new varieties using traditional breeding and genetic engineering approaches to increase rice yield and tolerance to biotic and abiotic conditions.

To increase rice production in a sustainable manner, farmers need rice strains that are optimised for different environmental conditions. During the last decades, diverse hybridisation and genetic engineering approaches have been carried out. Regarding salinity tolerance, there are many approaches for rice breeding. Inbreeding is one of the options and consists of crossing between two or more different rice varieties and subsequent selection through multiple cycles of self-pollination. Rice hybridisation is an approach which consists of crossing two genetically distinct parents. A third approach consists in the introduction of a foreign gene(s) through genetic engineering (transgenic plants) techniques. Furthermore, there are approaches such as pure-line and pedigree selection methods, bulk and backcross methods as well as recombinant inbred lines (RILs) and generation of near isogenic lines (NILs) which have been extensively used in rice. There are limitations and advantages to all these different approaches: for example, crossing rice varieties may take years. Moreover, producing and consuming transgenic rice is still a controversial topic.

There are thousands of rice varieties across the globe. According to IRRI (2016), there are more than 127,000 rice accessions and wild relatives. However, only a small proportion of natural accessions has been assessed for traits in saline conditions using different screening approaches and experimental designs (Kumar *et al.*, 2015; Platten *et al.*, 2013). Recent findings have identified many salt tolerant cultivars from *O. glaberrima* and *O. sativa* species where the salt tolerance of accessions is related to low Na^+ and high K^+ concentrations in roots and shoots (Islam *et al.*, 2016; Rahman *et al.*, 2016; Sakina *et al.*, 2016). Some of the most recurrent salt tolerant cultivars are Pokkali and FL478, whereas salt sensitive cultivars are M-202, Nipponbare, IR50 and IR20 (Xu *et al.*, 2006b; Platten *et al.*, 2013; Zeng and Shannon, 2000). Within the known accessions there are thousands of hybrid rice cultivars. Huang *et al.* (2016) characterised quantitative traits such as grain yield, flowering time and plant architecture in over 10,000 hybrid lines. There are also classic elite cultivars used as recurrent parents such as IR64, Tequin and IR68552-55-3-2.

1.6 Physiological Responses to Salinity

1.6.1 Salinity effects on growth and development

The proliferation of plants in saline environments will depend on their ability to react quickly and efficiently to osmotic and ionic components of salinity. Several experiments have confirmed that salinity delays seed germination and can cause temporary growth cessation during vegetative and reproductive stages. Regarding seed germination, recent studies have shown that the Delayed Seed Germination (*OsDOG1*) and Histone Deacetylase (*OsHDA750*) genes play essential roles in rice seed germination (Kumar *et al.*, 2015; Vibhuti *et al.*, 2015; Zhao *et al.*, 2016a).

Salinity affects early and late plant growth and development. Saline conditions have detrimental effects on rice growth during the first 4 weeks (early growth stage) and between 11 and 15 weeks (reproductive stage) after germination (Krishnamurthy *et al.*, 2016; Ahmadizadeh *et al.*, 2016). Physiological studies have shown that salinity ceases shoot elongation after ~ 20 minutes of salt exposure and growth is reactivated (osmoregulation) after 24 hours (Yeo *et al.*, 1985; Fricke *et al.*, 1996). This reduction of growth is caused by the osmotic component of salinity (Shabala, 2000) and there may be hundreds of determinant genes for the osmoregulation. The osmoregulation in plants is achieved through the accumulation of ions such as K^+, Na^+ and Cl- as well as sucrose and proline as osmoprotectants.

1.6.2 Salinity effects on plant nutrition

Terrestrial plants require 14 essential nutrients at different proportions. These nutrients are classified into macronutrients and micronutrients which are needed in relatively high and low concentrations, respectively (Maathuis and Diatloff, 2013). Salinity causes direct toxicity and nutritional imbalance because of excessive accumulation of ions in tissues. Particularly, shoot Na^+ has been shown to be highly and negatively correlated with salinity tolerance in rice (Platten *et al.*, 2013; Munns *et al.*, 2006). Despite the fact that roots play crucial roles in the transport of ions, root tissues Na^+ is not correlated with salinity tolerance in rice (Zheng *et al.*, 2015). Furthermore, high Na^+ concentration in soil is thought to disturb K^+ acquisition by competing with binding sites in transport systems that mediate K^+ uptake (Hasegawa *et al.*, 2000). Therefore, salinity leads to low K^+ in plant tissues and in turn to the reduction of growth, delay in flowering and low grain yield. Recent findings have shown that application of manure and application of proline increase mineral uptake in rice grown in saline conditions and as such promote tolerance (Dhar *et al.*, 2016). Furthermore,

feeding rice with NO_3^- and silicate (Si) increases salt tolerance through promoting high plant biomass and reducing long distance transport of Na^+ to shoots (Gao *et al.*, 2016; Flam-Shepherd, 2016).

1.6.3 Salinity effects on stomatal conductance

Salinity in the root zone decreases the osmotic potential of the soil solution. Under low water potential, abscisic acid (ABA) is produced from biosynthesis in plant roots to be transported through the xylem vessels to shoot tissues (Mizrahi *et al.*, 1970; Jia *et al.*, 2002; Wolf *et al.*, 1990). ABA binds the receptors in the plasma membrane of paired-guard cells for stomatal closure to optimise the balance of water loss by transpiration and carbon dioxide (CO_2) uptake (Umezawa *et al.*, 2010). The paired-guard cells form the leaf stoma, the shape of which change with the collective pressure of water, K^+, chloride (Cl^-) and malate pushing the plasma membrane against the cell wall, thereby modifying the open state of the stoma. There are genes such as inward-rectifying and outward K^+ channels (Li *et al.*, 1998; Nakamura *et al.*, 1995) and voltage-dependent anion channels (VDACs) which may have an essential role in this process (Keller *et al.*, 1989).

1.6.4 Salinity, oxidative stress and antioxidant responses

Research has extensively shown that reactive oxygen species (ROS) function as a common signalling molecule in both developmental and plant stress signalling. ROS include hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen ($^1O_2$*) and the hydroxyl radical (•OH). The cell organelles are the main sources of ROS: Chloroplasts produce ROS as a by-product of photosynthesis and ROS production in mitochondria results from reduction of oxygen in the respiratory chain complex. Furthermore, metabolic processes of peroxisomes and endoplasmic reticulum also produce ROS (El-Osta and Circu, 2016; Inupakutika *et al.*, 2016; Fan *et al.*, 2015). A balance of ROS production and scavenging is essential for plant stress signalling, plant growth and development. In saline conditions, ROS drastically increase. This may point to tissue damage and many studies have shown that salt tolerance of plants is related to maintaining low levels of ROS in plants (Badran *et al.*, 2015; Zhu *et al.*, 2007). ROS overproduction alters metabolic processes which may lead to oxidative bursts that can cause damage to proteins, DNA, and lipids and result in cell death (Baxter *et al.*, 2014; Apel and Hirt, 2004; Miller *et al.*, 2010).

Plant cells are equipped with enzymatic and non-enzymatic defence mechanisms against ROS. Scavenging of ROS through enzymes include cytosolic ascorbate peroxidases (APXs), chloroplastic and cytosolic glutathione reductases (GRs), superoxide dismutases (SODs), cytochrome P450 reductase enzymes, etc. (Miller *et al.*, 2007; Hu *et al.*, 2005; Alscher *et al.*, 1997; Hanukoglu, 2006). Recent data revealed that high activities of SOD, catalase and peroxidase enzymes enhance

salt tolerance in Arabidopsis under saline conditions (Zaidi *et al.*, 2016). SODs catalyse the reduction of O_2^- to H_2O_2, and then H_2O_2 is converted to H_2O through the further action of catalases and peroxidases. Other mechanisms of H_2O_2 removal are carried out by ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase.

1.6.5 Salinity and plant senescence

Plant senescence is a natural process and is accelerated under stressful conditions. Physiological studies in plants have extensively covered that senescence is related to high production of ABA, ethylene and ROS as well as reduction of photosynthesis and cytokinin levels (Talla *et al.*, 2016; Kao and Yang, 1983; Jajic *et al.*, 2015). Different approaches have identified and characterised several gene families either accelerating or delaying senescence in rice. Senescence associated genes (SAGs) are genes related to natural senescence in different conditions and their expressions vary between plant organs (Lee *et al.*, 2001). The expression of SAGs can be induced by ABA and ROS controlled by different genes (Rosenvasser *et al.*, 2006; Liang *et al.*, 2014). For example, phytochrome-interacting TFs (PIFs) and several no-apical meristem (NAM), Arabidopsis transcription activation factor (ATAF) and cup-shaped cotyledon (CUC) NAC domain TFs have been shown interacting with SAGs (Zhao *et al.*, 2016b; Sakuraba *et al.*, 2014). Specifically, SAG113 is sensitive to ABA and is related to delayed senescence in Arabidopsis, whereas SAG18 was highly expressed in ozone treatment but has yet to be characterised for saline conditions (Zhang *et al.*, 2012; Miller *et al.*, 1999). The expression of SAGs and related genes to plant senescence vary along a plant's life cycle (Lee *et al.*, 2001). For example, Rice Early Flowering3.1 (*OsELF3.1*) has been shown to have a role in rice senescence only during heading date stage (Sakuraba *et al.*, 2016).

Delaying leaf senescence increases plant tolerance to drought and salinity conditions. Overexpression of a CCCH-tandem zinc finger protein (*OsTZF1*) and S-Domain Receptor-Like Kinase (*OsSIK2*) have been shown to delay leaf senescence and improve salt tolerance in rice (Jan *et al.*, 2013; Chen *et al.*, 2013). Moreover, overexpression of NAM, ATAF and CUC (NAC) TFs have also been shown to play roles in drought and salinity tolerance in rice by delaying senescence (Liu *et al.*, 2016b).

Plant hormones play essential roles in the process of plant senescence during biotic and abiotic stress conditions. Decades ago, research showed that premature leaf senescence is one of the results of the reduction of cytokinin production in roots (Sitton *et al.*, 1967). Further studies have confirmed the importance of this hormone for promoting accumulation of biomass, enhancing grain yield and plant tolerance to adverse conditions (Zahir *et al.*, 2001; Chakrabarty *et al.*, 2009; Peleg *et*

al., 2011). Moreover, recent findings have shown that cytokinin-O-glucosyltransferase was downregulated in arsenate conditions but its response in saline conditions is yet to be characterised (Chakrabarty *et al.*, 2009).

1.7 Na^+ Uptake and Distribution

1.7.1 Na^+ uptake

It is well established that Na^+ can enter through non-selective ion channels such as glutamate-like receptors (GLRs) (Tapken and Hollmann, 2008) and cyclic nucleotide gated channels (CNGCs) (Guo *et al.*, 2008). Moreover, Na^+ can also enter root cells through two classes of high-affinity K^+ transporters (HKTs). The HKTs have two functions: (i) to take up Na^+ from the soil solution to reduce K^+ requirements in conditions of K^+ starvation and (ii) to reduce the excess of Na^+ through loading and unloading of this ion in xylem sap (Rodríguez-Navarro and Rubio, 2006).

HKTs are subdivided into two groups: The HKT1 and HKT2 are different in terms of amino acids in the first pore loop of their proteins i.e. gene members of HKT1 have a serine residue and gene members of HKT2 have a glycine residue (Mäser *et al.*, 2002; Garciadeblás *et al.*, 2003). Furtermore, the HKT1 members specifically mediate Na^+ transport. For example, *OsHKT1;3* is a selective Na^+ transporter and is highly expressed in root cortex and vascular tissues (Jabnoune *et al.*, 2009; Rosas-Santiago *et al.*, 2015). HKT2 members can mediate Na^+ and/or K^+ transport (Fu and Luan, 1998; Golldack *et al.*, 2002; Horie *et al.*, 2001; Rubio *et al.*, 1995; Schachtman and Schroeder, 1994). As examples, *OsHKT2;1*, *OsHKT2;2*, *OsHKT2;3* and *OsHKT2;4* mediate the transport of Na^+ to partially substitute the role of K^+ in K^+-starved conditions (Horie *et al.*, 2007; Miyamoto *et al.*, 2015).

1.7.2 Long distance transport and distribution of Na^+

Once Na^+ is inside the root vascular stele, it is transported through xylem vessels to shoots. Many physiological studies have shown that the efficient control of Na^+ loading and unloading in xylem sap is related to salinity tolerance (James *et al.*, 2006; Suzuki *et al.*, 2016). The loading and unloading of Na^+ in xylem sap is mediated through HKTs, shaker-like outward channels (SKORs, KORCs and NORCs), cation proton exchangers (CHXs) and salt overly sensitive 1 (*SOS1*) (Katschnig *et al.*, 2015; Platten *et al.*, 2013; Wegner and De Boer, 1997; Olías *et al.*, 2009). For example, *OsHKT1;4* and *OsHKT1;5* have been shown to be determinant genes encoding transport of Na^+ in xylem sap (Ren *et al.*, 2005; Suzuki *et al.*, 2016; Platten *et al.*, 2013). In Arabidopsis plants, *AtCHX21* and *AtHKT1;1* play a role in the regulation of Na^+ in xylem sap (Hall *et al.*, 2006; Davenport

et al., 2007). The potassium channel SKOR has been shown to have roles in loading and unloading K^+ in xylem sap in plant species and may lead to high values of K^+ and Na^+ ratio (Shabala *et al.*, 2010; Domingo *et al.*, 2016; Hu *et al.*, 2016c).

1.7.3 Vacuolar Na^+ compartmentation and Na^+ efflux to the apoplast

Na^+ compartmentation in vacuoles and removal of Na^+ to the apoplast are related to salinity tolerance. One classic example for a protein involved in Na^+ compartmentation is the tonoplast Na^+/H^+ exchanger 1 (*NHX1*). This gene is activated by the complex formed between the calcineurin B-like protein (CBL10) and CBL-interacting serine/threonine-protein kinase 24 (CIPK24). The *NHX1* is localised in the tonoplasts of plant cells and moves Na^+ from the cytoplasm into vacuoles (Bassil *et al.*, 2011; Kim *et al.*, 2007; Lv *et al.*, 2012). Recent findings have shown that the expression of the NHX1 is controlled by TCP, cysteine-rich (CXC) and RWP-RK domains of transcription factors (Almeida *et al.*, 2016). Meanwhile, the removal of Na^+ to the apoplast is also an essential mechanism for salt tolerance. The salt overly sensitive Na^+/H^+ antiporter (*SOS1*) has been extensively studied its role for salinity tolerance in different plant species (Yue *et al.*, 2012; Yang *et al.*, 2009; Feki *et al.*, 2011). The *SOS1* is activated after a transient increase of Ca^{2+} in the cytosol which is detected by CBL4. The CBL4 binds Ca^{2+} and then interacts with CIPK24. Subsequently, the formed CBL4-CIPK24 complex phosphorylates the *SOS1* (Halfter *et al.*, 2000; Liu *et al.*, 2015a; Yang *et al.*, 2009).The *SOS1* mediates the removal of Na^+ from cytoplasm to the apoplast (Gao *et al.*, 2016).

1.8 Genetic Approaches to Identify Salt Tolerance Genes

Securing genetic diversity and selecting efficient progeny are the most important factors in plant breeding. To identify genes related to a trait, there are many approaches such as microarrays, forward and reverse genetic screening as well as genetic and physical mapping. Microarrays use genetic DNA markers to quantify the relative level of gene expression while forward and reverse genetic screens use mutants. Genetic mapping uses information from a second-generation population of crossed lines and physical mapping uses natural accessions.

1.8.1 Microarray analysis

The microarray approach provides a format for the measurement of the expression of thousands of genes in a single experiment. By using complementary DNA (cDNA), microarrays have identified hundreds of genes related to various traits including responses to biotic and abiotic stress conditions (Edwards *et al.*, 2008; Hossain *et al.*, 2016; Wu *et al.*, 2016). For example, Liu *et al.* (2016a) have recently identified the Constans (CO)-like gene (*OsGHd2*) which has been shown to

increase rice grain yield in optimal conditions. Regarding salinity, this approach has also identified over two hundred candidate genes for K^+ and Na^+ homeostasis in plant cells (Hossain *et al.*, 2016).

Moreover, this apprach has identified genes related to signal tranduction such as trancription factors which may have specific roles in saline conditions. For example, Jangam *et al.* (2016) identified a disease resistance protein (*Os*RGA1) that has been shown to have pleitropic functions in rice and Arabidopsis (Chakraborty *et al.*, 2015). Furthermore, prototypic member of the DEAD-box protein family (*Os*Psp68) and stress asscociate little protein 1 (*Os*SALP1) were identified through microarrays and may have roles in ABA and ROS plant-stress signalling pathways (Banu *et al.*, 2015; Yuan *et al.*, 2016).

1.8.2 Forward and reverse genetic screening

Forward and reverse genetic screening approaches have identified genes that are determinants in biotic and abiotic conditions. Forward-genetic screening consists of the exposure of mutants to a condition and the identification of different phenotypes compared to a wild-type plant (Stamatiou *et al.*, 2013; Kurowska *et al.*, 2011). In contrast, reverse-genetic screening consists of identifying a gene function and then identifying the associated phenotype (Sessions *et al.*, 2002). Both forward and reverse genetic screening have limitations when identifying genes with pleiotropic functions and detection of small genes because of their low effectiveness for mutagenesis (Krysan *et al.*, 1996; Till *et al.*, 2004). Furthermore, forward-genetic screening is laborious and time consuming with most of the phenotypes being the result of more than one gene (Vidaurre and Bonetta, 2012; Wang and Sherwood, 2011). These approaches have been extensively used for Arabidopsis and rice T-DNA mutants, and have identified novel determinant genes related to the tolerance of biotic and abiotic stress conditions (Page and Grossniklaus, 2002; Koh *et al.*, 2007).

A classic example of a forward screen is the discovery of *sos1* mutants which showed hypersensitivity to Na^+ and further studies confirmed that *SOS1* enhances salt tolerance by the extrusion of Na^+ from the cytoplasm to the apoplast (Wu *et al.*, 1996; Yang *et al.*, 2009). Moreover, some members of CBLs, CIKPs, calcium-dependent protein kinases (CPKs) and other genes have also been identified through these approaches (Xu *et al.*, 2006a; Ahmad *et al.*, 2016; Hwang *et al.*, 2016; Zhang *et al.*, 2006).

1.8.3 Quantitative trait locus (QTL)

Genomic mapping, or quantitative trait locus (QTL) mapping, measures associations between genetic markers and traits. Over many years, QTL analysis has improved in efficiency and robustness using different statistical models, genetic markers and crossed plant populations (Wang *et al.*, 1999;

Huang *et al.*, 1997; Lafitte *et al.*, 2004). Rice genomic mapping has successfully identified alleles using random amplified polymorphic DNA (RAPD), sequence tagged sites (STSs), restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs) markers (Masood *et al.*, 2004; Yu *et al.*, 2004; Shimizu *et al.*, 2004; Li *et al.*, 2006). Using single nucleotide polymorphisms (SNPs) as molecular markers for genotypic data is becoming a common technique.

The type and the number of genetic markers may have a direct effect on the resolution of the association between genotype and phenotype (Mei *et al.*, 2005; Harushima *et al.*, 1998; Kurata *et al.*, 1994). In general, the QTL approach statistically identifies genomic regions that are hypothetically responsible for genetic variation in a trait. These specific regions in a genome are thought to harbour genetic variants that make a significant contribution to the expression of complex traits (Hu *et al.*, 2012; Pandit *et al.*, 2010).

The QTL approach has identified hundreds of minor and major QTLs for rice grown in saline conditions (Waziri *et al.*, 2016). To illustrate the efficiency of the QTL approach, recent outcomes have identified many QTLs related to salt tolerance index, plant growth, and for K^+ and Na^+ concentrations in roots and shoots (Tiwari *et al.*, 2016; Gimhani *et al.*, 2016; Lin *et al.*, 2004). The main limitation of QTL mapping is that the approach only determines approximations of the QTLs within big genomic regions which can contain hundreds of genes. Moreover, genomic mapping uses populations created by crossing natural accessions to produce a set of lines in which the alleles of the parents segregate and, depending on the species, backcrossing may take months or even years (Vaughan, 2015; Weigel, 2012; Cai and Morishima, 2002).

1.8.4 Genome wide association studies (GWAS)

Genome wide association study (GWAS) or physical mapping was developed as an innovative method for studying associations between genotypes and phenotypes. A main difference to traditional genomic mapping is the ability of GWAS to use SNPs as molecular markers which precisely position genetic markers in the genome (Si *et al.*, 2016). Moreover, GWAS can handle up to approximately one million SNPs and 10,000 natural accessions as a mapping population (Lee *et al.*, 2015; Lipka *et al.*, 2012; Huang *et al.*, 2010; Chen *et al.*, 2014b). GWAS identifies SNP markers that are significantly associated with a trait of interest across a diverse range of natural accessions. For species such as rice, Arabidopsis, maize, wheat, barley and other crops, GWAS has contributed to revealing rich genetic architectures underlying complex traits (McCouch *et al.*, 2016; Atwell *et al.*, 2010; Xue *et al.*, 2013; Chen *et al.*, 2016; Pasam *et al.*, 2012). Furthermore, GWAS has revealed association signals and identified genes related to rice domestication, panicle architecture, grain-yield

and grain-quality as well as determinant genes involved in biotic and abiotic conditions (Crowell *et al.*, 2016; Zhao *et al.*, 2011; Kang *et al.*, 2015; Ueda *et al.*, 2015).

GWAS is conducted through a free software package using different statistical models and principal component analyses. Genome Association and Prediction Integrated Tool (GAPIT) and GenABEL are open-source packages that perform GWAS. By default, GAPIT uses compressed mixed linear model (CMLM) and population parameters previously determined (P3D) (Lipka *et al.*, 2012). GenABEL can be added to different statistical models such as naïve and Family-Based Score Test for Association (FASTA). The naïve statistical model in GWAS does not consider population structure adjustment which can reveal false positive associations (Zhao *et al.*, 2011; Li *et al.*, 2014; Hayes, 2013). However, FASTA uses a mixed linear model to correct false positive associations (Chen and Abecasis, 2007). Depending on the nature of the population, there are other statistical models that can adjust false positive associations on GWAS (Svishcheva *et al.*, 2012; Hu, 2015).

1.9 Determinant Genes for Salinity Tolerance

This section highlights the relatively well-known genes identified through different approaches, while summarising the effectiveness of determinant genes for salinity tolerance in transgenic agricultural crops. During the last decade, scientists across the world have identified and characterised hundreds of genes related to sensing saline conditions, plant adaptation and senescence, together with an emphasis on determinant genes for K^+ and Na^+ uptake, transport and ion homeostasis in plant cells. However, few genes have been used for genetic engineering to create salt tolerant crops.

1.9.1 Gene sensors for saline conditions

Plant cells can perceive stimuli through multiple and complex sensors. Histidine kinases (KHs), mitogen-activated protein serine/threonine kinases (MAPKs), and receptor-like kinases (RLKs) are well-known osmotic sensors (Passricha *et al.*, 2016; Le Gall *et al.*, 2015; Kasprowicz, 2011). Cell wall-associated kinases (WAKs), leucine-rich repeat protein kinases (RPKs) and stress activated protein kinases (SAPKs) may also have roles in sensing these stimuli (de Oliveira *et al.*, 2014; Kohorn and Kohorn, 2012b; Xu *et al.*, 2013). On the other hand, Na^+ sensors have yet to be identified and characterised. Two decades ago, Wu *et al.* (1996) suggested that the long C-terminal of *SOS1* might sense Na^+. Recently, Nagarajan *et al.* (2016) identified a Na^+ binding site of root anion-permeable transporter (Bot1) which could also be a candidate gene for Na^+ sensor.

1.9.2 Determinant genes for the uptake and transport of K^+ and Na^+

Much research has been carried out to characterise determinant genes for the uptake, transport and distribution of K^+ and Na^+. Some members of the HKTs (Rodríguez-Navarro and Rubio, 2006), CNGCs (Guo *et al.*, 2008), NSCCs (Demidchik and Tester, 2002; Essah *et al.*, 2003; Maathuis and Sanders, 1993; Tyerman *et al.*, 1997), GLRs (Tapken and Hollmann, 2008) and TPCs (Kintzer and Stroud, 2016; Guo *et al.*, 2016; Peiter *et al.*, 2005) are relatively well characterised for Na^+ uptake.

Characterised genes for K^+ uptake include inward rectifying K^+ channel (*AKT1*), which is expressed in root hairs, epidermis, cortex and endodermis of the mature roots (Ahmad *et al.*, 2016; Desbrosses *et al.*, 2003), as well as certain members of high-affinity K^+ transporters (HAKs) which mediate K^+ uptake (Qi *et al.*, 2008). For long distance transport of ions, there are certain members of the HKTs (Suzuki *et al.*, 2016; Ren *et al.*, 2005; Platten *et al.*, 2013), CHXs (Hall *et al.*, 2006), HAKs (Shen *et al.*, 2015) and stellar K^+ outward rectifying channels (SKORs) (Gaymard *et al.*, 1998; Hu *et al.*, 2016c). The best-known Na^+ compartmentation and efflux proteins are NHXs and *SOS1* but there are many ion exchanger family genes such as calcium/hydrogen Ca^{2+}/H^+ antiporters (CAXs) and magnesium/hydrogen Mg^{2+}/H^+ and sodium/calcium Na^+/Ca^{2+} exchangers that might have roles in ion homeostasis in plant cells in saline conditions. Moreover, there are several cation and anion channels that play essential roles in the regulation of stomata opening in plants. For example, shaker type K^+ channels (KATs) mediate the transport of K^+ in guard cells for stomatal opening (Li *et al.*, 1998; Hwang *et al.*, 2013) which is important for osmotic regulation.

1.9.3 Developing salt-tolerant crops: using single and multiple genes

Securing genetic diversity and selecting efficient progeny are the most important factors in plant breeding. In addition, the combination of genetic engineering and conventional breeding programmes allows useful traits to be introgressed into commercial crops in a lesser amount of time. Researchers have used microarrays, forward and reverse genetic screening, and genetic and physical mapping to identify thousands of genes that may have roles in saline conditions. So far, only a few introgressed genes in elite rice cultivars have been successfully established in field conditions, and the agronomical traits have been shown to be unstable. The low effectiveness of the introgression of genes into elite rice cultivars is due to structural differences between chromosomes, sterility of seeds, germplasm with genetic variability and salinity tolerance is polygenic (Gaikwad *et al.*, 2014; Singh *et al.*, 2016b; Zhu, 2000). By using genes from halophytes and glycophytes, the NHX family gene is the most frequently used gene for rice transformation for salinity tolerance. Although genes such as vacuolar H^+-ATPase subunit cl (*SaVHAc1*), dehydration-responsive element binding (*AaDREB1*),

Capsicum annuum phospholipase A1 (*CaPLA1*) and others have shown their effectiveness in saline conditions. The effectiveness of transgenic plants for grain yield under controlled and field conditions is limited for rice and other agricultural crops (Table 1.1 and 1.2).

Table 1.1. Determinant genes for salinity tolerance in rice. Genes isolated from either halophytes or glycophytes to agricultural crops.

Gene	Foreign gene from	Remarks
[1]*AgNHX1*	*Atriplex gmelini*	14-day old plants were exposed to 100 mM NaCl during 3 days and recovery (no salt). High survival rate and low Na^+ uptake and transport to shoots.
[2]*SsNHX1*	*Sauceda salsa*	5-week old plants were exposed to salt conditions increasing NaCl concentration by 50 mM/3 days to reach 30 mM. High net photosynthesis rate and water retention. High cytosolic K^+/Na^+ ratio, Ca^{2+} and Mg^{2+}.
[3]*SaNHX1*	*Spartina anglica*	20-day old plants were exposed to 150 mM NaCl between 15 and 30 days. Healthy plants.
[4]*PutNHX*	*Puccinellia tenuiflora*	5-day old plants were exposed to 100, 300 and 1000 mM NaCl during 5 days. High shoot K^+ and low shoot Na^+
[4]*PutNHA*		
[5]*SaVHAc1*	*Spartina alterniflora*	4-week old plants were exposed to 100 and 200 mM NaCl during 3 days. High plant biomass, high relative water content, reduced stomata density. High K^+/Na^+ ratio.
[6]*AaDREB1*	*Adonis amurensis*	2-week old plants were exposed to 150 mM NaCl during 16 days. High salt tolerance at early growth stage and high chlorophyll content.
[7]*OsPP1a*	*Oryza sativa*	14-day old plants were exposed to 150 mM NaCl during 3 days. High plant height and growth rate. High activity of genes mediating ROS scavenging.
[8]*PDH45*	*Pisum sativum*	15-day old plant were exposed to 100 and 200 mM NaCl during 1 day. Regulation of Na^+ and ROS production.
[9]*BrCIPK1*	*Brassica rapa*	2-week old plants were exposed to 130 mM NaCl during 1 and 2 days. High plant biomass, water content, proline and sucrose for plant adaptation.
[10]*OsPEX11*	*Oryza sativa*	10-day old plants were exposed to 200 mM NaCl during 1 day. Regulation of genes encoding HKTs, *OsSOS1*, *OsNHX1* and *AKT1* as well antioxidant defence.
[11]*SsNHX1/AVP1*	*Saueda salsa and Arabidopsis*	5-week old plants were exposed to 300 mM NaCl during 3 days. Higher salt tolerance and K^+/Na^+ ratio in double gene insertion compared to single. High photosynthesis rate and low ROS production.
[12]*PtCYP714A3*	*Populus trichocarpa*	3-week old plants were exposed to 150 mM NaCl during 12 days and 10 days of recovery (no salt). High salt tolerance with high root and shoot Na^+ concentrations.

References: [1](Ohta *et al.*, 2002), [2](Zhao *et al.*, 2006b), [3](Lan *et al.*, 2011), [4](Kobayashi *et al.*, 2012), [5](Baisakh *et al.*, 2012), [6](Zong *et al.*, 2016), [7](Liao *et al.*, 2016), [8](Nath *et al.*, 2016), [9](Abdula *et al.*, 2016), [10](Cui *et al.*, 2016), [11](Zhao *et al.*, 2006a), [12](Wang *et al.*, 2016a).

Table 1.2. Determinant genes for salinity tolerance in agricultural crops. Genes isolated from either halophytes or glycophytes to agricultural crops.

Gene	Foreign gene from	Expressed in	Remarks
[1]*TaNHX2*	*Triticum aestivum*	*Capsicum annuum*	Plants were exposed to 50, 100 and 200 mM NaCl during two weeks. Salt tolerance was related to high proline, antioxidants for ROS scavenging and relative water content.
[2]*HVA1*	*Hordeum vulgare*	*Zea mays*	Plants were exposed to 100 and 200 mM NaCl during 10 days. Salt tolerance was related high plant biomass.
[3]*MdSOS2L1*	*Malus pumila*	*Solanum lycopersicum*	8-week old plants were exposed to 200 mM NaCl twice a week for 3 weeks. Salt tolerance in plants was related to high concentrations of antioxidants for ROS scavenging.
[4]*OsPGK2-P*	*Oryza sativa*	*Nicotiana tabacum*	15-day old plants were exposed to 100 mM NaCl until maturity. Transgenic plants showed high plant biomass and seed yield, besides enhancement of proline accumulation and ion homeostasis.
[5]*AtNHX1*	*Arabidopsis thaliana*	*Triticum aestivum*	Plants were grown in field saline conditions with an EC between 10.6 and 13.7 dS m-1 during 30 days. High salt tolerance and grain yield through low shoot Na^+ and high root and shoot K^+.

References: [1](Bulle *et al.*, 2016), [2](Nguyen and Sticklen, 2013), [3](Hu *et al.*, 2016a), [4](Joshi *et al.*, 2016), [5](Xue *et al.*, 2004).

1.10 Aims and Objectives

Rice is a highly-demanded food source in many countries; however, it is highly sensitive to saline conditions. Therefore, there is a concern for future rice production due to the increasing salinisation of agricultural land areas and it is crucial to identify novel genes and alleles associated with salinity tolerance in diverse accessions to build a foundation of knowledge which could safeguard the production of rice. By using different approaches, many scientists have identified and characterised over a hundred different determinant genes for salinity tolerance. However, salinity is a complex trait that may implicate thousands of genes during a plant's life cycle. For this reason, the main aims of this project are (a) to identify well-known genes and (b) to identify novel determinant candidate genes for salinity tolerance using GWAS and forward genetic screening. The current project divides across three research chapters using rice and Arabidopsis species as studies, to fulfil specific objectives in each section.

For the first research chapter, the objectives are (a) to assess a correlation between osmotic and salinity tolerance in rice cultivars and (b) to identify common physiological responses of rice cultivars to osmotic and saline conditions. For the second research chapter, the objectives are to identify relatively well-known and novel determinant genes for osmotic and ionic components of salinity through GWAS. This study also assesses the efficiency of GWAS to get insights of amino acid changes of SNP positions, and evaluates correlations between genetic and physical mapping approaches. For the third research chapter, the objective is to identify novel determinant genes for salt tolerance using a forward genetic screen.

Chapter 2

Physiological Characterisation of Rice Cultivars Exposed to Long and Short Terms of Osmotic and Saline Conditions

2.1 Introduction

Rice is the most popular cereal grain worldwide, accounting for 22% of the total energy intake of the human population. The demand of this cereal will increase due to rising human population. The production of rice grown either in lowland or highland conditions is affected by drought and salinity. Lowland refers to paddy fields, whereas highland refers to the direct sowing and growth of seeds in non-flooded aerobic conditions (Bernier *et al.*, 2008). Drought and salinity are considered the two major constraints that reduce grain yield of rice. Drought is caused either when there is poor precipitation or when there is no overlap between crop cultivation and the rainy season. Salinity is caused by excessive accumulation of Na^+ in soil due to natural geology, seawater ingression or improper agricultural management. One of the common effects of drought and salinity is an osmotic condition created from two different sources (Verslues *et al.*, 2006).

Osmotic and salinity conditions affect many aspects of plants growth and development. An osmotic condition refers to a situation where living cells and tissues are less than fully turgid. An osmotic condition can be created by using different solutes such as Polyethylene Glycol 4000 (PEG), mannitol and sorbitol. Regarding salinity, NaCl in relatively in high concentrations creates osmotic and ionic conditions. In osmotic conditions ≥ 0.2 MPa, plant growth, ion homeostasis and water relations are adversely affected and in extreme conditions, plants die. Much research has been carried out in rice cultivars responding to either PEG or NaCl conditions.

2.1.1 Osmotic and salinity responses in cereal crops

Several physiological traits are affected in rice when exposed to osmotic and saline conditions. Physiological characterisation in response to osmotic and saline conditions has been well measured with studies using a single cultivar and others using over a hundred different rice cultivars. The reduction of growth is the most common effect of PEG and NaCl conditions in cereal crops (Lu and Neumann, 1998; Matoh *et al.*, 1986). A reduction in water loss by transpiration is another physiological response that is observed in these conditions (Cabuslay *et al.*, 2002). This is the result of the partial closure of stomata leading to the reduction of net photosynthesis rate of plants (Moradi and Ismail, 2007). Studies have shown that there are correlations between plant growth and

physiological responses such as stomata conductance, photosynthesis and water loss. Meanwhile, K^+ is essential for plant nutrition, playing an important role in plant adaptation in osmotic and saline conditions (Wei *et al.*, 2013; Lin *et al.*, 2004; Chen *et al.*, 2007). Reactive oxygen species (ROS) have been shown to be produced continuously as a by-product of oxidative plant aerobic metabolism of different cellular compartments. A balance between ROS production and scavenging is essential for plant growth and development, and stress signalling in plants. An overproduction of ROS has been shown to occur in plants when they are exposed to osmotic and saline conditions and is related to low tolerance to these conditions (Badran *et al.*, 2015; Zhu *et al.*, 2007).

2.1.2 Additional effects of salinity: Na^+ ionic

Salinity not only affects plants in an osmotic way but also in an ionic way. Na^+ in low concentrations is beneficial for plants, particularly in conditions with limited K^+ supply (Maathuis, 2013; Box and Schachtman, 2000; Elzam and Epstein, 1969). However, in high saline conditions (>4 dS), most plants are unable to complete their life cycle. Because rice is a salt-sensitive species, it is crucial to understand its physiological response to salt exposure. Plants have three main mechanisms to cope with salt stress: low Na^+ uptake, compartmentation of Na^+ into vacuoles, and exclusion of Na^+ from the cytosol to the apoplast where Na^+ is less toxic (Munns and Tester, 2008).

2.1.3 Gene responses to osmotic and salinity conditions

Much study has been done towards gene identification and characterisation of determinant genes for osmotic and saline conditions. Several gene families have been identified and characterised for roots and shoots of rice cultivars. For example, the expression of plasma membrane intrinsic proteins (PIPs) were quantified in L-type lateral roots of rice in two rice cultivars exposed to osmotic conditions. The PIP genes are related to water uptake which may have direct effect on the transpiration rate in shoots (Matsunami *et al.*, 2016). In saline conditions, nonselective cation channels (NSCCs) and high-affinity K^+ transporters (HKTs) have roles in Na^+ uptake and in reduction of Na^+ through loading and unloading of this ion in the xylem sap (Demidchik and Tester, 2002; Rodríguez-Navarro and Rubio, 2006). Particularly, the HKT2 members can mediate Na^+ and/or K^+ transport, which might also have roles in osmotic stress tolerance. Inward-rectifying K^+ channel (*OsAKT1*) is one of the main K^+ uptake components of rice. The expression of *OsAKT1* has been shown to correlate with K^+ uptake and tissue levels in rice exposed to saline and osmotic conditions (Ahmad *et al.*, 2016; Fuchs *et al.*, 2005).

2.1.4 Elite rice cultivars: background and drought tolerance

There are over 127,000 different rice accessions across the world from *Oryza sativa* and *Oryza glaberrima* backgrounds. To determine the drought tolerance of relatively newly released elite rice cultivars, Table 2.1. summarises the genetic background and drought tolerance characterisation of 12 rice cultivars, some of which cultivars have already been cultivated due to their high grain yield. In regard to cultivars with *O. sativa* backgrounds, 7 *O. sativa indica* and 2 *O. sativa japonica* have been well characterised for grain yields and tolerance to drought conditions. When reviewing the grain yield of rice *indica*, cultivars IR74371-54-1-1 and Apo have been shown to be the highest rice yielding lines, followed by R77298-14-1-2-B-10 and IR77298-5-6-B-18, while IR64 cultivar has the lowest grain yield group (Venuprasad *et al.*, 2011; George *et al.*, 2002; Venuprasad *et al.*, 2007). Regarding *japonica* cultivars, Nipponbare is the highest yielding cultivar, followed by Curinga (Arbelaez *et al.*, 2015; Katsura *et al.*, 2008). For the new rice for Africa (Nerica lines), Jones *et al.* (1997) started developing these lines for rainfed highland interspecific hybridization between African rice *O. glaberrima* Steud. and elite Asian *O. sativa* L. cultivars. The highland Nerica lines have shown promising high yields (~ 4 tons per hectare), short life cycles and some members of this group have high tolerance to drought conditions (Jibrin *et al.*, 2010; Oikeh *et al.*, 2009).

Many researchers have undertaken the task to characterise their responses to different conditions and other traits. By using different experimental designs, the drought tolerance of cultivars is summarised in Table 2.1. However, data for salinity tolerance characterisation of these cultivars is only partially available. Therefore, the aim of this study was to (a) assess if there is a correlation between osmotic and salt tolerance in rice and (b) to identify physiological changes in rice that are common in response to osmotic and saline conditions.

Table 2.1. Rice cultivars used in the present study and their reported cross background and drought tolerance.

Cultivar	Drought/salinity tolerance	Population	Cross
IR74371-54-1-1	T[1]/U	*O. sativa indica*	IR55419-04* Way Rarem
Apo	M[2]/U		-
IR77298-14-1-2-B-10	T[3]/U		IR64*4/Adday Sel
IR77298-5-6-B-18	T[4]/U		IR64*4/Adday Sel
IR64	S[5]/S[6]		IR2061-465-1-5-5*IR5657-33-2-1
Nipponbare	S[7]/S[8]	*O. sativa japonica*	-
Curinga	T[8]/U		-
Nerica L-19	T[10]/U	*O. glaberrima *O. sativa indica*	TOG 5681/3*IR64
Nerica L-20	T[10]/U		TOG 5681/3*IR64
Nerica L-23	U/U		TOG5681/2*IR 64//IR31851-96-2-3-2-1
Nerica L-24	U/U		TOG 5681/2*IR 64//IR31851-96-2-3-2-1
Nerica L-25	U/U		TOG 5681/2*IR 64//IR31851-96-2-3-2-1

NOTE: T=tolerant, M=moderate, S=sensitive and U=Unknown. References for drought and salinity tolerances of rice cultivars: [1](Venuprasad *et al.*, 2007; George *et al.*, 2002), [2](Venuprasad *et al.*, 2009), [3](Sharoni *et al.*, 2012; Moumeni *et al.*, 2011); [4](Moumeni *et al.*, 2011), [5](Verulkar *et al.*, 2010), [6](Castillo *et al.*, 2007), [7](Degenkolbe *et al.*, 2013), [8](Sobahan *et al.*, 2012), [9](Sakai *et al.*, 2010), and [10](Bocco *et al.*, 2012).

2.2 General Methods

Experiments were carried out in hydroponic-glasshouse conditions. The temperature of the glasshouse was on average 28 °C during the day and 24 °C at night with a 12-hour (h) photoperiod. Seeds of rice cultivars were germinated in a clay based terragreen substrate for 15 days and then transferred to a hydroponic standard medium for another 15 days (Yoshida *et al.*, 1976). The hydroponic medium consists of macronutrients and micronutrients as mentioned in Table 2.2. During the experiments, the hydroponic medium was renewed each week.

Table 2.2. Composition and concentration of elements of hydroponic medium. Macronutrient stock solutions were prepared by elements, whereas micronutrient stock solution was prepared in a single stock using deionised water. The pH of the final hydroponic medium was adjusted to 5.5 using either H_2SO_4 or HCl.

	Element	Formula	Stock solution (g/l)	Stock solution/hydroponic medium	Element concentration in Hydroponic medium (mM)
Macronutrient	N	NH_4NO_3	9.14	1.25	2.9
	P	$NaH_2PO_4{\cdot}2\ H_2O$	40.3		0.3
	K	K_2SO_4	71.4		1.0
	Ca	$CaCl_2$	88.6		1.0
	Mg	$MgSO_4{\cdot}7\ H_2O$	324.0		1.6
Micronutrient	Mn	$MnCl_2{\cdot}4\ H_2O$	1.5	1.25	0.01
	Mo	$(NH_4)_6{\cdot}Mo_7O_{24}{\cdot}4H_2O$	0.074		0.001
	B	H_3BO_3	0.934		0.2
	Zn	$ZnSO_4{\cdot}7\ H_2O$	0.035		0.0002
	Cu	$CuSO_4{\cdot}5\ H_2O$	0.031		0.0002
	Fe	$FeCl_3{\cdot}6\ H_2O$	7.7		0.04
	Silica	Na_2SiO_3	0.18		

2.2.1 Long term experiments: physiological characterisation of rice cultivars

In this study, 12 rice cultivars from *O. sativa* and *O. glaberrima* backgrounds were characterised for physiological responses to long term control, osmotic and salinity conditions. Parallel experiments were carried out in rice cultivars exposed to hydroponic standard medium "control", and hydroponic medium plus either osmotic or salinity inducers for a period of 30 days (long term experiments). The hydroponic medium was modified by adding 9% PEG for osmotic treatment and 50 mM NaCl for salt treatment. The osmotic pressure for both conditions was around

0.25 MPa. Based on relative growth rate reduction (RGR-reduction) traits, osmotic and salinity tolerances of rice cultivars were allocated to three clusters ("tolerant", "moderate" and "sensitive").

2.2.2 Short term experiments: physiological characterisation rice cultivars

To find common physiological responses of rice cultivars exposed to short term osmotic and salinity conditions, a cultivar was selected to represent each cluster. Using the long term experiment findings, IR77298-5-6-B-18, IR77298-14-1-2-B-10 and Nipponbare were selected as representative cultivars. The "short term" experiments consisted of the exposure of rice plants for 1, 3 and 6 days to control hydroponic medium, 9% PEG and 50 mM NaCl. The selected rice cultivars were analysed for their K^+ and Na^+ concentrations in roots and shoots as well as in xylem sap. Moreover, measurements of water loss by transpiration (WL), water used efficiency (WUE) and reactive oxygen species (ROS) were evaluated.

2.2.3 Measurement of growth

To determine relative growth rate (RGR), the fresh plant weight of the 30-day old rice plants was recorded. Subsequently, plants were then submitted to control, osmotic and salinity conditions for another 30 days. The initial and final plant biomass (IPB and FPB) of rice cultivars was recorded and the RGR was calculated using the formula of Evans (1972) and multiplied by 100 to express the RGR in % day^{-1} (Formula 2.2.1). Furthermore, RGR-reduction (% day^{-1}) was calculated following the Formula 2.2.2.

Formula 2.2.1

RGR = ((ln FPB - ln IPB) / (t)) x 100, where:

RGR is the relative growth rate of plants (% day^{-1}), ln is the natural logarithm, IPB is the absolute initial plant biomass (g) at the start of the experiment, FPB is the absolute final plant biomass (g) at the end of the experiment. Regarding time, t is the duration of the experiment (days).

Formula 2.2.2

RGR-reduction = (RGRtp / RGRcp) x 100, where:

RGR-reduction is the relative growth rate reduction (% day^{-1}), RGRtp is the RGR of treated plants (% day^{-1}) and RGRcp is the RGR of plants exposed to standard hydroponic medium (% day^{-1}).

2.2.4 Measurements of K^+ and Na^+ concentrations in roots and shoots

For measurements of K^+ and Na^+ ion concentrations, roots and shoots were sampled for rice plants exposed to control, 9% PEG and 50 mM NaCl. Fresh tissues were dried at 80 °C during 3 days, then the dry tissue weights were recorded, tissue was placed in 15 ml falcon tubes and 10 ml of CaCl2 at 20 mM was added to each sample. Samples were stored for three days at room temperature and then analysed for K^+ and Na^+ ion concentrations using a flame photometer. The flame photometer was calibrated using standard concentrations of KCl and NaCl at 0.1, 0.25, 0.5 and 1.0 mM. For the readings of K^+ and Na^+ registered by flame photometer, a linear regression was carried out for each ion and the slope was used to calculate the concentration of ions in umol per gram of dry weight of rice tissues (Formula 2.2.3).

Formula 2.2.3

TIC = (((FPR / SV) / 1000) / TDW) x (V), where:

TIC is the ion concentration in tissues (umol gDW^{-1}), FPR is the flame photometer reading, SV is the slope value of calibration from the linear regression of standard concentrations of either KCl or NaCl. TDW is the tissue dry weight (g) and V is the volume of $CaCl_2$ (μl).

2.2.5 Collection of xylem sap and measurements of K^+ and Na^+ concentrations

To determine variation of K^+ and Na^+ concentrations in xylem sap of rice cultivars, plants were sampled at different time points. After 1, 3 and 6 days of osmotic and saline conditions, 6 rice plants per treatment and cultivar were cut 80 mm above the root-shoot junction. The cut roots were mounted in a pressure chamber by inserting the plant stem though the chamber's head. Around 20 kPa of pressure was applied which exceeded the osmotic pressure of the external solution. Between 20 and 30 microliters of xylem sap was collected, diluted 100 times with deionised water and then analysed for K^+ and Na^+ ion concentrations using a flame photometer. The concentrations K^+ and Na^+ in xylem sap were determined following the flame photometer calibration described above and using the Formula 2.2.4.

Formula 2.2.4

XSKNa = (FPR / SV) x (V), where:

XSKNa is the concentration of either K^+ or Na^+ in xylem sap (mM), FPR is the photometer reading for either K^+ or Na^+, SV is the slope value of the calibration from the linear regression of standard concentrations of either KCl or NaCl and V is the times dilution of samples.

2.2.6 Measurement of water loss

To determine water loss by transpiration (WL) of rice cultivars, rice cultivars were submitted to 50 mL falcon tubes containing known weights of standard hydroponic medium control, hydroponic medium at 9% PEG and 50 mM NaCl. Whereas evaporation of hydroponic medium was concerned, a set of 50 ml tubes, without plants, containing known weights of standard hydroponic medium control, hydroponic medium at 9% PEG and 50 mM NaCl were included. After 1, 3 and 6 days, the FPB and hydroponic medium weights were recorded and the WL was calculated following the Formula 2.2.5.

Formula 2.2.5

WL = ((IHW – FHW)) - (E) / (FPB x t), where:

WL is the water loss by transpiration (g FW^{-1} day^{-1}), IHW is the initial hydroponic weight (g), FHW is the final hydroponic weight (g). Moreover, E is the total evaporation (g) of hydroponic medium without plants. To standardise water loss of plants, the WL was divided by the final plant biomass (FPB in grams) after treatment exposure and t is the time treatment exposure (days).

2.2.7 Measurement of water used efficiency

To measure the water used efficiency (WUE) of plants exposed to control, osmotic and salinity conditions, FPB and IPB of plants were recorded. For hydroponic volume, the initial and final hydroponic medium volumes were recorded and the WUE was calculated following the Formula 2.2.6.

Formula 2.2.6

WUE = (FPB - IPB) / (IHV - FHV), where:

WUE is the water use efficiency of plants (mg FW^{-1} ml^{-1} H_2O), FPB is the final plant biomass (mg), IPB is the initial plant biomass (mg), IHV is the initial hydroponic volume (ml) and FHV is the final hydroponic volume (ml) at the end of each experiment.

2.2.8 Measurement of reactive oxygen species

To semi-quantitatively determine reactive oxygen species (ROS) in portions of root tissues of rice cultivars, 1 cm portions of roots were fixed onto a petri dish and were loaded with 2',7'-dichlorofluorescein diacetate (H2DCFDA) and visualised using a fluorescence microscope. For dye loading, the 1 cm roots were fixed onto a petri dish using tape and the moisture of the roots was

maintained with deionised water. Then, the deionised water was removed and the roots were incubated for ten minutes in 40 mM KCl (pH 7.4). Then, roots were loaded with DCFDA at 100 umol and incubated in the dark for ten minutes.

After dye loading, the portions of roots were rinsed using deionised water and then observed using a fluorescence microscope (Leica MZ FLIII). Pictures were recorded at 5x zoom-in using a charge coupled device (CCD) camera with 0.1 second of light exposure. The ROS detected at this stage was considered time zero. The portions of roots fixed onto petri dishes were then rinsed with deionised water and exposed to either control, 9% PEG or 50 mM NaCl treatments. Dye loading, photographing, and exposing portions of roots to treatments were repeated for three times at 1, 3 and 6 days after osmotic and salinity conditions. The number of replicates was six sections of roots from different plants per treatment and cultivar.

The quantification of ROS was carried out using Image J software (Rasband, 2008). Each set of collected images was loaded into the software and the green channel pixel intensity was determined. Each set of images comprised of six sections of roots from different plants. The ROS signals (DCFDA fluorescence) quantified from plant roots exposed to control condition were considered as the normal oxidation. To determine the ROS levels purely induced by osmotic and saline conditions, ROS was calculated following the Formula 2.2.7.

Formula 2.2.7

ROS = (ROStr - ROScr) where:

ROS is the production of reactive oxygen species in plant roots (DCFDA fluorescence), ROStr is the production of reactive oxygen species of treated roots and ROScr is the production of reactive oxygen species of control roots.

2.2.9 Statistical analysis

Statistical analyses for rice cultivar traits were carried out using statistical software packages. The growth rates, K^+ and Na^+ concentrations, WL, WUE and ROS were analysed using statistical analysis system SAS 9.3 and R software packages (R Development Core Team, 2016; SAS Institute, 2011). To determine the effects of treatment (A), of cultivar (B) and the interaction between treatment and cultivar (A x B), a factorial design analysis was carried out for the physiological traits of rice cultivars. In addition, one-way ANOVA was performed to different data bases. To test the differences between the means of the different data sets, Tukey's honest significant test (Tukey HSD; $P<0.05$) was used where significant differences were detected by ANOVAs. To assess the relationships

between K^+ and Na^+ concentrations in roots and shoots, several correlations analyses were performed for different data sets. Furthermore, to group the cultivars into tolerant, moderate and sensitive clusters based on RGR-reduction traits, a hierarchical cluster analysis was performed using JMP SAS 10 and standardised data method Ward of hierarchical cluster analysis (Ward Jr, 1963; JMP®, 2012).

2.3 Results

2.3.1 Plant growth in long term control conditions

By using absolute and relative values of growth and K^+ in roots and shoots, the common effects of osmotic and salinity conditions were assessed in 12 rice cultivars. To assess the effects of the IPB in long term control conditions, several analyses were carried out using absolute and relative growth rates. The variation of plant growth exposed to control conditions may be attributed to the genetic diversity of the cultivars and measuring different traits. Figure 2.1 shows the average plant biomass of rice cultivars at the beginning of the experiment and there was no significant difference between treatments. Further analysis showed that the IPB was significantly different between rice cultivars at the beginning of the experiment, while the Nerica L-25 cultivar showed the highest values for this trait (Figure 2.2).

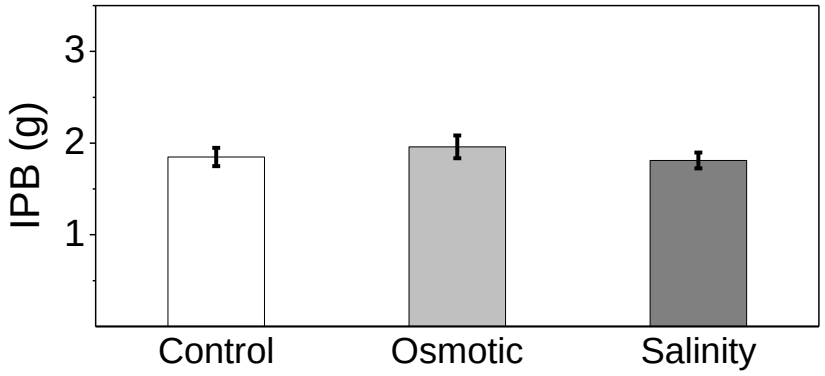


Figure 2.1. Initial plant biomass of rice cultivars in control, osmotic and salinity conditions. Average IPB of 12 rice cultivars at 30-day old. Bars show the mean ±SE of 36 plants.

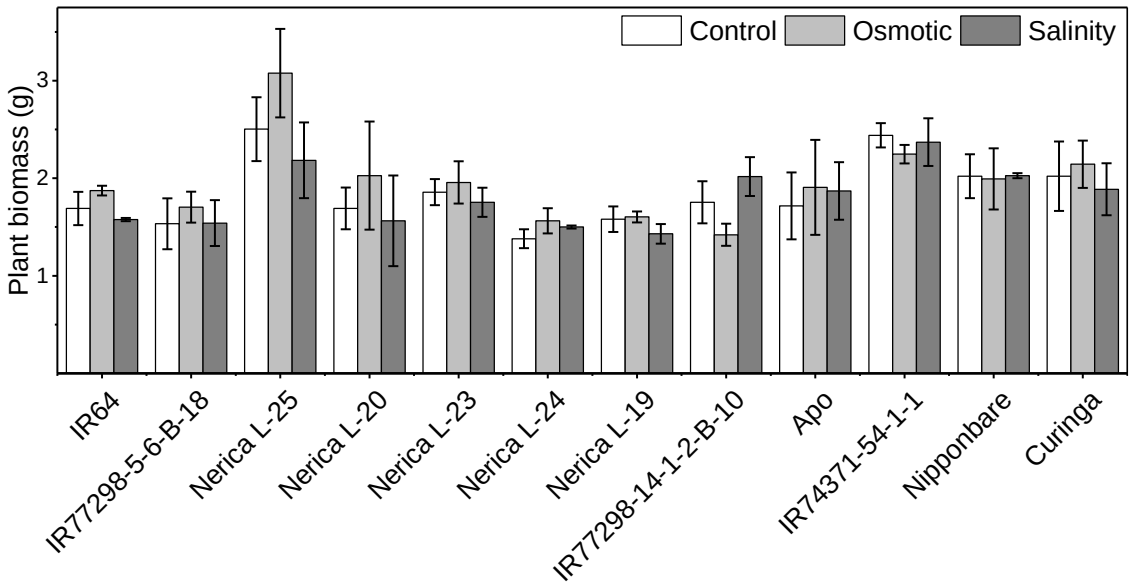


Figure 2.2. Initial plant biomass of rice cultivars in control, osmotic and salinity conditions. IPB of rice cultivars at 30-day old. Bars show the mean ±SE of three plants.

Using absolute and relative growth values of plants after treatment can lead to different conclusions. At the end of the experiment, the FPB of rice cultivars was recorded, whereas the RGR was calculated. Figure 2.3 shows the FPB and RGR of rice cultivars grown standard in hydroponic medium. When using the absolute values, the FPB of plants was significantly different between cultivars. The Nerica L-19 and Nipponbare cultivars showed the highest values for this trait, whereas the Nerica L-23 had the lowest FPB (Figure 2.3A). However, when using the RGR, there was no significant difference between rice cultivars (Figure 2.3B). This controversy between FPB and RGR is because the FPB could be the result of the small differences of plant sizes at the beginning the experiment. However, correlation analyses showed that the IPB did not have an effect on either the FPB or RGR of rice cultivars grown in control conditions (Figure 2.4). For subsequent analyses of growth traits, the RGR and RGR-reduction will have more emphasis due to representing purely the effects of the treatment.

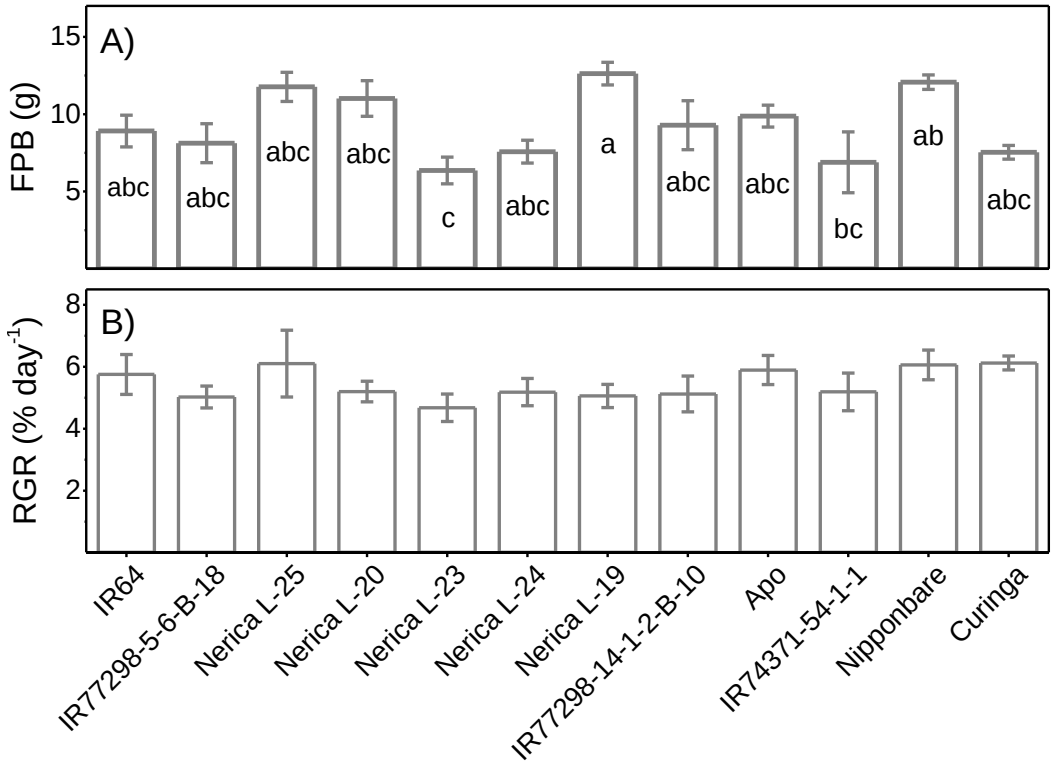


Figure 2.3. Absolute final plant biomass and relative growth rate of rice cultivars. (**A**) FPB of rice cultivars. (**B**) RGR of rice cultivars. Plants were grown during 30 days in standard hydroponic medium. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of three plants.

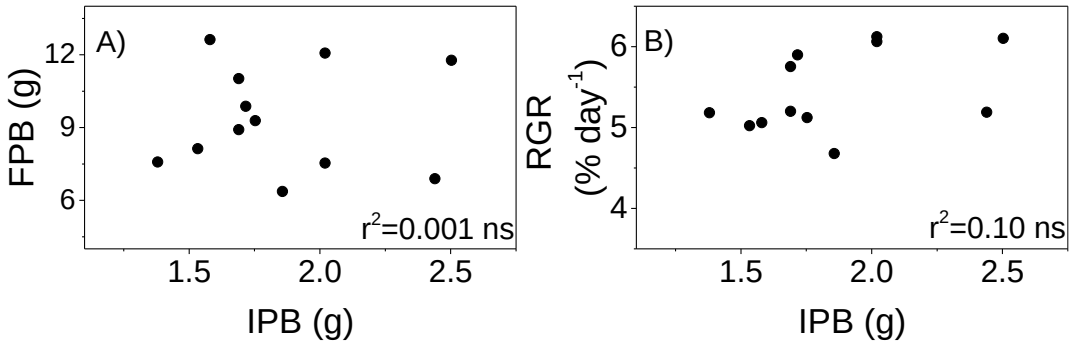


Figure 2.4. Correlations between absolute and relative growth values of rice cultivars exposed to control conditions. (**A**) Correlation between IPB and FPB. (**B**) Correlation between IPB and RGR. No significant correlations (ns) were identified at the 0.05 level (two-tailed).

2.3.2 Plant growth in osmotic and salinity conditions

Regarding treatment effects, there were significant differences between control, osmotic and salinity conditions for FPB, RGR and RGR-reduction traits (Table 2.3 and Figure 2.5). Plants exposed to osmotic and saline conditions showed leaf rolling, young leaf growth reductions and mature leaf senescence, which are common symptoms of osmotic and salinity effects on plants.

Table 2.3. Multiple ANOVA analyses for absolute and relative growth traits of rice cultivars exposed to long term treatments.

Effect	*P*-values		
	FPB	RGR	RGR-reduction
TREATMENT	< 0.0001	0.0001	0.0287
CULTIVAR	< 0.0001	< 0.0013	< 0.0001
TREATMENT x CULTIVAR	0.0080	0.0740	0.5302

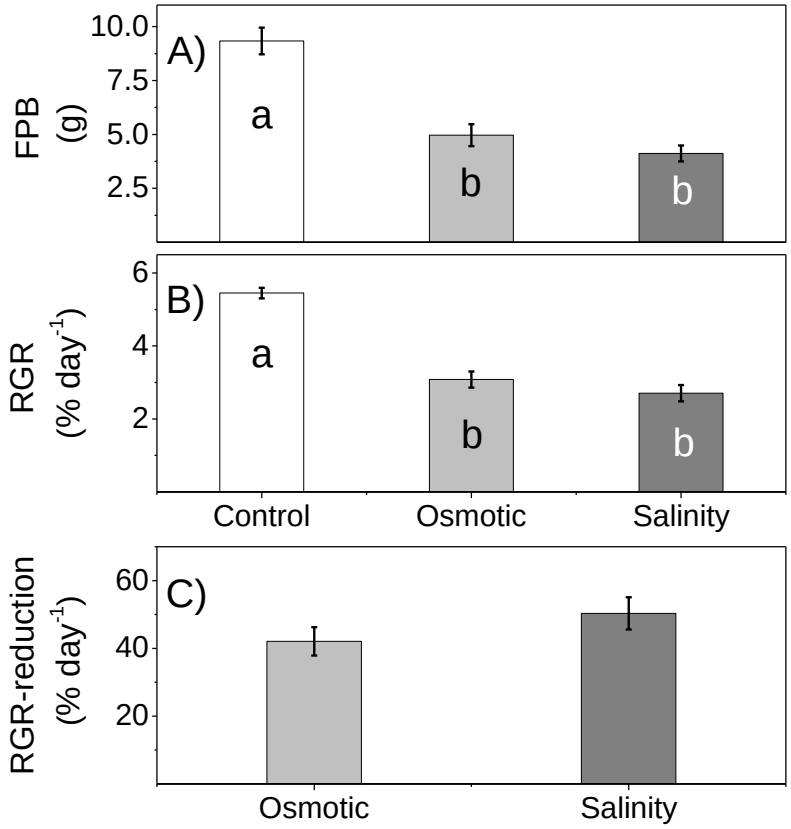


Figure 2.5. Absolute plant biomass and relative growth rates of rice cultivars. (**A**) Absolute plant biomass. (**B**) Relative growth rate. (**C**) Relative growth rate reductions. Means followed by different letters are significantly different between treatments (Tukey's honest significant test HSD, P<0.05). Bars show the mean ±SE of 12 rice cultivars.

Where cultivar effects were concerned, there were significant differences between cultivars for FPB, RGR and RGR-reduction traits of rice cultivars exposed to osmotic and salinity conditions (Table 2.3 and Figure 2.6). Thus, there were consistent results using either absolute or relative growth values of rice cultivars. For further analyses, the RGR-reduction traits should show more differences due to these representing purely the effects of the osmotic and salinity treatments over a period of time. Moreover, the RGR-reduction integrates the effects of RGRcp and RGRtp values of rice cultivars. Therefore, by using RGR-reduction, a wider range of resilience phenotypes were indicated between rice cultivars. In osmotic conditions, RGR-reduction of rice cultivars ranged from 24% to 67%. The Apo cultivar showed the highest RGR-reduction value, whereas the Nerica L-25 showed the lowest value of this trait (Figure 2.6A). When comparing osmotic tolerance across 12 rice cultivars with different genetic backgrounds, Table 2.4 shows that the RGR-reduction was significantly different between *indica*, *japonica* and Nerica lines (cross between *O. sativa indica* and *O. glaberrima*).

In saline conditions, the RGR-reduction of the cultivars was between 24% and 77%. The Curinga cultivar showed the highest sensitivity to saline conditions, whereas IR64 and IR77298-5-6-B-18 cultivars showed the highest tolerance to this condition (Figure 2.6B). In general, the IPB of rice cultivars did not show significant effects on FPB, RGR or RGR-reduction of plants exposed to long term osmotic and salinity conditions (Table 2.5). Furthermore, there were no correlations between control-RGR and either osmotic-RGR or salinity-RGR of rice cultivars (Figure 2.7). However, the highest IPB was in the Nerica L-25 cultivar, which was favoured for its tolerance to osmotic and saline conditions, whereas the lowest IPB was in the Nerica L-24 did not show big effect on the response to osmotic and saline conditions.

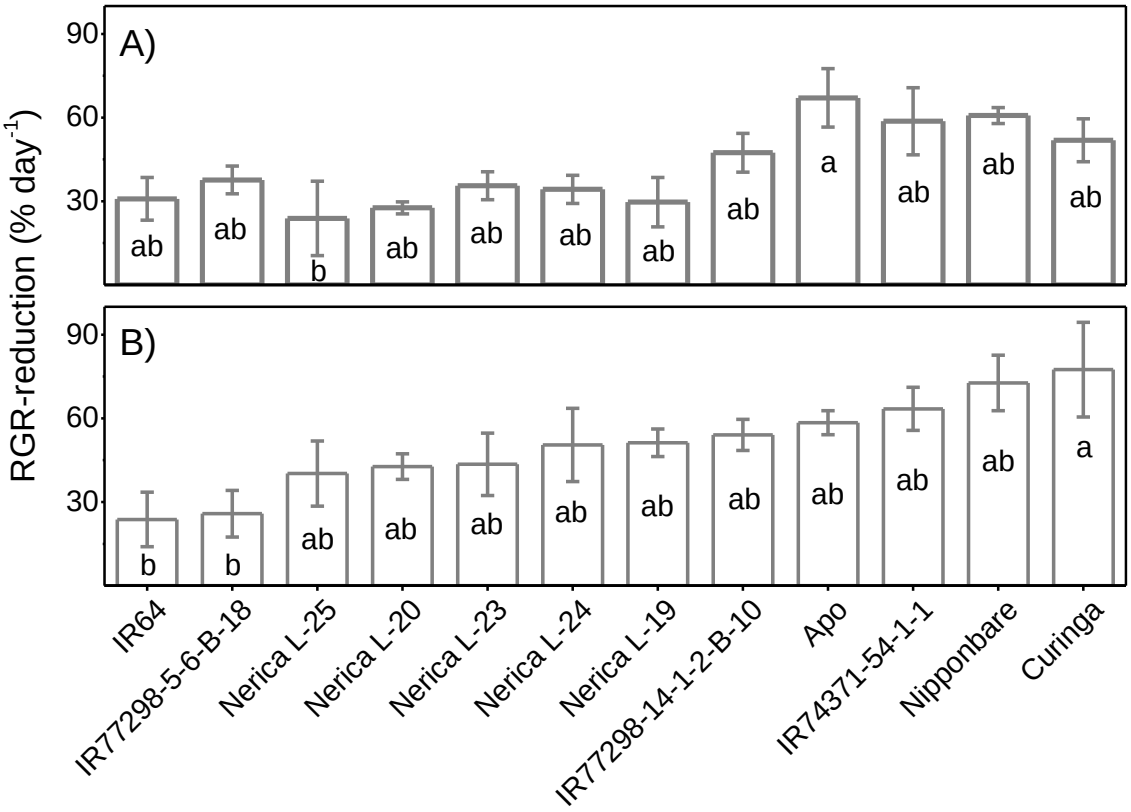


Figure 2.6. Relative growth rate reduction of rice cultivars. (**A**) RGR-reduction of rice cultivars exposed to osmotic conditions. (**B**) RGR-reduction of rice cultivars exposed to salinity conditions. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of three plants.

Table 2.4. Multiple ANOVA analyses for absolute and relative growth values of rice cultivars. ANOVA analyses were carried out by treatment and population.

Treatment	Background	FPB	RGR	RGR-reduction
Osmotic	*O. sativa indica*	3.6 a	2.5 a	52.5 a
	O. sativa japonica	5.6 a	3.1 a	45.8 ab
	O. sativa indica x *O. glaberrima*	6.1 a	3.6 a	30.2 b
Salinity	*O. sativa indica*	3.3 a	2.5 a	55.8 a
	O. sativa japonica	4.6 a	2.9 a	48.2 a
	O. sativa indica x *O. glaberrima*	4.7 a	2.9 a	45.6 a

NOTE: Nerica lines are the results of crosses between *O. sativa indica* and *O. glaberrima*.

Table 2.5. Correlations between absolute and relative growth values of rice cultivars.

Treatment	Trait	FPB	RGR	RGR-reduction
Osmotic	IPB	0.293	0.299	-0.127
		0.356	0.346	0.695
Salinity	IPB	-0.552	-0.457	0.487
		0.063	0.135	0.109

NOTE: Initial plant biomass (IPB), final plant biomass (FPB), relative growth rate (RGR), relative growth rate reduction (RGR-reduction).

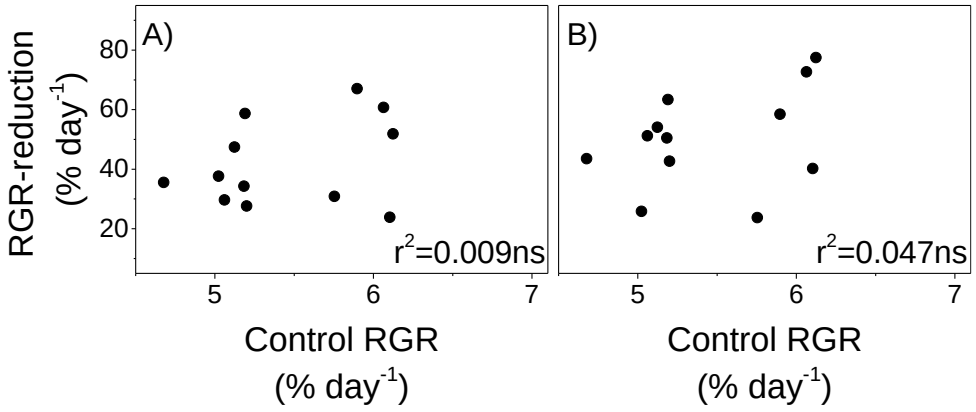


Figure 2.7. Correlations between control-RGR and treated-RGR-reduction of rice cultivars. (**A**) Correlation between control-RGR and osmotic-RGR-reduction. (**B**) Correlation between control-RGR and salinity-RGR-reduction. No significant correlations (ns) were identified at the 0.05 level (two-tailed).

Concerning the interaction effects between treatment and cultivar, there was significant difference for the FPB but not for the RGR and RGR-reduction traits (Table 2.3). In addition, the RGR-reduction was not significantly different between osmotic and salinity inducers for most of the rice cultivars (Figure 2.8). This insignificant difference confirms that the effects of osmotic and salinity conditions was similar on the relative growth rates of rice cultivars. Based on RGR-reduction, Curinga and Nipponbare were the most sensitive cultivars, whereas IR77298-5-6-B-18 and IR64 were the most tolerant cultivars to both conditions. Furthermore, there were positive correlations between the osmotic and salinity effects on the RGR and RGR-reductions of plants (Figure 2.9).

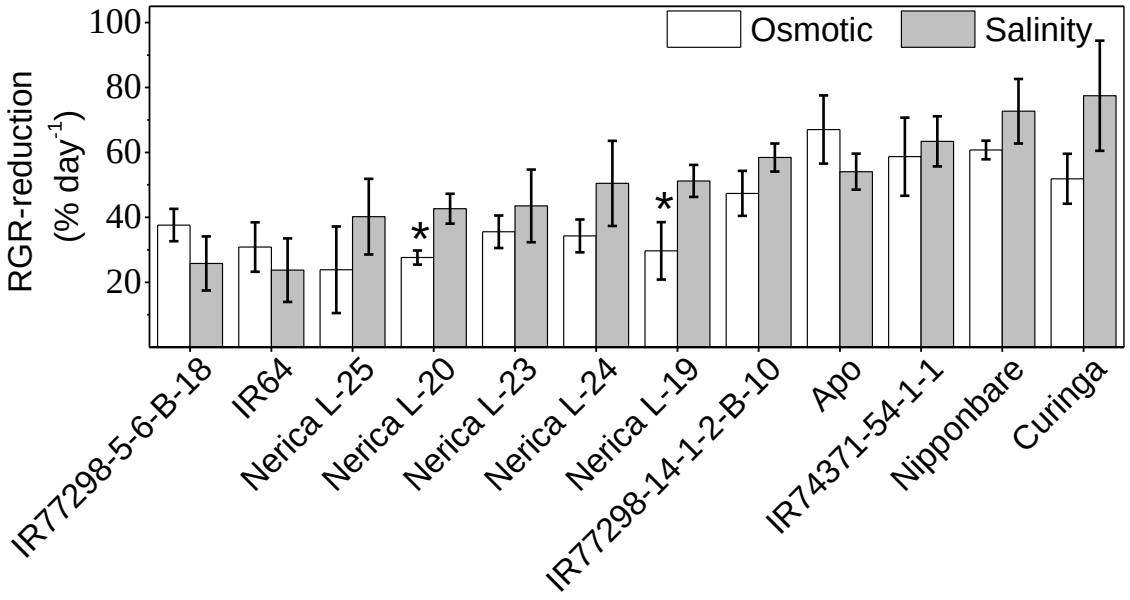


Figure 2.8. RGR-reduction t-tests of rice cultivars exposed osmotic and salinity conditions. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. The significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of three plants.

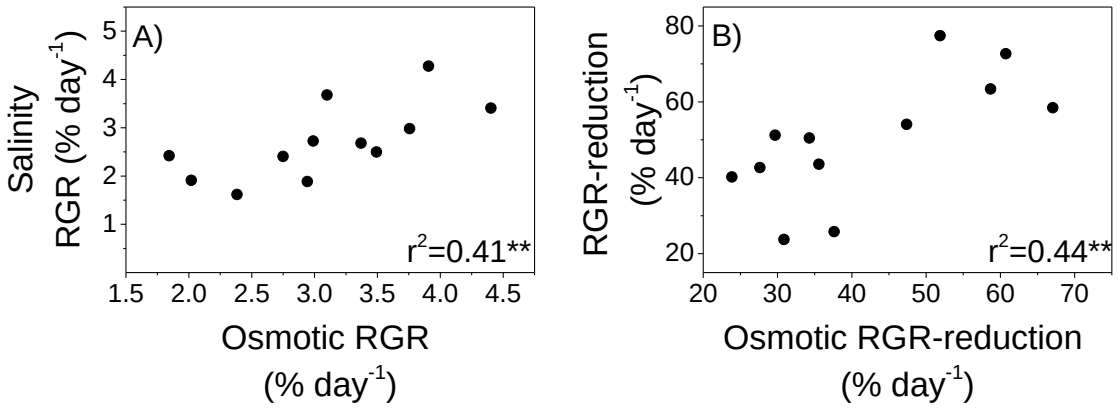


Figure 2.9. Correlations between osmotic and salinity inducers on relative growth rates of rice cultivars. (**A**) Correlation between osmotic-RGR and salinity-RGR. (**B**) Correlation between osmotic-RGR-reduction and salinity-RGR-reduction. **Correlation is significant at the 0.01 level (two-tailed).

For further analysis, the osmotic and salinity tolerance of rice cultivars were ranked based on the percentage of RGR-reductions. By using a hierarchical cluster analysis method by Ward Jr (1963), three clusters were identified for osmotic and salinity conditions. The plant's RGR-reductions are described as follows: cluster 1 contained tolerant, cluster 2 moderate and cluster 3 sensitive cultivars. The osmotic-tolerant cluster contained higher number of cultivars compared to the salinity-tolerant

cluster. Whereas moderate cluster concerns, the osmotic-moderate cluster contained lower number of cultivars compared to the salinity-moderate cluster (Figure 2.10 and Figure 2.11).

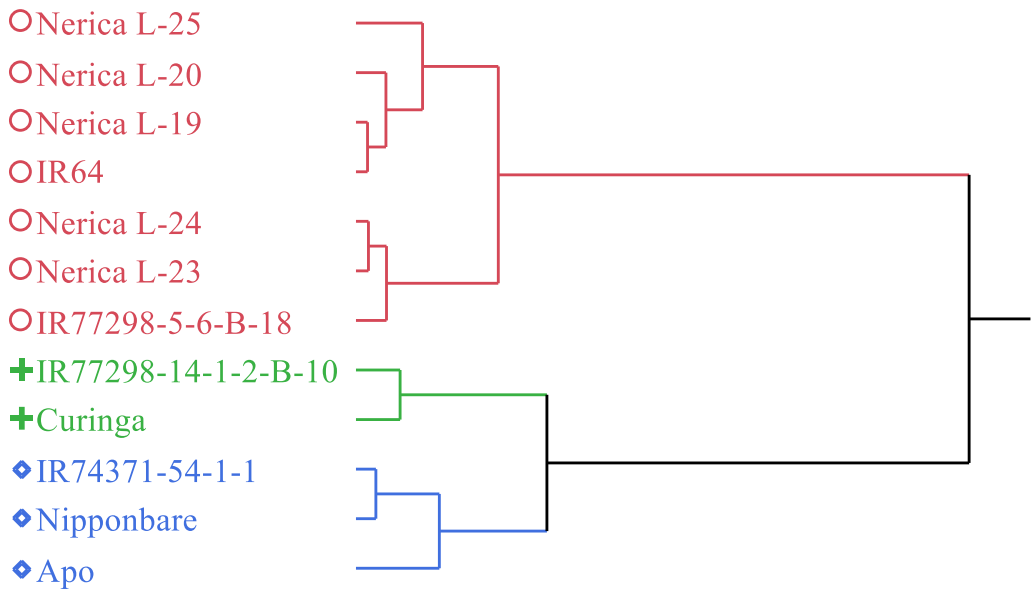


Figure 2.10. Dendrogram of the clustering of 12 rice cultivars exposed to osmotic conditions. Cluster analysis were carried out using RGR-reduction data of plants exposed to long term osmotic conditions. Osmotic-sensitive (◊; more than 60% RGR-reduction), osmotic-moderate (+; between 40% and 60% RGR-reduction) and osmotic-tolerant (o; less than 40% RGR-reduction) rice cultivars.

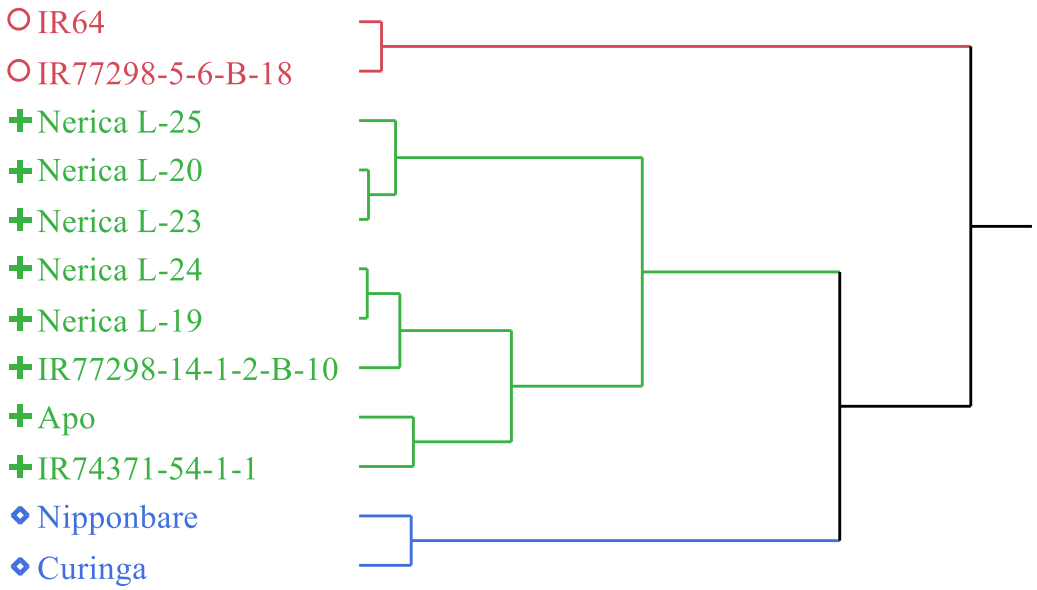


Figure 2.11. Dendrogram of the clustering of 12 rice cultivars exposed to salinity conditions. Cluster analysis were carried out using RGR-reduction data of plants exposed to long term osmotic conditions. Osmotic-sensitive (◊; more than 60% RGR-reduction), osmotic-moderate (+; between 40% and 60% RGR-reduction) and osmotic-tolerant (o; less than 40% RGR-reduction) rice cultivars.

2.3.3 Osmotic and salinity effects on K^+

In osmotic and saline conditions, the maintenance of high K^+ in roots and shoots is related to high tolerance to these conditions (Lee et al., 2003). To determine the treatment, cultivar and treatment x cultivar effects on root K^+ and shoot K^+, several statistical analyses were carried out. Regarding purely treatment effects on K^+, statistical analysis showed that there was a significant difference between control, osmotic and salinity conditions on the root K^+ (Table 2.6 and Figure 2.12). Further analysis showed that the root K^+ was not significantly different between cultivars (Table 2.6 and Figure 2.13). Even though the root K^+ was not significantly different between cultivars, most of the rice cultivars showed significant reductions of the root K^+ when plants were exposed either to osmotic or saline conditions. For the shoot K^+, there were no significant differences between treatments or cultivars (Table 2.6 and Figure 2.14). Regarding treatment x cultivar effects, statistical analyses did not show significant differences for K^+ in roots and shoots (Table 2.6).

Table 2.6. Multiple ANOVA analysis results for root K^+ and shoot K^+ of rice cultivars exposed to long term treatments.

Effect	*P*-values	
	Root K^+	Shoot K^+
TREATMENT	<0.0001	0.7215
CULTIVAR	0.6412	0.4584
TREATMENT x CULTIVAR	0.6313	0.5507

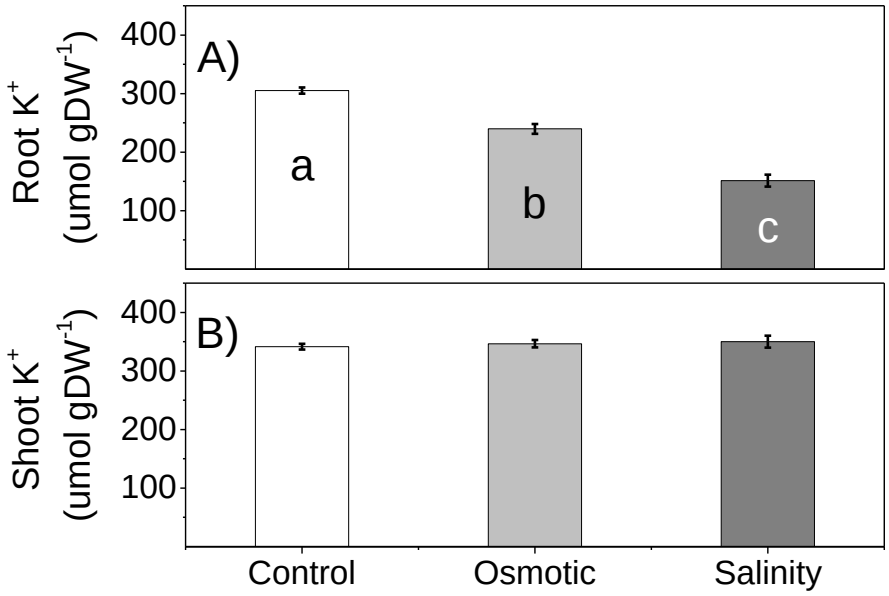


Figure 2.12. K^+ concentrations in roots and shoots of rice cultivars. (**A**) Root K^+ of plants exposed to different treatments. (**B**) Shoot K^+ of plants exposed to different treatments. Bars show the mean ±SE of 12 rice cultivars.

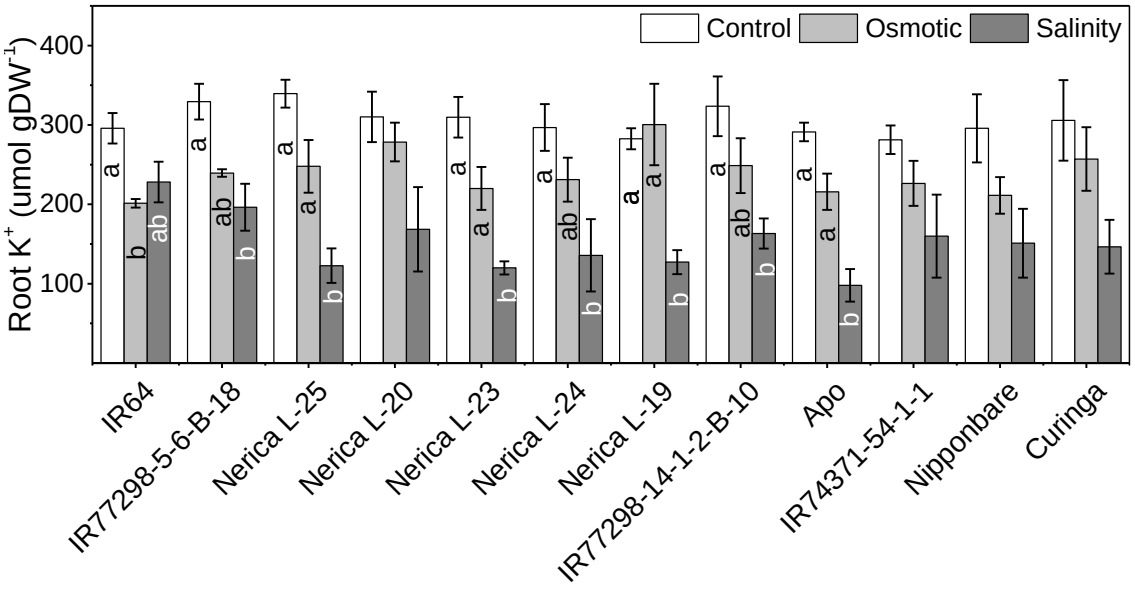


Figure 2.13. K^+ concentrations in roots of rice cultivars. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of three plants.

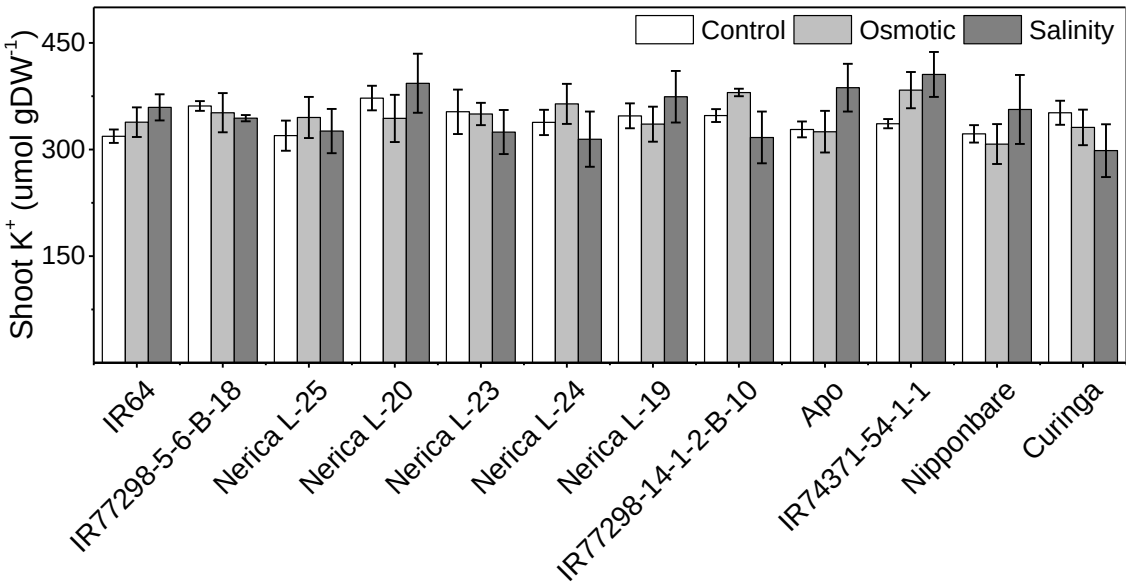


Figure 2.14. K^+ concentrations in shoots of rice cultivars. Bars show the mean ±SE of three plants.

2.3.4 Correlation between osmotic and salinity effects on root K^+

To determine a correlation between osmotic and salinity effects on the root K^+, t-test analyses were carried out. Figure 2.15 shows that 8 of the 12 cultivars did not show significant differences between osmotic-induced and salinity-induced effects. The Nerica L-19, Nerica L-23, Nerica L-25 and Apo showed significant differences between osmotic and salinity effects on root K^+. Further analyses showed that the root K^+ did not correlate with the osmotic and salinity tolerance of plants based on RGR-reduction (Figure 2.16).

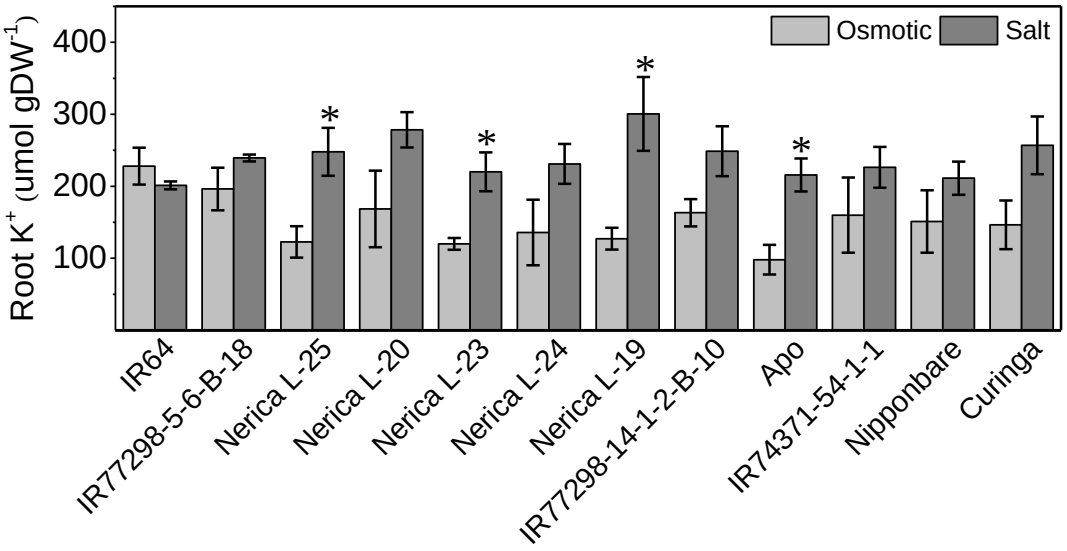


Figure 2.15. Root K^+ of rice cultivars exposed to long term osmotic and salinity conditions. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced effects. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of three replicates.

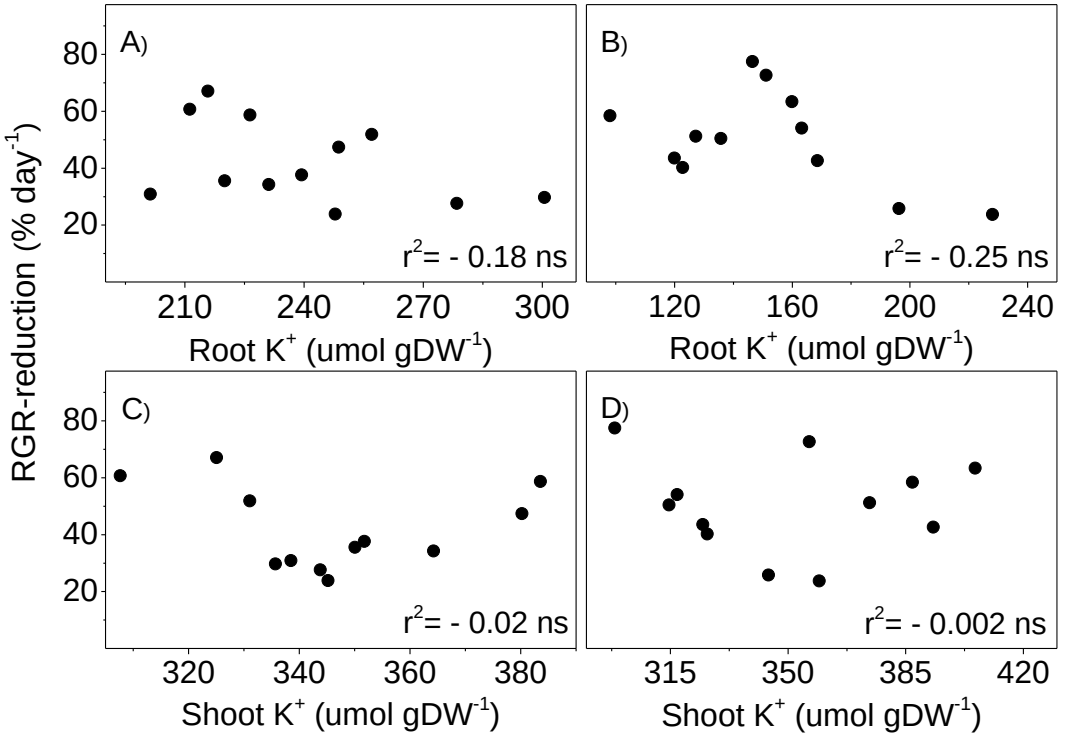


Figure 2.16. Correlations between K^+ concentrations and RGR-reduction of rice cultivars. (**A**) Correlation between root K^+ and RGR-reduction of plants exposed osmotic conditions. (**B**) Correlation between root K^+ and RGR-reduction of plants exposed to saline conditions. (**C**) Correlation between shoot K^+ and RGR-reduction of plants osmotic conditions. (**D**) Correlation between shoot K^+ and RGR-reduction of plants exposed to salt conditions. No significant correlations (ns) were identified at the 0.05 level (two-tailed).

2.3.5 Short term experiments

This section presents the results of physiological characterisation of the selected tolerant, moderate and sensitive rice cultivars. Based on the three clusters identified in the long term experiments, representative tolerant, moderate and sensitive cultivars were selected for more detailed physiological characterisation.

2.3.6 Osmotic and salinity effects on ROS

To determine the level of ROS production in roots of plants exposed to different treatments, 1 cm root sections from different plants were exposed to control, 9% PEG and 50 mM NaCl solutions. Figure 2.17 shows ROS signals recorded using a CCD camera, and there was clear evidence of low ROS production by plant roots exposed to control conditions. In contrast, the ROS signals were elevated in treated plants and the ROS signals in the sensitive rice cultivar showed higher ROS production compared to the moderate and tolerant cultivars. Further analysis showed that the quantified ROS in root sections exposed to control treatment was not significantly different between rice cultivars (Figure 2.18A). Because ROS is produced in roots when exposed to control conditions, these values were subtracted from the quantified ROS of plant roots exposed to osmotic and saline conditions (General methods, section 2.2.8). Regarding treated root sections, the ROS levels were significantly different between cultivars at different time points of treatments. The sensitive cultivar showed higher ROS degrees compared to the moderate and tolerant cultivars exposed to osmotic and saline treatments (Figures 2.18B and C). After 6 days growing in treatments, the sensitive cultivar showed ~ 70% higher ROS levels in comparison with the moderate and tolerant cultivars.

To determine whether different external stimuli affected the level of ROS production in root sections, t-test analyses were applied to different data sets. The ROS in the tolerant cultivar was significantly different between osmotic and salinity inducers after 6 days of treatment (Figure 2.18D). Remarkably, the ROS levels in roots of the moderate cultivar were significantly different between osmotic and salinity inducers at different time points (Figure 2.18E). Likewise, the sensitive cultivar showed significant difference in ROS production from 1 day to 3 days from the onset of treatment (Figure 2.18F).

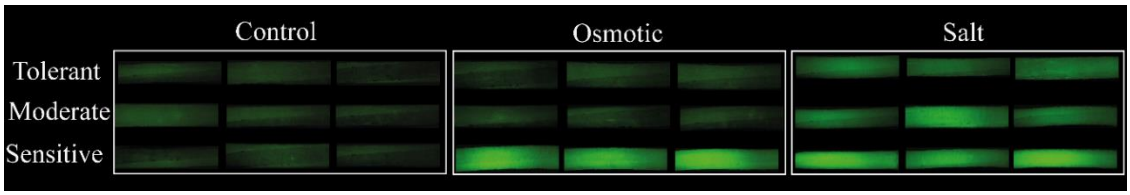


Figure 2.17. Reactive oxygen species signals in root sections of the selected rice cultivars. 1 cm roots at 5x zoom-in pictures were recorded after 6 days of treatments. To illustrate the reproducibility of ROS production, the roots were sampled from three independent rice plants.

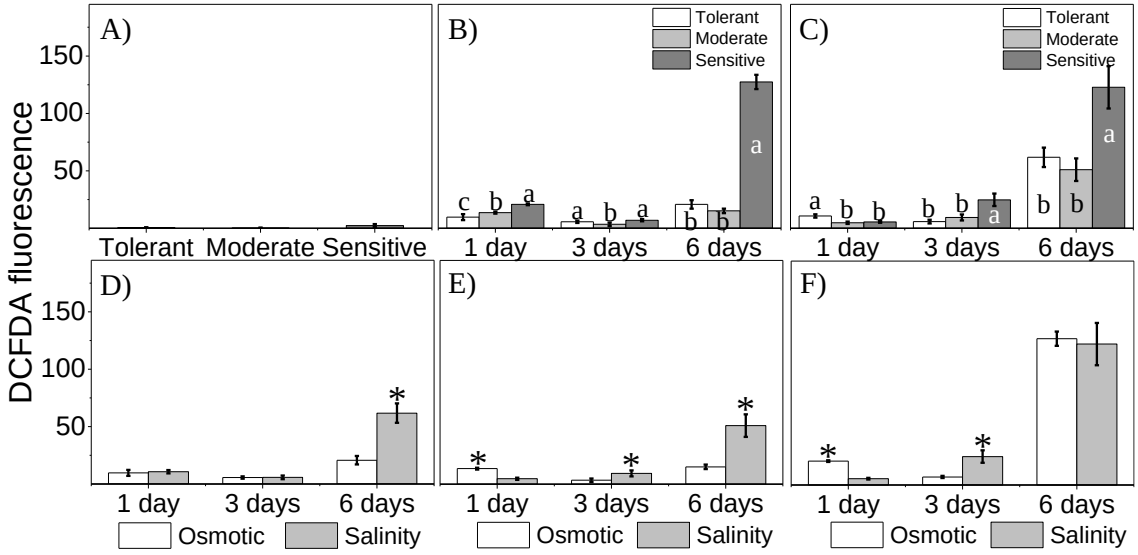


Figure 2.18. Reactive oxygen species quantificated in root sections of the selected rice cultivars. (**A**) ROS levels in root sections of rice plants exposed to control conditions. (**B**) ROS levels in root sections of rice plants exposed to osmotic conditions. (**C**) ROS levels in root sections of rice plants exposed to saline conditions. Significance was calculated by one-way ANOVA. Means followed by different letters show significant difference between cultivars (Tukey's honest significant test HSD, $P<0.05$). (**D**) ROS levels of tolerant cultivar. (**E**) ROS levels of moderate cultivar. (**F**) ROS levels of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of six sections of roots from different plants.

2.3.7 Osmotic and salinity effects on WL

A reduction of transpiration of plants is a common response to osmotic and saline conditions (Romero-Aranda *et al.*, 2001; Liu and Stützel, 2002). To assess the effects of treatments on the WL, data sets were analysed by treatments and cultivars. Regarding the rate of evaporation, 50 ml falcon tubes containing standard hydroponic medium showed an evaporation of 1 ml per day. Whereas the evaporation in hydroponic medium with either 9% PEG or 50 mM NaCl, these values were 0.25 ml and 0.40 ml per day, respectively. Furthermore, these values were subtracted from the WL of plants exposed to either control, osmotic or saline conditions (General methods, section 2.2.6). Statistical

analysis showed that the WL was significantly different between rice cultivars when exposed to standard hydroponic medium (Figure 2.19A). For plants exposed to osmotic and saline treatments, WL was significantly different between cultivars. In general, the sensitive rice cultivar showed ~25% more WL in comparison to moderate and tolerant cultivars (Figures 2.19B and C).

To determine whether different external stimuli affected the degree of WL in plants, t-test analyses were applied to different data sets. Interestingly, the tolerant and moderate cultivars did not show significant differences between osmotic and salinity effects on the WL for most of the time points (Figures 2.19D and E). Whereas the sensitive cultivar concerns, the WL was significantly different between osmotic and salinity inducers during the first 3 days of treatment (Figure 2.19F).

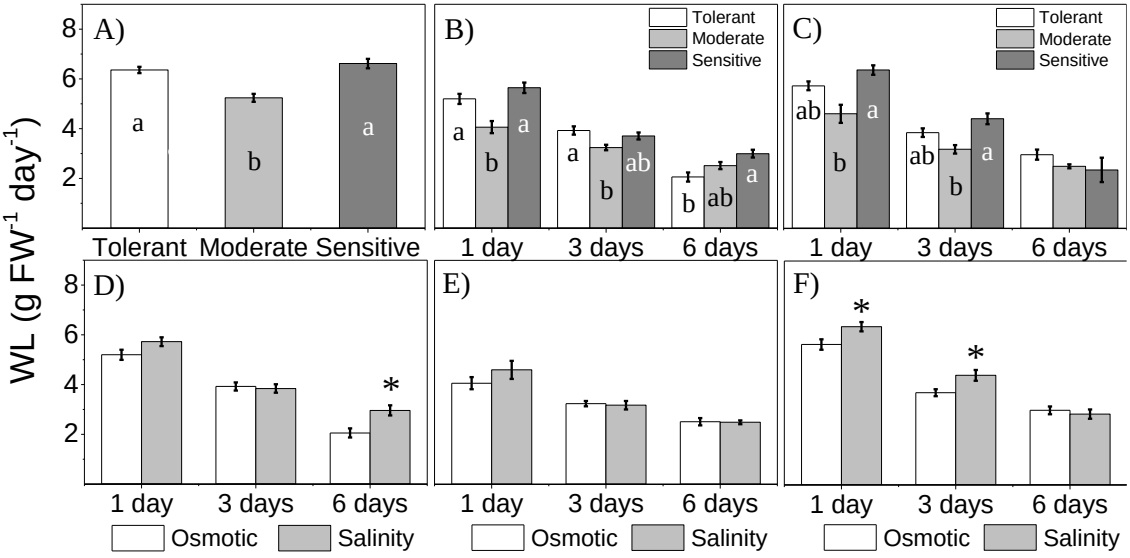


Figure 2.19. Water loss by transpiration of selected rice cultivars. (**A**) WL of rice cultivars exposed to control conditions. (**B**) WL of rice cultivars exposed to osmotic conditions. (**C**) WL of rice cultivars exposed to saline conditions. Significance was identified by one-way ANOVA. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). (**D**) WL of tolerant cultivar. (**E**) WL of moderate cultivar. (**F**) WL of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of six plants.

2.3.8 Osmotic and salinity effects on WUE

High tolerance to osmotic and saline conditions of plants is related to high WUE (Al-Karaki, 2000; Flowers *et al.*, 1988). To assess the effects of treatments on the WUE, data sets were analysed by treatments and cultivars. Figure 2.20A shows the WUE of rice cultivars exposed to standard hydroponic conditions, and the sensitive cultivar showed higher WUE compared to moderate and tolerant cultivars. When plants were exposed for 3 days to osmotic conditions, the tolerant cultivar

showed higher WUE in comparison to the moderate and sensitive cultivars (Figure 2.20B). Regarding plants exposed to saline conditions, the tolerant cultivar showed higher WUE compared to the moderate and tolerant lines (Figure 2.20C). After 6 days of saline treatment, the tolerant cultivar showed around 45% higher WUE in comparison to sensitive and moderate cultivars.

To determine whether different external stimuli affected the degree of WUE in plants, t-test analyses were applied to different data sets. In general, the tolerant cultivar did not show significant differences between osmotic and salinity treatments (Figure 2.20D). However, for the moderate and sensitive cultivars, the WUE was affected differently by osmotic and salinity treatments (Figures 2.20E and F). Plants showed about 45% higher WUE in osmotic conditions compared to the plants in saline conditions.

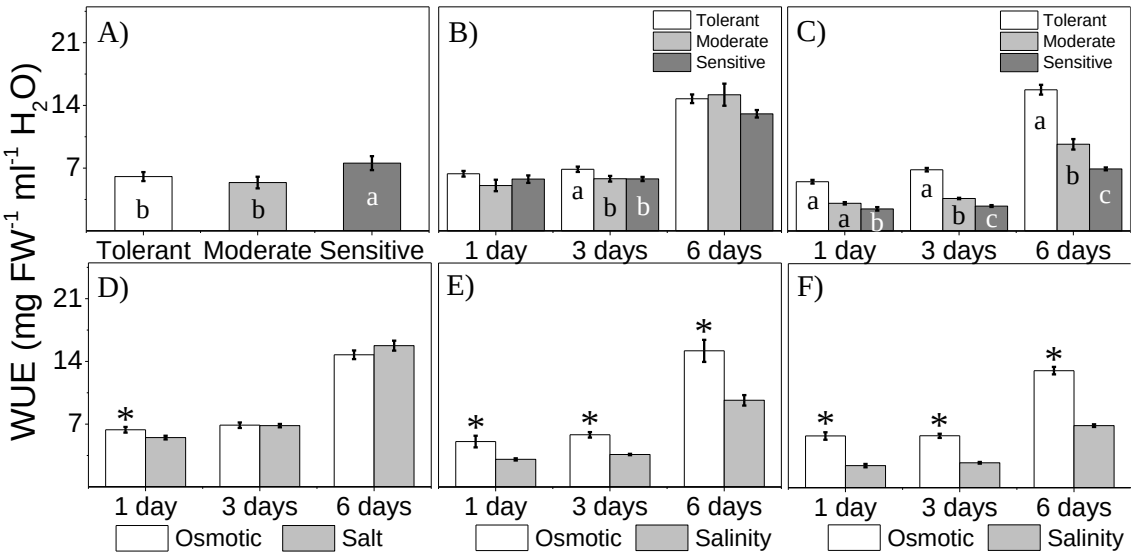


Figure 2.20. Water use efficiency of the selected rice cultivars. (**A**) WUE of rice cultivars exposed to control conditions. (**B**) WUE of rice cultivars exposed to osmotic conditions. (**C**) WUE of rice cultivars exposed to saline conditions. Significance was identified by one-way ANOVA. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). (**D**) WUE of tolerant cultivar. (**E**) WUE of moderate cultivar. (**F**) WUE of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of six plants.

2.3.9 Osmotic and salinity effects on root K^+

K^+ is important for early plant adaptation to osmotic and saline conditions. To assess the effects of treatments on the root K^+, data sets were analysed by treatments and cultivars. Figure 2.21A shows that the root K^+ was significantly different between cultivars when grown in control hydroponic conditions. In this condition, the sensitive and moderate cultivars showed higher root K^+ in comparison to tolerant cultivar. For plants exposed to either osmotic or salinity conditions, the root K^+ was not significantly different between cultivars (Figures 2.20B and C). Despite the fact that the root K^+ was not significantly different between cultivars overall, there was a reduction of K^+ in roots over time of exposure to the treatments. After 6 days in salinity, the root K^+ of the tolerant, moderate and sensitive lines had reduced by around 30%, 45% and 43% to starting content, respectively (Figures 2.20B and C). To determine whether different external stimuli affected the root K^+ in plants, t-test analyses were applied to different data sets. In general, the root K^+ was not significantly affected by osmotic and salinity conditions except for the sensitive cultivar after 6 days of treatment (Figures 2.20D-F).

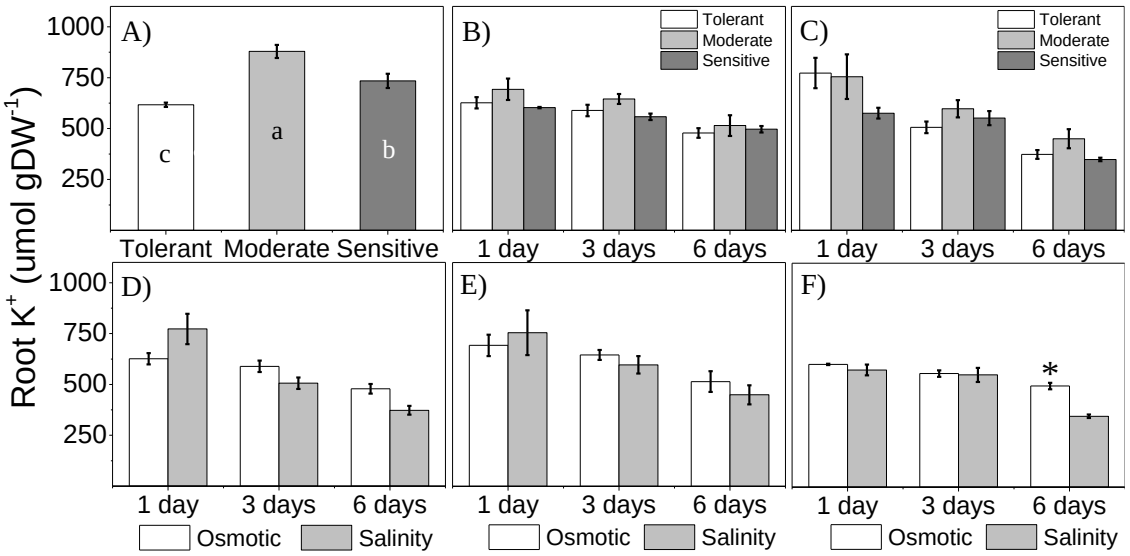


Figure 2.21. Root K^+ of the selected rice cultivars. (**A**) Root K^+ of rice cultivars exposed to control conditions. (**B**) Root K^+ of rice cultivars exposed to osmotic conditions. (**C**) Root K^+ of rice cultivars exposed to saline conditions. Significance was calculated by one-way ANOVA. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). (**D**) Root K^+ of tolerant cultivar. (**E**) Root K^+ of moderate cultivar. (**F**) Root K^+ of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of six plants.

2.3.10 Osmotic and salinity effects on K^+ in xylem sap

The degree of K^+ loading and unloading in xylem sap of plants may be disrupted by osmotic and salinity conditions. To assess the effects of treatments on K^+ in xylem sap, data sets were analysed by treatments and cultivars. In control conditions, K^+ in xylem sap was not significantly different between cultivars (Figure 2.22A). Regarding osmotic conditions, the sensitive cultivar xylem sap showed lower K^+ compared to the moderate and tolerant cultivars (Figure 2.22B). In saline treatments, the K^+ in xylem sap was significantly different between cultivars after 6 days of treatment (Figure 2.22C). Following 6 days of osmotic and saline treatments, K^+ in xylem sap was reduced by around 40% in the selected rice cultivars. To determine whether different external stimuli affected K^+ in xylem sap of plants, t-test analyses were applied to different data sets. After 6 days of treatment, K^+ in xylem sap was significantly affected by osmotic and salinity conditions (Figures 2.22C-D). K^+ in xylem sap of plants was reduced about 20% more in saline conditions compared to osmotic conditions.

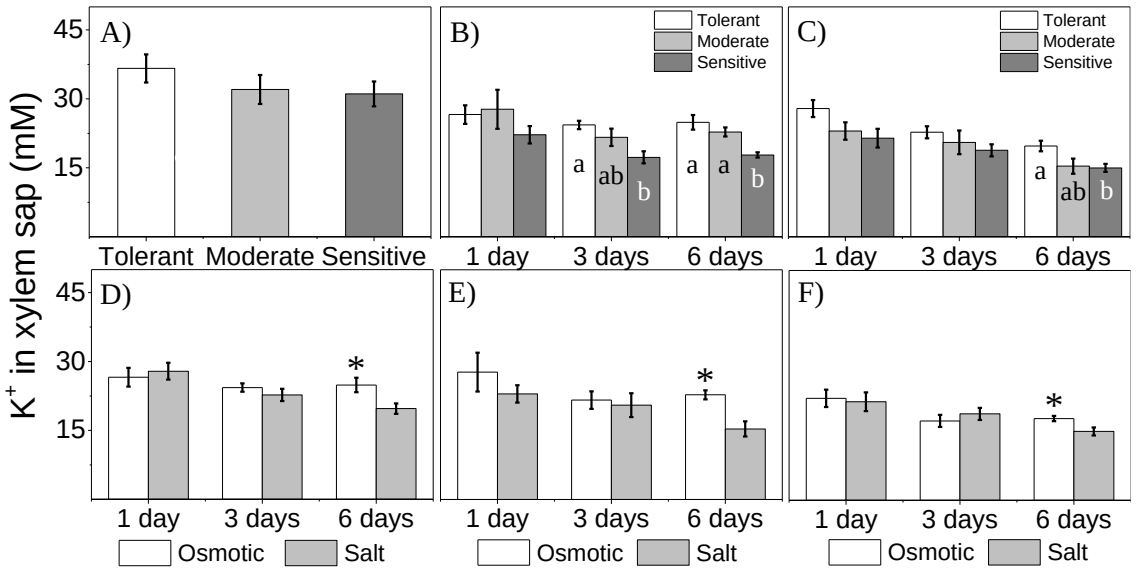


Figure 2.22. Xylem sap K^+ of the selected rice cultivars. (**A**) Xylem sap K^+ of rice cultivars exposed to control conditions. (**B**) Xylem sap K^+ of rice cultivars exposed to osmotic conditions. (**C**) Xylem sap K^+ of rice cultivars exposed to saline conditions. Significance was calculated by one-way ANOVA. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). (**D**) Xylem sap K^+ of tolerant cultivar. (**E**) Xylem sap K^+ of moderate cultivar. (**F**) Xylem sap K^+ of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of six plants.

2.3.11 Osmotic and salinity effects on shoot K^+

K^+ is essential for plant adaptation and is related to osmotic and salinity tolerance in cereal crops (Wei *et al.*, 2013; Lin *et al.*, 2004; Chen *et al.*, 2007). To assess the effects of the treatments on the shoot K^+, data sets were analysed by treatments and cultivars. The shoot K^+ of plants grown in standard hydroponic medium was not significantly different between cultivars (Figure 2.23). Where osmotic treatments were concerned, the shoot K^+ of the sensitive cultivar was significantly lower compared to moderate and tolerant cultivars exposed to 1 and 6 days of treatment (Figure 2.23B). After 6 days of osmotic treatment, the shoot K^+ of the tolerant, moderate and sensitive cultivars reduced by about 25%, 11% and 31%, respectively.

In saline conditions, the shoot K^+ of the sensitive cultivar was lower in comparison to the tolerant cultivar after exposure to 3 days of 50 mM NaCl (Figure 2.23C). However, after 6 days of treatment, the reduction of shoot K^+ of the moderate cultivar was about 18% compared to sensitive (27%) and tolerant (31%) cultivars. To determine whether different external stimuli affected the shoot K^+ in plants, t-test analyses were applied to different data sets. Statistical analyses showed that shoot K^+ was not affected by different osmotic and salinity inducers (Figures 2.23D-F).

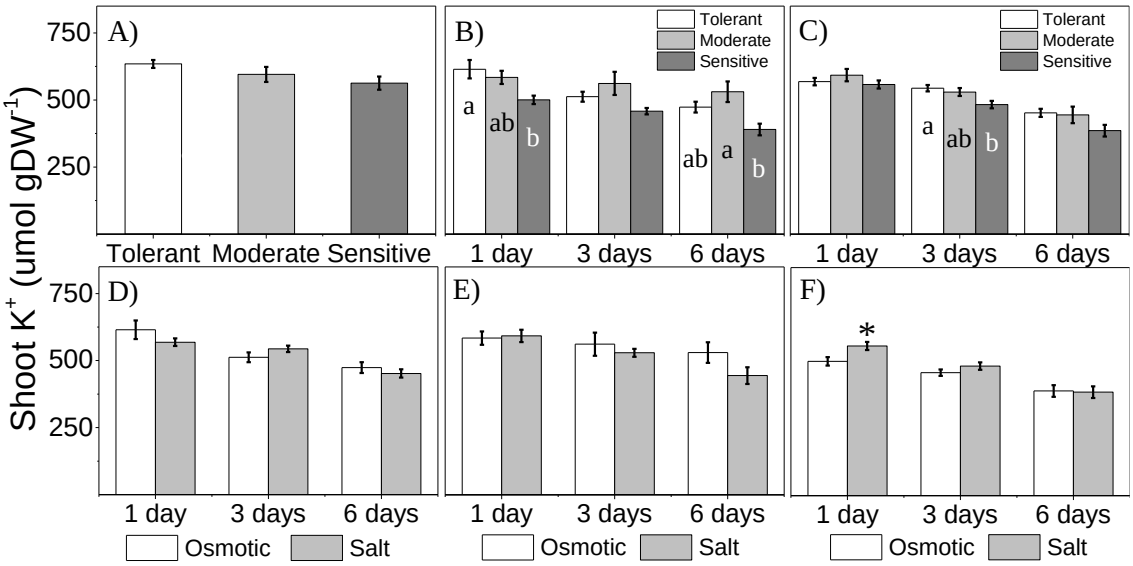


Figure 2.23. Shoot K^+ of the selected rice cultivars. (**A**) Shoot K^+ of rice cultivars exposed to control conditions. (**B**) Shoot K^+ of rice cultivars exposed to osmotic conditions. (**C**) Shoot K^+ of rice cultivars exposed to saline conditions. Significance was calculated by one-way ANOVA. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). (**D**) Shoot K^+ of tolerant cultivar. (**E**) Shoot K^+ of moderate cultivar. (**F**) Shoot K^+ of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test ($P<0.05$). Bars show the mean ±SE of six plants.

2.3.12 Correlation between WL and K^+ concentrations in plant tissues

It is well established in the literature that the rate at which ions are taken up by plant roots and transported to shoot tissues may be considerably affected by the rate of water loss via transpiration (Russell and Shorrocks, 1959; Olyaei *et al.*, 2016). To determine whether WL plays a direct role in the process of uptake, transport and distribution of K^+ in plant tissues, correlation analyses were carried out between the WL and K^+ concentrations in plant tissues. Figures 2.24A-C show positive correlations between the WL and root K^+ of plants exposed to osmotic and salinity conditions. Further analyses showed that the WL of plants did not correlate with K^+ in xylem sap of rice cultivars exposed to osmotic conditions. However, in saline conditions, there were positive correlations between the WL and K^+ in xylem sap of tolerant and sensitive cultivars (Figures 2.24D-F). For correlations between the WL and shoot K^+ of plants, Figures 2.24G-I show that there were positive correlations between the WL and shoot K^+ of most plants, except for the moderate cultivar, exposed to osmotic conditions.

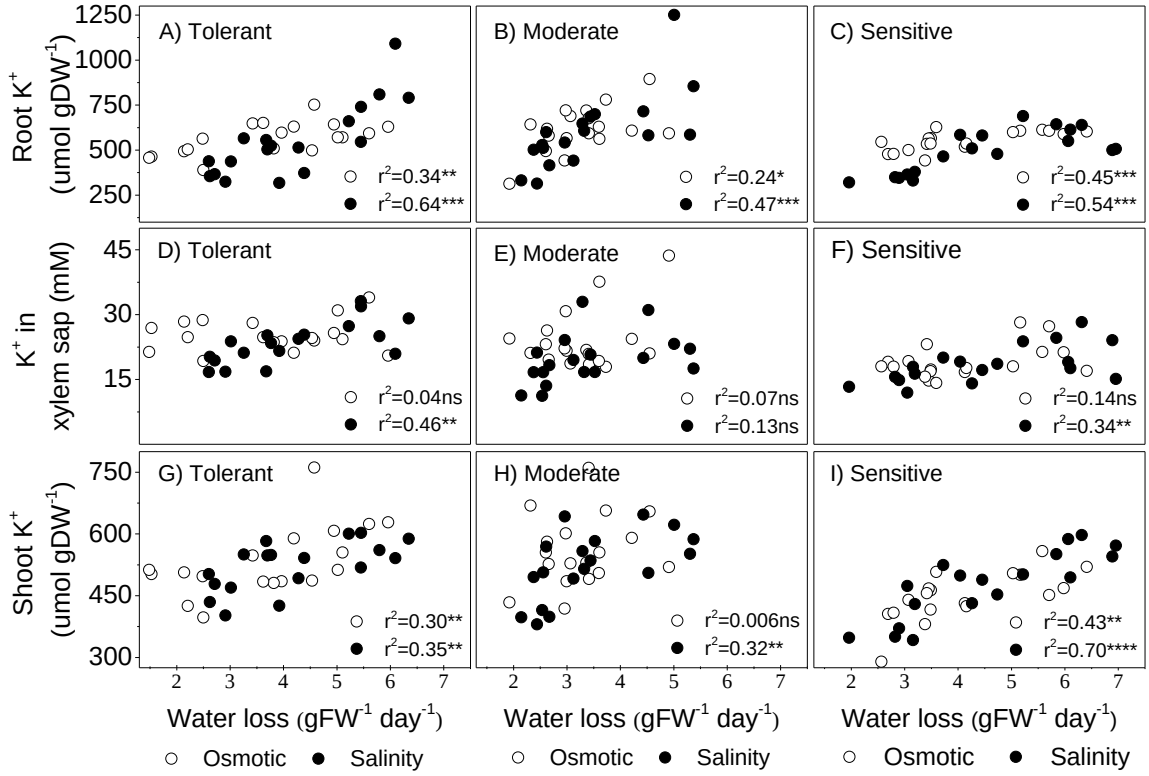


Figure 2.24. Correlations between water loss and K^+ concentrations in plant tissues of rice cultivars. (**A-C**) Correlations between WL and root K^+ of tolerant, moderate and sensitive rice cultivars exposed to osmotic and salinity conditions. (**D-F**) Correlations between WL and xylem sap K^+ of tolerant, moderate and sensitive rice cultivars exposed to osmotic and salinity conditions. (**G-I**) Correlations between WL and shoot K^+ of tolerant, moderate and sensitive rice cultivars exposed to osmotic and salinity conditions. Rice plants were sampled after1, 3 and 6 days of osmotic (9% PEG) and salt (50 mM NaCl) stress conditions. ^{ns}indicates the correlation is not significant at the 0.05 level (two-tailed). *indicates the correlation is significant at the 0.05 level (two-tailed). **indicates the correlation is significant at the 0.01 level (two-tailed). ***indicates the correlation is significant at the 0.001 level (two-tailed). ****indicates the correlation is significant at the 0.0001 level (two-tailed).

2.3.13 Correlation between K^+ concentrations in plant tissues

Much study has shown that there is a correlation between the root K^+ and shoot K^+ of cereal species exposed to osmotic and saline conditions (Chen *et al.*, 2005). To assess whether (a) the root K^+ correlates with the shoot K^+ and (b) K^+ in xylem sap of plants correlates with shoot K^+, correlation analyses were performed on these data sets. Figures 2.25A-C show that there were positive correlations between the root K^+ and shoot K^+ of plant exposed to osmotic and saline conditions. However, there was no correlation between K^+ in xylem sap and the shoot K^+ of plants exposed to osmotic conditions. Meanwhile, K^+ concentrations in xylem sap positively correlated with the shoot K^+ of the tolerant and moderate rice cultivars when exposed to saline conditions (Figures 2.25D-F).

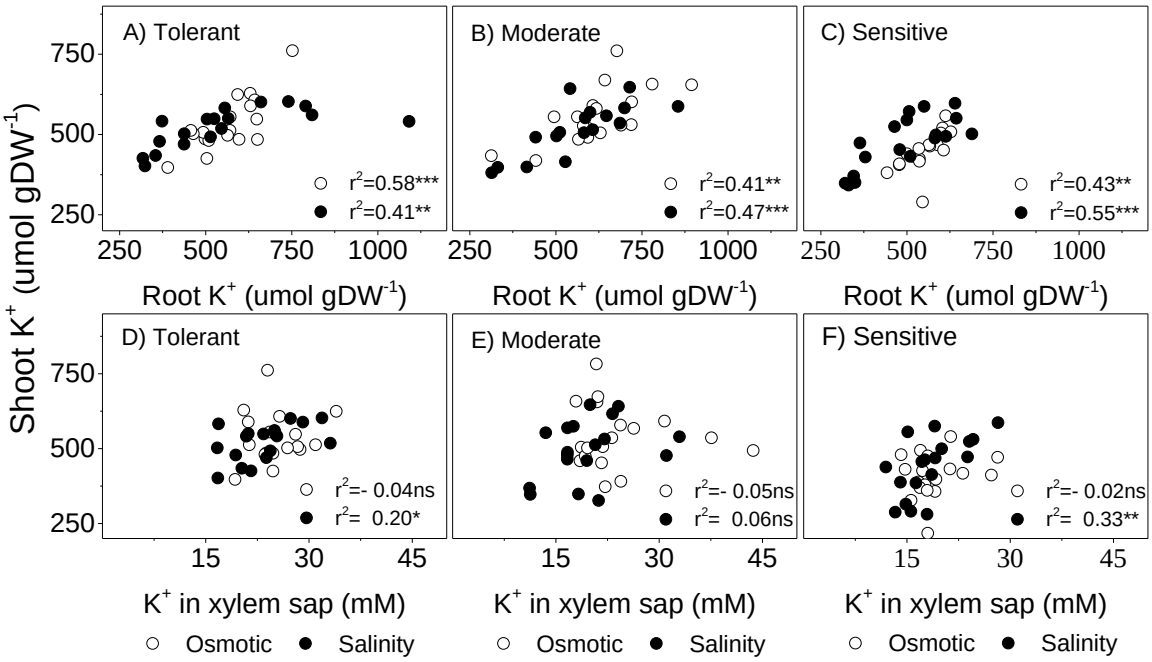


Figure 2.25. Correlations between root K^+, xylem sap K^+ and shoot K^+ of rice cultivars. (**A-C**) Correlations between root K^+ and shoot K^+ of tolerant, moderate and sensitive rice cultivars exposed to osmotic and salinity conditions. (**D-F**) Correlations between xylem sap K^+ and shoot K^+ of tolerant, moderate and sensitive rice cultivars exposed to osmotic and salinity conditions. Rice plants were sampled at 1, 3 and 6 days of osmotic (9% PEG) and salt (50 mM NaCl) stress conditions. ^{ns}indicates the correlation is not significant at the 0.05 level (two-tailed). *indicates the correlation is significant at the 0.05 level (two-tailed). **indicates the correlation is significant at the 0.01 level (two-tailed). ***indicates the correlation is significant at the 0.001 level (two-tailed).

2.3.14 Long term experiments: Na^+ ionic, the additional effect of salinity

The maintenance of low Na^+ in roots and shoots of rice plants is related to high tolerance to saline condition (Kavitha *et al.*, 2012). To assess whether (a) the root Na^+ is different between cultivars and (b) the root Na^+ correlates with the RGR-reduction of plants exposed to long term saline conditions, ANOVA and correlation analyses were performed using these data sets. There was no significant difference between cultivars for root Na^+ (Figure 2.26A) and no correlation between the root Na^+ and the RGR-reduction of plants (Figure 2.26B). However, the Na^+ in shoots of plants exposed to long term saline conditions was significantly different between the cultivars (Figure 2.27A). Interestingly, the shoot Na^+ positively correlated with the RGR-reduction of plants (Figure 2.27B). Further analyses showed that the tolerant cultivars exhibited lower shoot Na^+ compared to moderate and sensitive cultivars (Figures 2.27C and D).

To determine whether Na^+ concentration in shoots was affected by growth, the shoot Na^+ was divided by the mean dry weight of the plants to show how much Na^+ was taken up per plant. As shown in Figure 2.28A, there were significant differences between cultivars for shoot Na^+ based on plant dry weight. Most of the cultivars had low relative shoot Na^+ concentration because of high dry plant biomass, and further analysis showed that there was a negative correlation between the shoot Na^+ and plant dry biomass (Figure 2.28B).

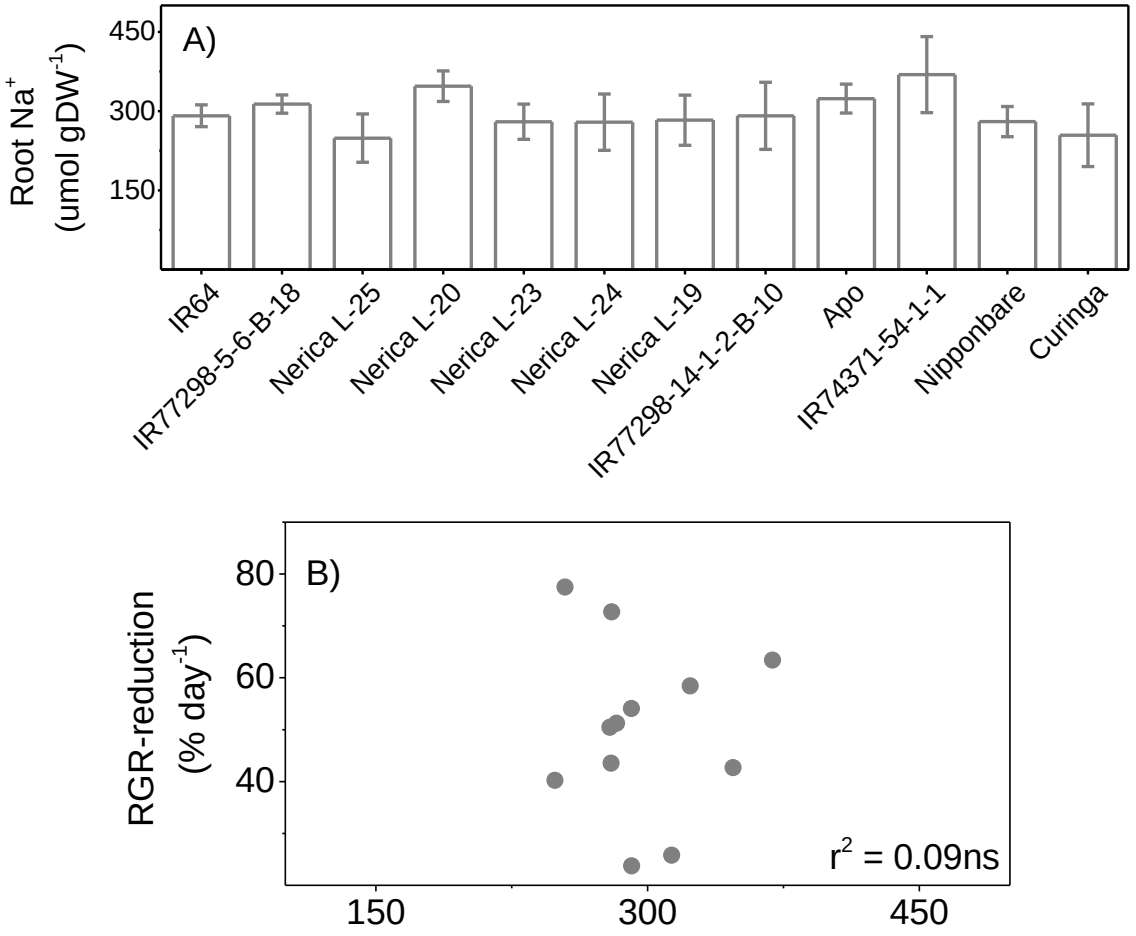


Figure 2.26. Root Na^+ and correlation between root Na^+ and RGR-reduction of rice cultivars. (**A**) Root Na^+ of rice cultivars exposed to long term saline conditions. Bars show the mean ±SE of three plants. (**B**) Correlation between root Na^+ and RGR-reduction of rice cultivars. No significant correlations (ns) were identified at the 0.05 level (two-tailed).

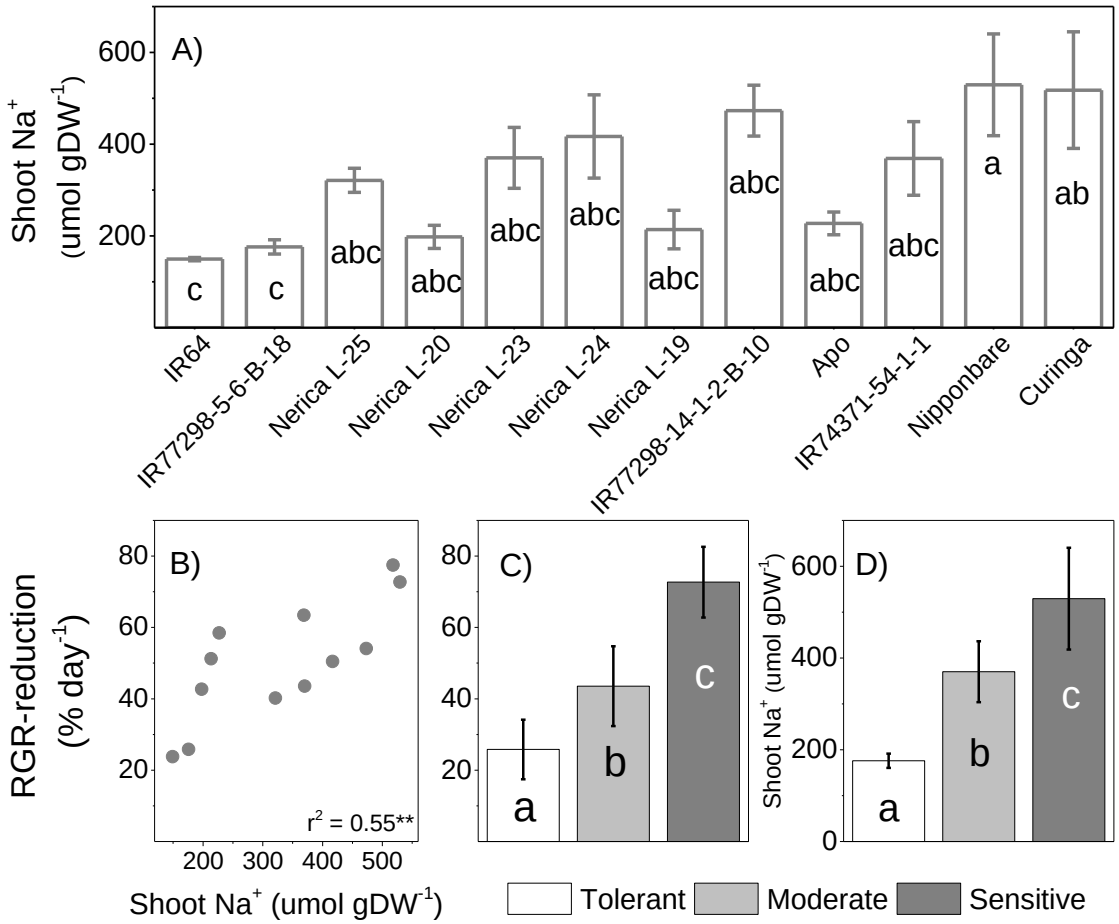


Figure 2.27. Shoot Na^+ of rice cultivars and correlation between shoot Na^+ and RGR-reduction. (**A**) Shoot Na^+ of rice cultivars. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of three plants. (**B**) Correlation between shoot Na^+ and RGR-reduction. **indicates the correlation is significant at the 0.01 level (two-tailed). (**C**) RGR-reduction traits of salt tolerant, moderate and sensitive rice cultivars. (**D**) Shoot Na^+ traits of salt tolerant, moderate and sensitive rice cultivars. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of two, eight and two plants of salt tolerant, moderate and sensitive cultivars.

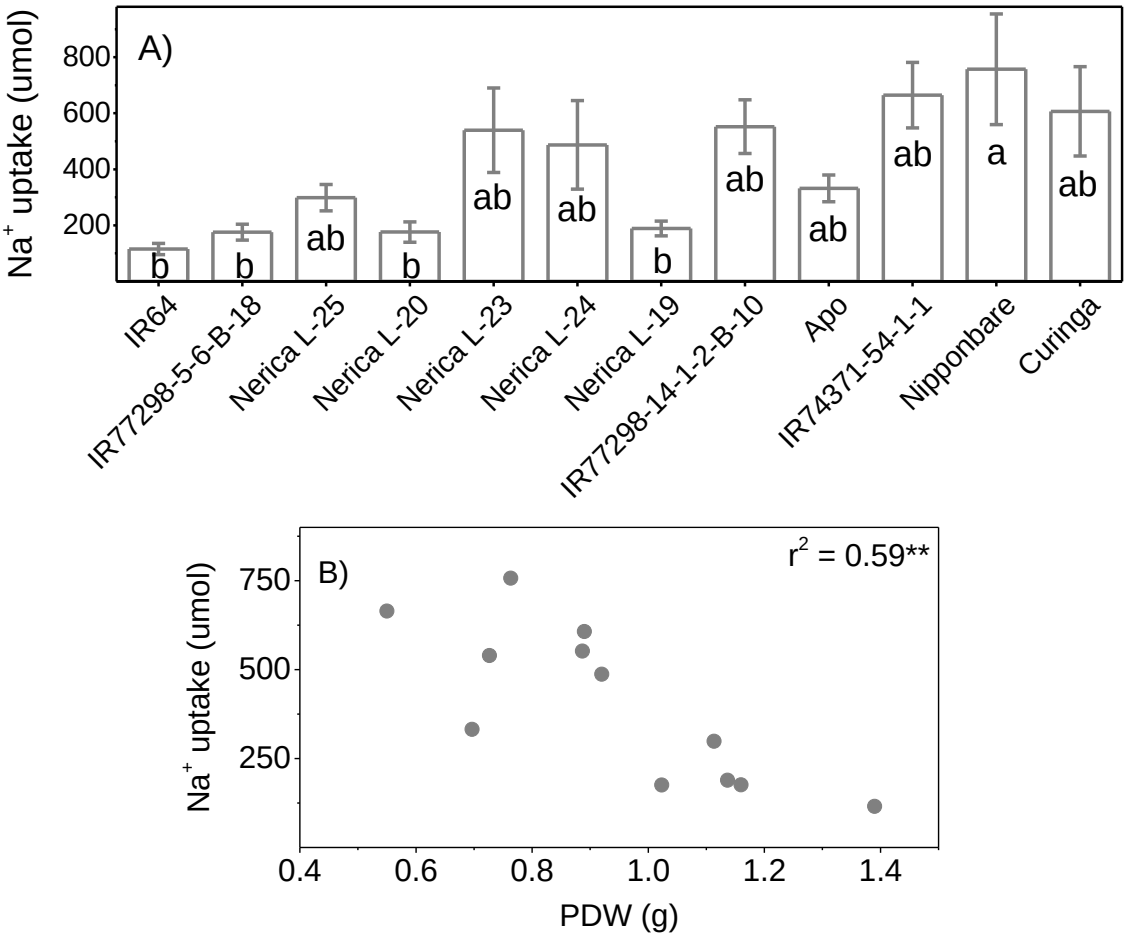


Figure 2.28. Shoot Na^+ concentration related to growth and its correlation with dry plant biomass. (**A**) Shoot Na^+ concentration related to dry plant biomass. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of three plants. (**B**) Correlation between related shoot Na^+ to growth and dry plant biomass. **indicates the correlation is significant at the 0.01 level (two-tailed).

2.3.15 Short term experiments: salinity effects on Na^+ of plant tissues

Based on the three clusters identified in the long term salinity conditions, the selected tolerant, moderate and sensitive cultivars (see above) were characterised by their physiological responses to short term saline conditions. Maintenance of low root Na^+ and efficient loading and unloading of Na^+ in xylem sap may avoid a high concentration of Na^+ in shoots of plants exposed to saline conditions (Zhu *et al.*, 2016b; Chen *et al.*, 2007). To assess the effects of saline treatments on the Na^+ in roots, xylem sap and shoots of plants, data sets were analysed by cultivars and time points. After 1 and 6 days of saline treatment, the concentrations of Na^+ were significantly different between cultivars (Figure 2.29A). Na^+ in xylem sap of plants increased with time of salt exposure and the sensitive

cultivar showed significantly higher Na^+ concentration after 6 days of treatment (Figure 2.29B). Likewise, the shoot Na^+ increased along treatment exposure and there were significant differences between cultivars. Interestingly, the shoots of the tolerant cultivar showed 10% more Na^+ after 6 days of salt treatment in comparison to the sensitive line in which the shoot Na^+ increased 50% during the same period (Figure 2.29C).

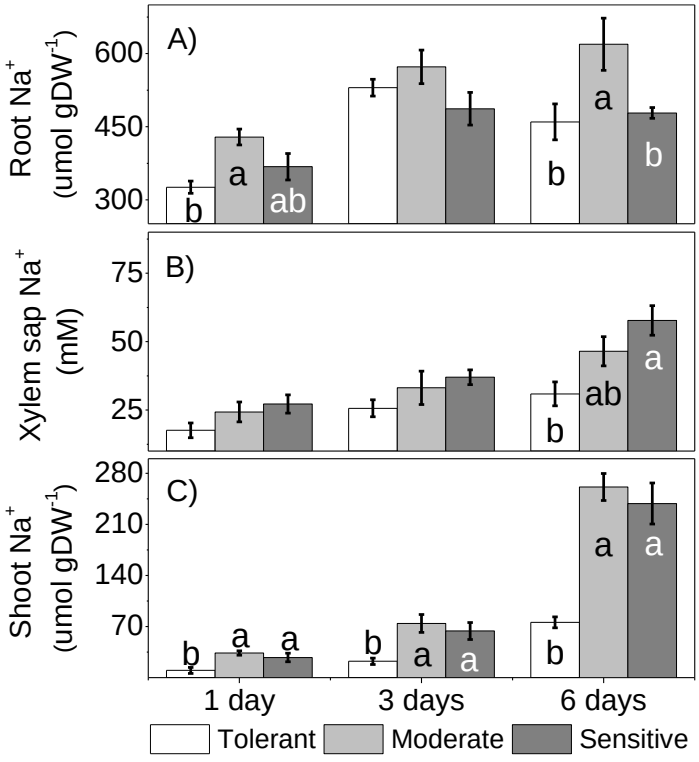


Figure 2.29. Na^+ concentrations in tissues of rice cultivars exposed to saline conditions. (**A**) Root Na^+ of rice cultivars. (**B**) Na^+ in xylem sap of rice cultivars. (**C**) Shoot Na^+ of rice cultivars. Significance was calculated by one-way ANOVA. Means followed by different letters are significantly different between cultivars (Tukey's honest significant test HSD, $P<0.05$). Bars show the mean ±SE of six plants.

2.3.16 Correlation of water loss and Na^+ in plant tissues

To determine whether WL plays a direct role in the process of uptake, transport and distribution of Na^+ in plant tissues, correlation analyses were carried out between the WL and Na^+ concentrations in plant tissues. As shown in Figures 2.30A-C, there were negative correlations between the WL and root Na^+ of plants. Further analyses showed that the WL of plants negatively correlated with Na^+ in xylem sap (Figure 2.30D-F). Moreover, the WL of plants correlated with shoot Na^+ of plants exposed to saline conditions (Figures 2.30G-I).

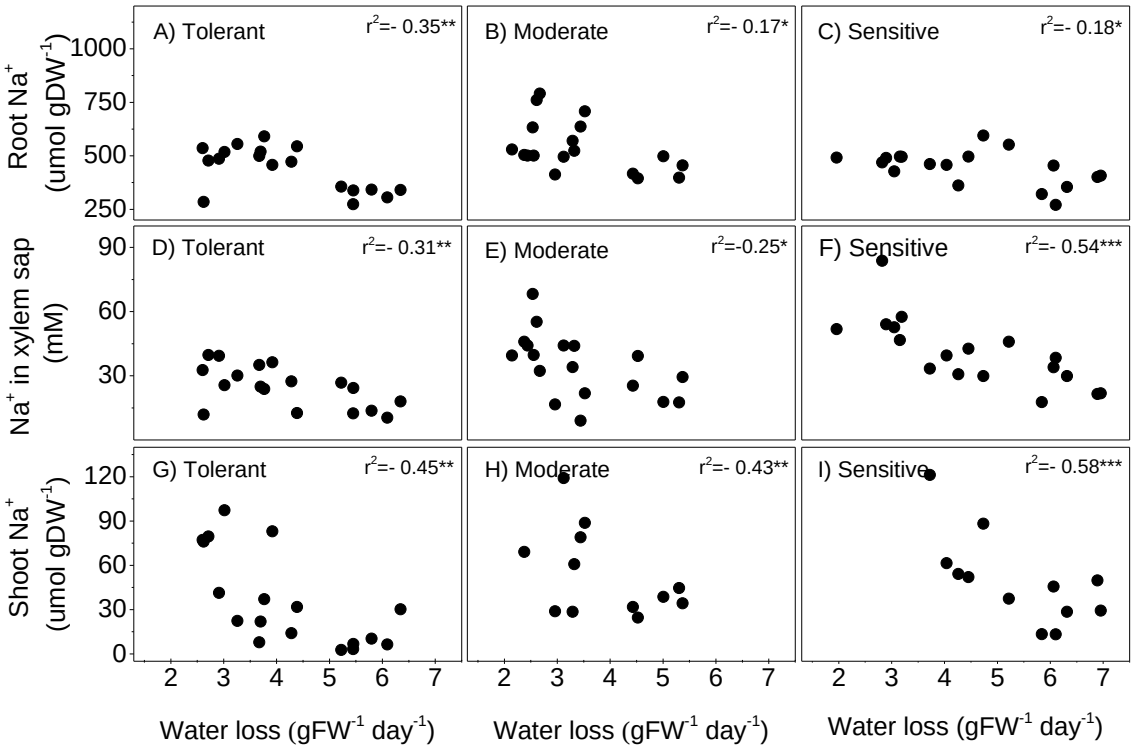


Figure 2.30. Correlations between water loss and Na^+ concentrations in plant tissues of rice cultivars. (**A-C**) Correlations between WL and root Na^+ of tolerant, moderate and sensitive rice cultivars exposed saline conditions. (**D-F**) Correlations between WL and xylem sap Na^+ of tolerant, moderate and sensitive rice cultivars exposed saline conditions. (**G-I**) Correlations between WL and shoot Na^+ of tolerant, moderate and sensitive rice cultivars exposed saline conditions. Rice plants were sampled at 1, 3 and 6 days of salt (50 mM NaCl) treatments. *indicates the correlation is significant at the 0.05 level (two-tailed). **indicates the correlation is significant at the 0.01 level (two-tailed). ***indicates the correlation is significant at the 0.001 level (two-tailed).

2.4 Discussion

2.4.1 Osmotic and salinity effects on growth

Rice is a species with over 127,000 different accessions. Such wide genetic diversity makes it possible to identify stress tolerant lines and use them in breeding programmes to combine biotic and abiotic tolerances with high grain yield. Many rice cultivars have been characterised for physiological responses to drought and saline conditions. Despite the fact that many rice cultivars have been well studied in these conditions, not much research has been conducted using *O. sativa* and *O. glaberrima* rice cultivars in parallel osmotic and salinity treatments. Therefore, the aim of this study was to (a) assess if there is a correlation between osmotic and salt tolerance in rice and (b) identify physiological changes in rice that are common in response to osmotic and saline conditions.

One interesting finding is that growth was significantly different between cultivars of plants grown in control conditions when using absolute growth values, but not when using relative growth values. These results support the idea that relative growth values are the most suitable parameters to measure the effect of a treatment on plant growth during the time of treatment imposition (Negrão *et al.*, 2016). Based on correlation analyses, the IPB did not correlate with the FPB or RGR of plants exposed to control conditions. Likewise, the IPB of treated plants did not show effects in the FPB, RGR and RGR-reduction. However, it was observed that the Nerica L-25 line's growth is vigorous in control conditions and is highly tolerant to osmotic and saline conditions. The most interesting finding was that there was a strong positive correlation between osmotic-tolerance and salinity-tolerance of rice cultivars based on absolute and relative growth values (Figure 2.3.9 and Supplementary data 2.1). Another important finding was that using RGR-reduction, the osmotic tolerance of plants was significantly different between *indica*, *japonica* and Nerica lines (cross between *O. sativa indica* and *O. glaberrima*).

Even though osmotic, saline and drought are different conditions, the tolerance of the 12 rice cultivars exposed to PEG and NaCl were comparable to the published drought tolerance. Eight cultivars used in this study were previously characterised for drought tolerance (Lafitte *et al.*, 2006; Moukoumbi *et al.*, 2011; Moumeni *et al.*, 2011; Rodenburg *et al.*, 2006; Sharoni *et al.*, 2012) and the reported ranking in tolerance correlated well with findings in this study. Nonetheless, this study classified three cultivars as drought tolerant and one as sensitive, properties which did not correlate with the degree of tolerance found in the literature for these cultivars (Anantha *et al.*, 2016; Moumeni *et al.*, 2011; Sakai *et al.*, 2010; Venuprasad *et al.*, 2007; Verulkar *et al.*, 2010). The difference between the published drought tolerance and the findings of this study may have been influenced by different experimental conditions such as weather, plant age, the severity of drought condition and the different traits that were used for drought tolerance index.

2.4.2 Effects of osmotic and salinity on K^+

It is well established in the literature that K^+ is essential for plant nutrition and is related to osmotic and salinity tolerance of crop cereals (Wei *et al.*, 2013; Lin *et al.*, 2004; Chen *et al.*, 2007). The results of this study show that K^+ concentrations in plant tissues varied between treatments and duration of treatment exposures. For plants exposed to short and long term saline conditions, the root K^+ of plants was lower in comparison to plants grown in osmotic and control conditions. This result may be explained by the fact that Na^+ competes with K^+ for uptake through common transport systems such as NSCCs and HKTs (Kader and Lindberg, 2005; Demidchik and Tester, 2002; Rodríguez-Navarro and Rubio, 2006). Furthermore, there were reductions of K^+ in roots of plants exposed to

long term osmotic and saline conditions. Meanwhile, the shoot K^+ of rice cultivars was not significantly different between treatments but there was a reduction of K^+ over time of exposure. These results must be interpreted with caution because there were significant differences between K^+ concentrations of 36-day and 60-day old plants grown in control conditions. These results are likely to be related to K^+ recirculation from shoots to roots, through phloem pathways (Cooper and Clarkson, 1989; Wolf and Dieter Jeschke, 1987). Moreover, the K^+ concentrations did not show correlation with the absolute or relative growth values of plants exposed to different treatments. Regarding K^+ in the xylem sap, there was observed that a clear loading and unloading of K^+ in the xylem sap of moderate and tolerant cultivars exposed to osmotic conditions but not in saline conditions.

2.4.3 Effects of osmotic and salinity on WL

Several reports have shown that WL in plants is reduced under osmotic and saline conditions (Álvarez and Sánchez-Blanco, 2014). The results of WL reduction found in this study indicate that there was a partial stomatal closure triggered by low water availability (Yoshida *et al.*, 2006). Research has shown that plants temporarily adapt to these stressful conditions using osmoprotectants, and ions such as K^+ or even Na^+ for osmoregulation (Le Rudulier *et al.*, 1984; Chen *et al.*, 2005). The adaptation is not permanent. Other environmental factors including weather conditions, balance of nutrients and strength of osmotic and salinity may accelerate or delay death (Hsiao *et al.*, 1976; Oliver *et al.*, 2010).

Low water potential is caused by osmotic and saline conditions and many studies have shown that water and ion uptake will decrease because of the change in water potential (Erlandsson, 1975). The current study found that the sensitive cultivar showed greater water loss compared to moderate and tolerant cultivars. This finding is contrary to that of Cabuslay *et al.* (2002) who found that tolerant cultivars exhibited higher transpiration. This inconsistency may be due to the duration of treatment exposures and possibly the sensitive cultivar having an imbalance between water uptake and water loss by transpiration (Oliver *et al.*, 2010). According to the results, it can be inferred that the sensitive cultivar exposed to long term osmotic and saline conditions may have lower growth, WUE and specifically lower concentrations of K^+ in plant tissues.

On the other hand, some research has shown that transpiration is not essential for long-distance transport of mineral elements in plants because there are other mechanisms such as the flow characteristics and diurnal dynamics of the phloem and xylem flow (Tanner and Beevers, 1990; Windt *et al.*, 2006). This event was observed with K^+ in xylem sap which did not correlate with the WL of

plants exposed to PEG and NaCl. A reduced water loss and K^+ in plant tissues was the common physiological response to short term osmotic and salinity conditions.

2.4.4 Effects of osmotic and salinity on ROS

Regarding ROS, the current study confirmed that plant roots produce low levels of ROS in control conditions, while in osmotic and saline conditions they are greatly increased (Vaidyanathan *et al.*, 2003; Raghavendra *et al.*, 2010; Chen *et al.*, 2009). ROS levels were the highest in the sensitive cultivar despite the same cultivar showing lower production of ROS during the first 60 minutes in either osmotic or salt conditions (Supplementary data 2.2). A possible explanation for this might be that the sensitive cultivar had a delayed response to the stress. However, when the stress continues, ROS production exceeds that of the other cultivars and likely causes greater damage to proteins, DNA and lipids (Miller *et al.*, 2010; Apel and Hirt, 2004).

2.4.5 Effects of saline conditions on Na^+ concentrations in plant tissues

Previous research has extensively shown that shoot Na^+ is related to salt tolerance in glycophytic plants. This study confirmed that salt tolerance correlated with shoot Na^+ using either absolute or relative growth values. Shoots are the main tissues where the plants accumulated Na^+. Another important finding was that salt tolerance of plants was achieved through high plant biomass i.e. the vigorous plants showed lower Na^+ concentrations. In short term saline conditions, there was no correlation between salt tolerance and the shoot Na^+. These results support the idea that excess accumulation of Na^+ in shoots occurs during long term stress.

Finally, these outcomes corroborated the idea of Lin *et al.* (2004), who suggested that the shoot K^+ negatively correlated with shoot Na^+ i.e. there was competition between Na^+ and K^+. This event was clearly observed in short term but not in long term saline conditions which might mean that Na^+ was over accumulated in plant tissues (Supplementary data 2.3).

2.5 Conclusion

The aims of this study were (a) to assess if there is a correlation between osmotic and salt tolerance in rice and (b) to identify physiological changes in rice that are common in response to osmotic and saline conditions. The research presented here has found strong correlations between osmotic and salinity tolerance in rice cultivars and these results are in agreement with those obtained in several previous studies. The research has also shown that rice cultivars exhibited multiple common physiological responses in short and long term osmotic and saline conditions. Reduced growth rates, changes in K^+ and WL as well as increase of ROS production were some of the common physiological responses of rice cultivars under osmotic and saline conditions.

Chapter 3

Genome Wide Association Studies to Identify Rice Tolerance Markers

3.1 Introduction

Rice breeders have been able to and need to maintain high genetic diversity for different biotic and abiotic stress conditions because rice is grown in over a 100 countries including many with limited resources and the demand for rice will increase with the increasing human population (Chang, 2003; Huang *et al.*, 2012b). There are estimated to be more than 12,700 natural accessions (IRRI, 2016) and of these only a few hundred have been investigated using genetic mapping approaches for morphological (Qu et al., 2008; Biscarini et al., 2016), physiological (Lin et al., 2004; Huang et al., 2010) and grain yield (Huang *et al.*, 2012b; Begum *et al.*, 2015) traits.

3.1.1 Rice genetic diversity

The genus *Oryza* belongs to the tribe *Oryzeae* of the family *Poaceae*. There are 12 genera within the *Oryzeae* tribe (Vaughan *et al.*, 2003). The genus *Oryza* contains approximately 22 species of which 20 are wild species and two, African *O. glaberrima* and Asian *O. sativa* are the cultivated species (Vaughan *et al.*, 2003). The African cultivated rice, *O. glaberrima*, is only grown on a small scale in West Africa (Khush, 1997). Studies have shown that *O. glaberrima* has five subpopulations based on genome-wide linkage disequilibrium (LD) and genome sequence approaches (Semon et al., 2005; Wang et al., 2014). Meanwhile, *O. sativa* derived from the wild species *Oryza rufipogon*, and is the most widely grown throught the world (Wu et al., 2014).

The domestication of *O. sativa* may have occurred between 12,000 and 7000 years ago with two main varieties of this species emerging which are the *indica* and *japonica* populations (Wang *et al.*, 2016b; Wu *et al.*, 2014). The domestication of the *indica* and *japonica* varieties probably originated in the foothills of the Himalayas in Eastern India and in the south of China, respectively (Londo et al., 2006; Sweeney and McCouch, 2007).

Using neighbour-joining and principal component analyses based on single nucleotide polymorphisms (SNPs), *indica* is divided into *australis*, *indica* and *admixed-indica* subpopulations,

while *japonica* is divided into *tropical-japonica*, *temperate-japonica*, *aromatic* and *admixed-japonica* (Wang *et al.*, 2016b; McCouch *et al.*, 2016; Huang *et al.*, 2012b).

3.1.2 Genomic mapping

In plant biology, genomic mapping is a useful approach for basic research and for application in plant breeding (Grotewold et al., 2015). Using quantitative trait loci (QTL) analyses, a gene's locus can be fairly accurately determined to a sub-region of a chromosome (Thomson et al., 2010), while using genome wide association study (GWAS) typically points to a more precise location of polymorphisms in the sequence of chromosomal DNA (Korte and Farlow, 2013).

In general, the impact of a specific genome region is determined by the strength of the association between a genotype and phenotype. The literature summarises that there are two types of maps: genetic and physical maps (Cherry et al., 1997; Chen et al., 2002). The genetic map is abstract, predicting the relative location of genetic markers on chromosomes (Lin et al., 2004) whereas the physical map can precisely position genetic markers in the genome (Si et al., 2016).

For a genomic mapping study, genetic markers and a population of organisms, such as humans, animals or plants are necessary. A genetic marker is a DNA sequence with a known location on a chromosome (Schuler et al., 1996). Using SNPs as molecular markers for genotypic data is becoming a common technique. This new generation of SNP molecular markers has helped to detect associations for a variety of human diseases but the approach has also been adopted for sequencing DNA of plant models such as Arabidopsis (Cao et al., 2011) and rice (Jackson, 2016) and is now part of many major breeding programmes for major crops. The details of QTL and GWAS analyses are further discussed below.

3.1.3 Quantitative Trait Loci (QTL) mapping

A quantitative trait locus (QTL) is a section of DNA or genomic region that correlates with variation in a phenotype. The QTL measures the association in terms of probabilities between phenotype and genotype. There are many QTL studies that have been carried out for biomass and yield (Marathi et al., 2012; Dixit et al., 2014; Matsubara et al., 2016) and for responses to biotic and abiotic stress conditions of *O. sativa* (Wada *et al.*, 2015; Liu *et al.*, 2015b; Wang *et al.*, 2013).

The number of QTLs for rice grown in salt stress conditions has shown relevance for genomic studies. There are nearly one hundred QTLs reported which are related to relative growth rate, salt tolerance index and K^+ and Na^+ concentrations in root and shoot tissues of rice exposed to saline conditions (Koyama et al., 2001; Lu et al., 2014; Zheng et al., 2015; Tiwari et al., 2016). Studies that

list major rice QTLs involved in salinity tolerance include those by Lin et al. (2004) who reported 8 QTLs for rice plants exposed to 140 mM NaCl. In addition, Cheng et al. (2011) reported 47 QTLs using introgression lines exposed to 140 mM NaCl and Hossain et al. (2015) reported 16 QTLs at the reproductive stage of rice plants exposed to 100 mM NaCl.

The main limitation of QTL mapping is that the method only determines approximations of the QTLs within big genomic regions which can contain hundreds of genes. In addition, QTL uses populations created by crossing natural accessions to produce a set of lines in which the alleles of the parents segregate (Vaughan, 2015) and depending on the species, backcrossing may take months or even years (Weigel, 2012; Cai and Morishima, 2002).

Therefore, QTL mapping has been partially replaced by Genome Wide Association Study (GWAS) approaches due to the higher accuracy of this method to identify the exact genomic region that correlates with the phenotypic variation. In contrast to QTL analyses, association mapping is based on the use of natural populations of diverse cultivars with multiple recombination events that contribute to a higher resolution.

3.1.4 Genome Wide Association Study (GWAS)

GWAS was developed as a new innovative method for studying associations between genotypes and phenotypes (Lee *et al.*, 2015). A key difference to the traditional methods (e.g. QTL) is the ability of GWAS to handle up to approximately one million SNPs simultaneously and 10,000 natural accessions as a mapping population (Lipka *et al.*, 2012; Huang *et al.*, 2010; Chen *et al.*, 2014a). In a broad sense, GWAS identifies single nucleotide polymorphism markers that are significantly associated with a trait of interest across a diverse population. For species with available genome sequences, GWAS has contributed to revealing rich genetic architectures of complex traits (Li et al., 2014), GWAS has had an extensive application for studies using humans (Bush et al., 2016), animals (Pértille et al., 2016; Zhang et al., 2016), plants (Kooke *et al.*, 2016; Liang *et al.*, 2016; Hu *et al.*, 2016b), and bacteria (Chen and Shapiro, 2015).

For rice cultivars, GWAS has revealed hundreds of candidate genes for rice domestication (Wang *et al.*, 2016b), yield (Liang et al., 2016), flowering time (Zhao et al., 2011), root (Biscarini et al., 2016) and panicle architectures (Bai et al., 2016), as well as grain size, length and shape (Si et al., 2016; McCouch et al., 2016; Feng et al., 2016). Where biotic and abiotic stress are concerned, a small number of candidate genes has been revealed by GWAS for the blast pathogen (Shinada et al., 2015), drought (Huang et al., 2010), ozone (Ueda et al., 2015), aluminium (Famoso et al., 2011) and salinity stress conditions (Kumar et al., 2015).

3.1.5 GWAS in saline conditions

Recently, researchers have shown the efficiency of GWAS in the identification of candidate genes for rice in saline conditions. Kumar *et al.* (2015) identified 20 association signals and 44 significant SNPs when using ~ 4000 SNPs, 220 accessions and genome association and prediction integrated tool (GAPIT). Their experimental design, used plants at 35-days old from wet bed nurseries transplanted into hills and fertilisation was carried out applying 120-60-60 kg of NPK/ha. Plants were exposed to saline conditions from 56-day old plants to the end of the plant's life cycle. Phenotypic data was recorded from 5 plants per accession along with treatment and the stress susceptibility index was calculated following Fischer and Maurer (Fischer and Maurer, 1978). For genotypic data, 6000 SNPs were selected for GWAS analysis. This selected SNP array contains 16.3 % biotic and 73.5% abiotic stress-responsive genes. The SNP distribution across the rice genome was about one SNP per 51 kb. The most relevant finding of this study was the identification of the *qSaltol* QTL, localised between two significant SNPs identified by GWAS. The *qSaltol* QTL has been well characterised to contain determinant genes for salinity tolerance in rice (Nutan *et al.*, 2017; Waziri *et al.*, 2016).

3.1.6 Identification of non-synonymous SNPs

Non-synonymous SNP (ns-SNP) is a variation in a single nucleotide that occurs at a specific position in the genome. The ns-SNPs are localised in the coding sequences and have been shown to be able to cause significant phenotypic variation by changing the amino acids (Singh *et al.*, 2015b; Ramensky *et al.*, 2002; Smyth *et al.*, 2006; Huyen *et al.*, 2013). For example, Ren *et al.* (2005) identified 4 non-synonymous SNPs for high-affinity K^+ transporter (*OsHKT1;5*), and the salt tolerant Nona Bokra cultivar showed higher Na^+ transport activity compared to the salt sensitive Koshihikari cultivar. Further research has also shown that *OsHKT2;1* has 5 non-synonymous SNPs and the Nona Bokra lacks the *OsHKT2;1* (Linh *et al.*, 2012). For this reason, several studies have attempted to predict functional impact on the amino acid changes and salt tolerance in plants.

3.1.7 Correlation between published QTLs and SPNs

Despite the fact that QTL and GWAS measure association signals in different scales, it is feasible to analyse their relationships using QTL published data and GWAS SNP position. Recent findings have shown that the published QTLs correlated with SNPs identified through GWAS for different physiological traits in rice (Si *et al.*, 2016; Zhao *et al.*, 2011; Kumar *et al.*, 2015). For example, the *qSaltol* QTL was identified between 2 SNP positions revealed by GWAS.

3.1.8 Aims

In this study, a collection of 306 genotyped rice accessions was exposed to saline conditions to identify relatively well-known and novel determinant genes for osmotic and ionic components of salinity through GWAS. This study also assesses the efficiency of GWAS to get insights of amino acid changes of SNP positions, and evaluates correlations between genetic and physical mapping approaches.

3.2 General Methods

3.2.1 Rice diversity panel and high-density rice array

McCouch *et al.* (2016) have recently launched an open resource for GWAS in rice. These resources consist of ~ 400 rice accessions and 700, 000 SNPs. The rice diversity panels consist of a collection of hundreds of natural accessions (Kang *et al.*, 2015; McCouch *et al.*, 2016). Based on SNPs and principal component analysis, the genetic background of the accessions is summarised into *O. sativa indica* and *O. sativa japonica* populations which derive from *O. rufipogon* (Kim *et al.*, 2016). Specifically, of the 306 accessions used in this study there were 105 *indica* accessions and 201 *japonica* accessions. To determine the diversity of *indica* and *japonica* populations, further analysis showed that the *indica* variety contained 46, 55 and 4 accessions of *australis, indica, admixed-indica* subpopulations, respectively, while, the *japonica* variety contained 10, 71 and 85 accessions of *aromatic, temperate-japonica* and *tropical-japonica* subpopulations, respectively. Moreover, 4 accessions of *Admixed-indica-japonica* subpopulations were identified.

Regarding the rice SNP arrays, there are two available SNP arrays for these rice accessions. The first consists of a 44,100 SNPs (44k SNPs) and the latter consists of high-density rice array of 700,000 SNPs (HDRA). These SNP arrays were developed by McCouch RiceLab, Cornell University (Zhao *et al.*, 2011; McCouch *et al.*, 2016). The HDRA was designed to efficiently capture most of the haplotype variation observed in a discovery panel consisting of 16 million SNPs. This was achieved by sequencing 128 wild and domesticated rice accessions at ~ 7 x genome coverage. Furthermore, the HDRA has an average call rate = 93.5% or ~ 1 SNP every 0.54 kb across the rice genome (McCouch *et al.*, 2016).

3.2.2 Experimental growth conditions

All experiments were carried out in glasshouse and hydroponic medium conditions. Rice seeds were germinated in clay based terragreen substrate and at 15 days old rice plants were transferred to control standard hydroponic "CSH" medium (Yoshida et al., 1976). During the experiments, the hydroponic medium was changed each week to maintain an optimal mineral condition for plant growth and development. 30 days after sowing, the rice plants were exposed to either control or saline conditions. Three different durations of salinity were imposed: short (6h), medium (7d) and long term (30d). Salinisation of the medium was achieved by the addition of 50 mM NaCl.

3.2.3 Growth measurement and tissue sampling for K^+ and Na^+

To determine growth, the initial plant biomass (IPB) of the 30-day old rice plants was recorded. Plants were then submitted to control or saline conditions for another 30 days. Final plant biomass (FPB) were recorded and relative growth rate (RGR) was calculated with the formula of Evans (1972) and RGR-reduction was calculated by dividing the RGR-salt treated plants by the RGR-control plants and expressed in percentage of RGR reduction (Chapter 2, formulas 2.2.1 and 2.2.2).

For measurements of K^+ and Na^+ ion concentrations, root and shoot tissues were sampled for rice plants exposed to control and 50 mM NaCl. Fresh tissues were dried at 80 °C during three days, then the dry tissue weights were recorded, tissue was placed in 15 ml falcon tubes and 10 ml of $CaCl_2$ at 20 mM was added to each sample. Samples were stored for three days at room temperature and then analysed for K^+ and Na^+ ion concentrations using a flame photometer. The flame photometer was calibrated using standard concentrations of KCl and NaCl at 0.1, 0.25, 0.5 and 1.0 mM. For the readings of K^+ and Na^+ registered by flame photometer, a linear regression was carried out for each ion and the slope was used to calculate the concentration of ions in umol per gram of dry weight of rice tissues (Chapter 2, formula 2.2.3). The K^+ and Na^+ concentrations of root and shoot tissues as well as the growth data of rice accessions were collected from three independent plants for each experiment, i.e. short, medium and long term saline conditions.

3.2.4 Statistical analysis: phenotype in saline conditions

The data was analysed using analysis of variance (ANOVA) and correlation analysis between the RGR and K^+ and Na^+ concentrations. The ANOVA and correlation analyses were achieved using statistical analysis system SAS 9.3 and R software packages (SAS, 1999; R Development Core Team, 2016). Tukey's Honest Significant Difference Test (Tukey HSD; $P<0.05$) was used to test the

differences between the means of the different data sets, wherever significant differences were detected by ANOVA.

3.2.5 GWAS analysis and quality control

To perform GWAS, the mean values of growth, and K^+ and Na^+ concentrations of roots and shoots were submitted to R scripts using GenABEL package (Aulchenko *et al.*, 2007). The phenotypic data consisted of 6 single traits (RGR, RGR-reduction and K^+ and Na^+ in roots and shoots) and 46 combined traits of rice plants to perform GWAS. The combined traits were obtained dividing either K^+ or Na^+ concentrations in roots and shoots by growth values and vice versa (Supplementary data 3.1). GenABEL is an open-source software package that can perform GWAS. McCouch *et al.* (2016) recently released a high number of SNP arrays allowing GWAS using 700,000 SNPs and the collected phenotypic data of the 306 accessions exposed to saline conditions. The GenABEL package uses the Family-Based Score Test for Association (FASTA) which uses mixed linear models to correct false positive associations (Chen and Abecasis, 2007). To maintain a certain quality control on GWAS, SNPs were removed from this study if their minor allele frequency was less than 5% across the panel or the SNP genotype was unknown for > 20 % of the cultivars. Population structure was controlled using a mixed linear model (MLM), with a kinship matrix used as a covariate. Furthermore, the top four principal components calculated from the kinship matrix were used as cofactors.

To determine associations between genotype and phenotype, the FASTA model for GWAS numerically calculates a lambda value "λ" which is graphically plotted as quantile-quantile or "QQ plot" (Neale et al., 2010). Lipka et al. (2012) suggested that a normal QQ plot shows only minor deviation from the null distribution, except in the upper tail of the distribution. This corresponds to the SNPs with the strongest evidence of association (Supplementary data 3.2). In addition to QQ plots, Manhattan plots illustrate the association between phenotype and genotype. The association is expressed in terms of $-\log_{10}$(*P*-values), with higher *P*-values denoting associations between phenotype and genotype with higher confidence. However, it is important to know that such associations will contain many false positives so measures need to be taken to reduce the incidence of false positive associations. In this analysis, a false discovery rate (Benjamini and Hochberg, 1995) of 5% was used to correct for multiple testing.

3.2.6 Identification of candidate genes and polymorphisms

To identify genes that are potentially associated with the measured phenotype parameters, a genomic area of 250 kb around the most significant SNP call was interrogated using the Phytozome-11 rice genome browser (https://phytozome.jgi.doe.gov/pz/portal.html). Genes annotated as

transposon, retrotransposon, protein expressed, hypothetical or unknown were excluded from the analysis. Figure 3.1A shows an example of a Manhattan plot with several *P*-values that exceed the significance threshold and genes within the SNP cluster that were assigned as candidate genes. To facilitate assignment of candidate genes, the genomic area surrounding the significant associations were zoomed-in to obtain a more detailed picture of the SNP cluster and to map major and minor peaks in the Manhattan plots (Figure 3.1B). The most significant SNP position in minor and major peaks were selected and the genomic area was searched in Phytozome version 11 rice genome browser for candidate genes within 125 kb on either side of the SNP position with the highest *P*-value (Figure 3.2).

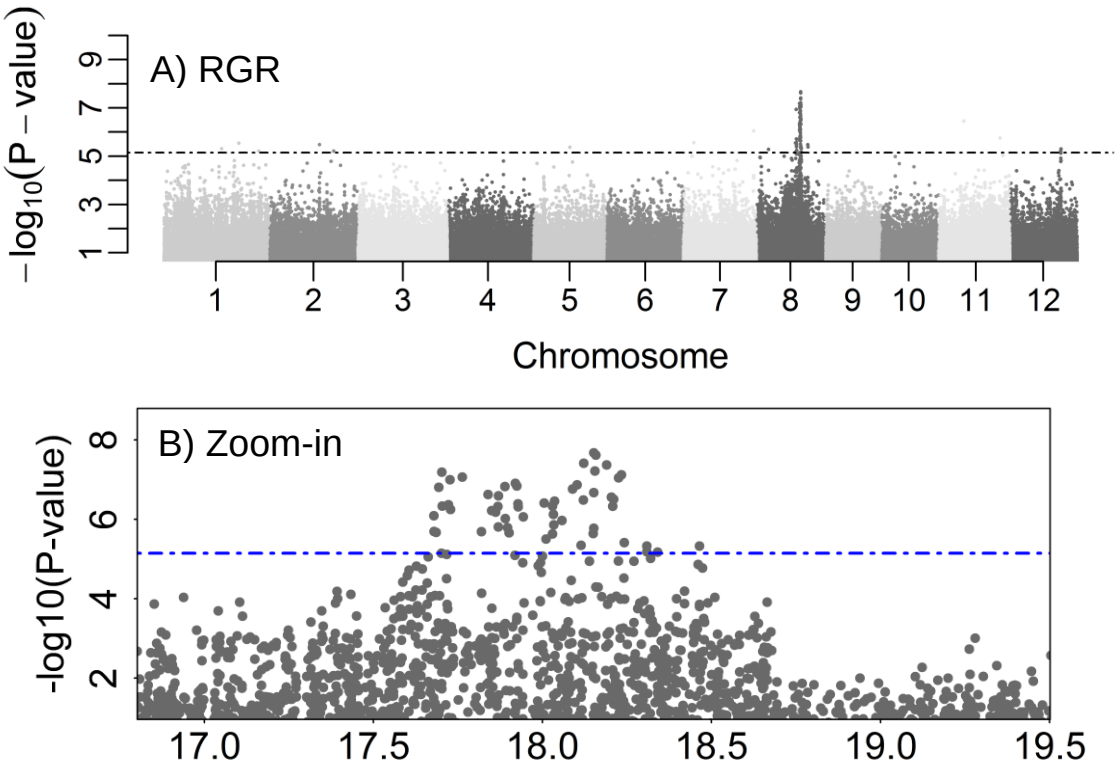


Figure 3.1. Manhattan plot showing the association signals. (**A**) GWAS analysis using RGR of the 306 accessions as phenotypic data. Plants were exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. (**B**) Zoom-in of regional association signals covering 2.5 Mb on chromosome 8 for the RGR of the 306 accessions exposed to long term saline conditions. The X-axis indicates the SNP positions between 16.9 and 19.4 Mb of the chromosome 8. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold.

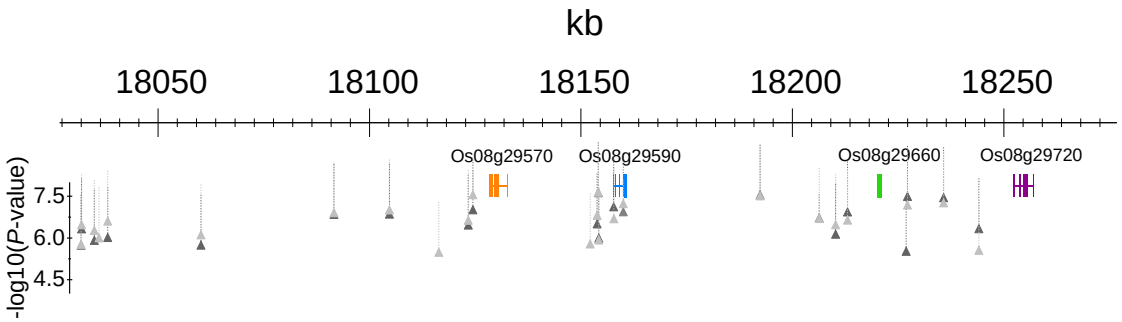


Figure 3.2. Candidate genes within a 250 kb rice genome browser window. Candidate genes were identified by GWAS using the RGR of the 306 accessions as phenotypic data. Plants were exposed to long term saline conditions (50 mM NaCl). The grey triangles with dashed lines representing significant SNP positions identified through GWAS. X-axis indicates the SNP positions across the zoom-in of rice genomic region. The y-axis shows the P-value for the association test at each locus on a log scale. Candidate genes are illustrated with different colours: ATP-binding cassette transporter OsABCG44 "orange"; zinc finger, C3HC4 type family protein "blue"; Transcription factor WRKY69 "green" and mitochondrial carrier "purple".

3.2.7 Identification of SNPs within coding regions of candidate genes

To gain insights into potential functional effects of the identified significant SNPs, bioinformatics analyses were carried out to identify SNPs in coding regions of candidate genes. Therefore, all SNPs that exceeded the significance criterion were assessed for their location in coding regions of candidate genes and subsequently for their potential impact on protein composition. These analyses were carried out using allele finder on the rice diversity website (https://ricediversity.org/tools/index.cfm) and rice genome browser (Bozeman, 2016). The coding sequences of all candidate genes were screened for the presence of non-synonymous SNPs (nsSNPs) to identify potential causative polymorphisms (Figure 3.3). In case relevant nsSNPs were found, allele frequencies were calculated and compared between a set of salt sensitive and salt tolerant accessions (60 in total) to determine correlations between the phenotype and genotype.

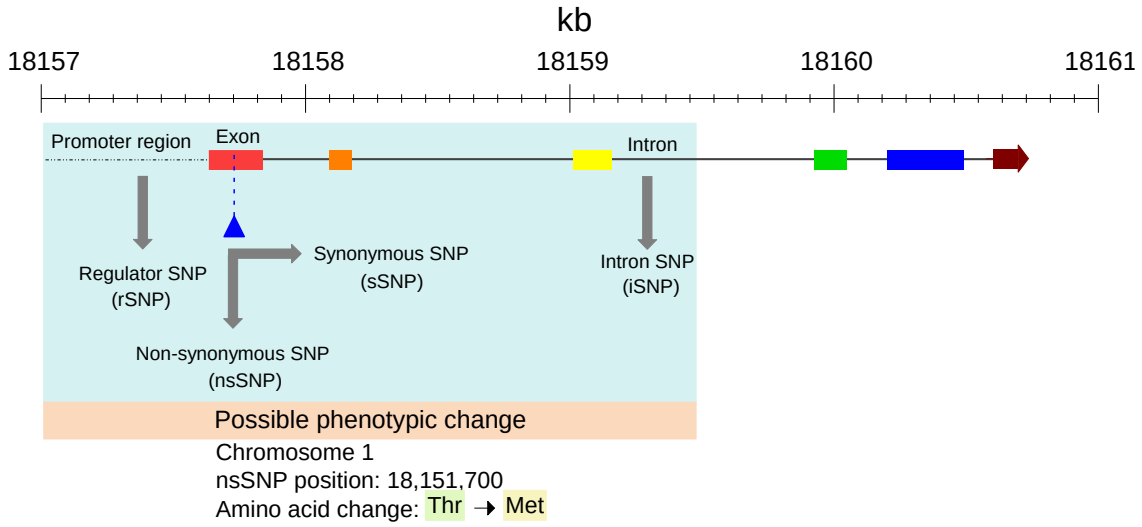


Figure 3.3. Single nucleotide polymorphism types within the genomic region of a candidate gene. The zinc finger, C3HC4 type family protein candidate gene was identified by GWAS using the RGR of the 306 accessions as phenotypic data of plants exposed to long term saline conditions (50 mM NaCl). Grey arrows illustrate the regulatory, intron, synonymous and non-synonymous SNPs across the genomic region of the candidate gene. The blue triangle with dashed lines represents one of the significant SNPs.

3.2.8 Relationship between published major QTLs and SNPs

To assess the potential relationship between genetic and physical mapping, an analysis of correlations between the major QTLs and SNP positions was carried out. By using Gramene and published data (Tello-Ruiz et al., 2015), the SNP positions identified by GWAS were correlated with the published minor and major QTLs of rice exposed to saline conditions.

3.3 Results

Salinity is a complex physiological trait limiting growth and causing accumulation of Na^+ (Negrão et al., 2013; Suzuki et al., 2016). Much study has shown that reduced rice growth is related to high Na^+ and low K^+ in plant tissues (Niones, 2004; Ammar et al., 2009; Wang et al., 2012). Therefore, in this study, salt tolerance based on ion concentrations and growth phenotypes of 306 accessions were used for the GWAS using 700,000 SNPs.

The outcomes will be presented in the order of growth phenotypes of 306 accessions exposed to long term control conditions, growth phenotypes of 306 accessions exposed to medium and long term saline conditions, and K^+ and Na^+ phenotypes of 306 accessions exposed to long term control,

short, medium and long term saline conditions. Likewise, GWAS results will be presented in the same order as phenotypic data.

3.3.1 Genetic variation for RGR of accessions grown in control conditions

The variation of plant growth exposed to control conditions can be attributed to the genetic diversity of the accessions. Before treatment exposures, the IPB showed significant difference between accessions, however, the average of the IPB of 306 accessions was not significantly different (Supplementary data 3.3 and 3.4). However, there was no reliable growth data obtained for the short (6h) exposure period. Growth in medium term control was assumed to be the same as in long term control because previous experiments showed that there was a strong correlation between medium-term-growth and long-term-growth of rice cultivars (Supplementary data 3.5). The RGR in long term control, non salinised condition, ranged from 2.7% to 6.6% and there was a significant difference between accessions (Supplementary data 3.6).

3.3.2 Medium and long term effects of salinity on RGR of accessions

To determine the effects of medium and long term saline conditions on growth, Negrão *et al.* (2016) suggested that the relative growth values of accessions are the most suitable parameters to measure the effect of a treatment on plant growth during the time of treatment exposure. For plants exposed to medium term saline conditions, the RGR ranged from 0.026% to 7.3% while the RGR of plants exposed to long term saline conditions was between 0.02% and 4.9%. For both medium-term-RGR and long-term-RGR, there were significant differences between accessions (Supplementary data 3.6). Additionally, there was a strong correlation between the medium-term-RGR and long-term-RGR of the 306 accessions (Supplementary data 3.7). However, there was no correlation between control-RGR and salt-RGR of the rice accessions exposed to different terms of saline conditions (Supplementary data 3.8). Meanwhile, the average RGR of the 306 accessions was significantly different between control, medium and long term saline conditions (Figure 3.4).

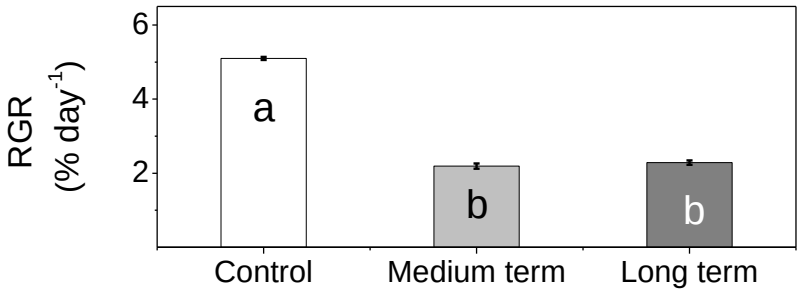


Figure 3.4. Relative growth rates of accessions exposed to control, medium and long term saline conditions. Means followed by different letters are significantly different between treatments (Tukey's honest significant test HSD, P<0.05). Bars show the mean ±SE of 306 accessions.

Moreover, the RGR-reduction of the 306 accessions exposed to medium term was between 1.7% and 24% while in long term saline conditions the RGR-reduction ranged from 10% to 99.4% (Supplementary data 3.9), further analysis showed that the average values of the RGR-reduction were significantly different between medium and long term saline conditions (Figure 3.5). Furthermore, there were negative correlations between the RGR and the RGR-reduction of the 306 accessions when exposed to either medium or long term saline conditions (Figure 3.6).

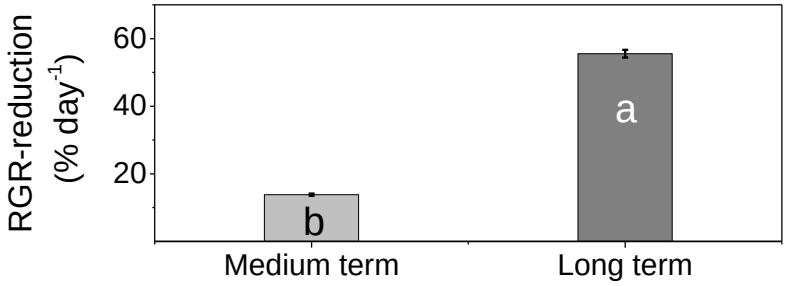


Figure 3.5. Relative growth rates of accessions exposed to control, medium and long term saline conditions. Means followed by different letters are significantly different between treatments (Tukey's honest significant test HSD, P<0.05). Bars show the mean ±SE of 306 accessions.

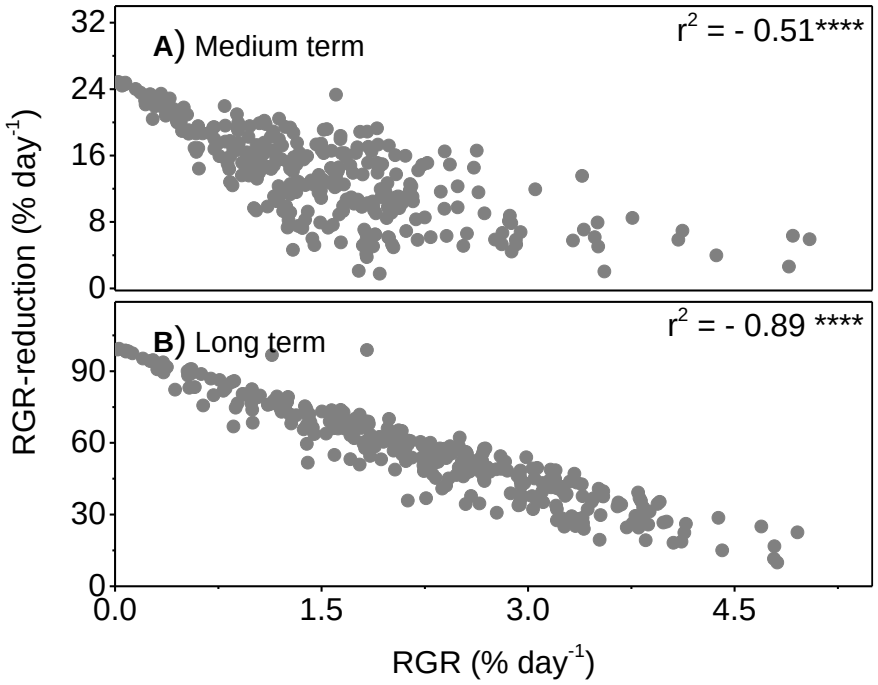


Figure 3.6. Correlations between RGR and RGR-reduction of 306 rice accessions exposed to saline conditions. (**A**) The correlation between RGR and RGR-reduction of accessions exposed to medium term salt stress. (**B**) The correlation between RGR and RGR-reduction of accessions exposed to long term salt stress. ****Correlation is significant at the 0.00001 level (two-tailed).

3.3.3 Phenotypic variation for K^+ and Na^+

For plants grown in long term control conditions, the K^+ concentrations in roots and shoots were determined and shown to be significantly different between accessions. The root K^+ values ranged from 240 to 888 umol gDW^{-1} and the shoot K^+ values were between 289 and 1009 umol gDW^{-1} (Supplementary data 3.10).

Na^+ uptake may contribute to osmotic adjustment of rice plants exposed to saline conditions (Yeo et al., 1991; Cramer, 2002). The K^+ and Na^+ concentrations in roots and shoots were significantly different between accessions. In short term saline treatments, the K^+ concentrations in roots and shoots ranged from 347 to 793 and 527 to 927 umol gDW^{-1}, respectively (Supplementary data 3.11). For Na^+ concentrations, the root Na^+ ranged from 148 to 546 umol gDW^{-1} and the shoot Na^+ ranged from 12 to 53 umol gDW^{-1} (Supplementary data 3.12).

High K^+ and low Na^+ in plant tissues is related to salt stress tolerance for many plant species (Chen et al., 2007; Hauser and Horie, 2010; Lin et al., 2004). In medium term saline conditions, the K^+ and Na^+ concentrations in roots and shoots were significantly different between accessions. The

root K^+ ranged from 249 to 1170 and the shoot K^+ ranged from 250 to 1093 umol gDW^{-1}, respectively (Supplementary data 3.13). On the other hand, the root Na^+ ranged from 235 to 894 umol gDW^{-1} and the shoot Na^+ was between 10 and 907 umol gDW^{-1}, and both were significantly different between accessions (Supplementary data 3.14).

For accessions exposed to long term saline conditions, the root K^+ concentrations ranged from 73 to 593 umol gDW^{-1} and the shoot K^+ concentrations were between 203 and 1209 umol gDW^{-1} of the salt treated plants. In addition, there were significant differences between accessions for the root K^+ and shoot K^+ concentrations (Supplementary data 3.15). Furthermore, the Na^+ concentrations in roots and shoots of rice accessions were significantly different between accessions. The root Na^+ ranged from 244 to 720 umol gDW^{-1} and the shoot Na^+ was between 63 and 1938 umol gDW^{-1} (Supplementary data 3.16).

3.3.4 K^+ and Na^+ concentrations along treatment exposures

To illustrate how K^+ and Na^+ in roots and shoots changed through the time of salt exposure, Table 3.1 shows root and shoot K^+ and Na^+ levels for control and each of the 3 treatment experiments. Tissue K^+ is increased to some extent by salinisation with the exception of root K^+ in the long term treatment which dropped from ~440 to ~314 umol gDW^{-1}. However, it is evident that tissue K^+ values between accessions remain fairly constant and variation is at most approximately 5-fold. In contrast, values of tissue Na^+ fluctuate by a factor of almost 100 suggesting a far larger genetic diversity for this trait compared to tissue K^+ contents.

In roots, 6 hours salt exposure caused a drastic increase in Na^+ levels (ranging from 149 to 546, with an average of 334 umol gDW^{-1}) but during the same period only a very small proportion of Na^+ was translocated to the shoot (ranging from 13 to 53, with an average 25 umol gDW^{-1}). The latter property may stem from the 'excluder' eco-physio-type of rice which generally leads to preferential accumulation of salt in roots and prevention of high Na^+ in photosynthetic tissues. In medium term saline conditions, the root Na^+ increased to an average of 502 umol gDW^{-1} (the range was 235-894) while it still remained relatively low in shoots (average 312 umol gDW^{-1}). The root Na^+ concentration of accessions did greatly change in long term saline conditions (457 umol gDW^{-1}), and this might prevent Na^+ translocation to shoots in many accessions. In the period from 7 to 30 days, the average shoot Na^+ more than doubled to around 700 umol gDW^{-1}. Interestingly, even after this prolonged exposure, around 10 accessions managed to limit shoot Na^+ to values below 100 umol gDW^{-1}, a value that roughly equates to 10 mM on FW basis (assuming a 10-fold FW^{-1}:DW^{-1} ratio).

Table 3.1. Summary of K^+ and Na^+ concentrations of accessions exposed to control and saline conditions. The table summarises the range, mean values and standard error for K^+ and Na^+ concentrations in root and shoot tissues of the 306 accessions.

Phenotype	Control	Short term	Medium term	Long term
Root Na^+	-	149 - 546; **334 ± 3.4**	235 - 894; **502 ± 8.5**	244 - 720; **457 ± 4.3**
Shoot Na^+	-	13 - 53; **25 ± 0.3**	10 - 907; **312 ± 11.1**	62 - 1939; **692 ± 20.8**
Root K^+	240 - 888; **440 ± 5.7**	346 - 793; **560 ± 4.6**	249 - 1170; **522 ± 10.5**	73 - 593; **314 ± 5.7**
Shoot K^+	289 - 1010; **529 ± 9.1**	527 - 927; **718 ± 3.6**	250 - 1093; **657 ± 8.9**	203 - 1208; **586 ± 13.9**

3.3.5 Relationships of K^+ and Na^+ with relative growth rate

Several studies have shown that K^+ and Na^+ correlate with growth of plant species exposed to saline conditions (Lin *et al.*, 2004; Cuin *et al.*, 2008). To assess how K^+ and Na^+ in roots and shoots relate to salt tolerance (either in the form of RGR or expressed as RGR-reduction), correlations were calculated. For plants exposed to medium term saline conditions, there were no correlations between the K^+ or Na^+ concentrations and RGR of the 306 accessions (Supplementary data 3.17). However, in long term saline conditions, either the root K^+ or root K^+:Na^+ ratio of accessions positively correlated with the RGR. Moreover, the shoot Na^+ negatively correlated with RGR, whereas the shoot K^+:Na^+ ratio positively correlated with the RGR (Figure 3.7). However, there were no correlations between the root Na^+ and RGR nor the shoot K^+ and the RGR (Supplementary data 3.18).

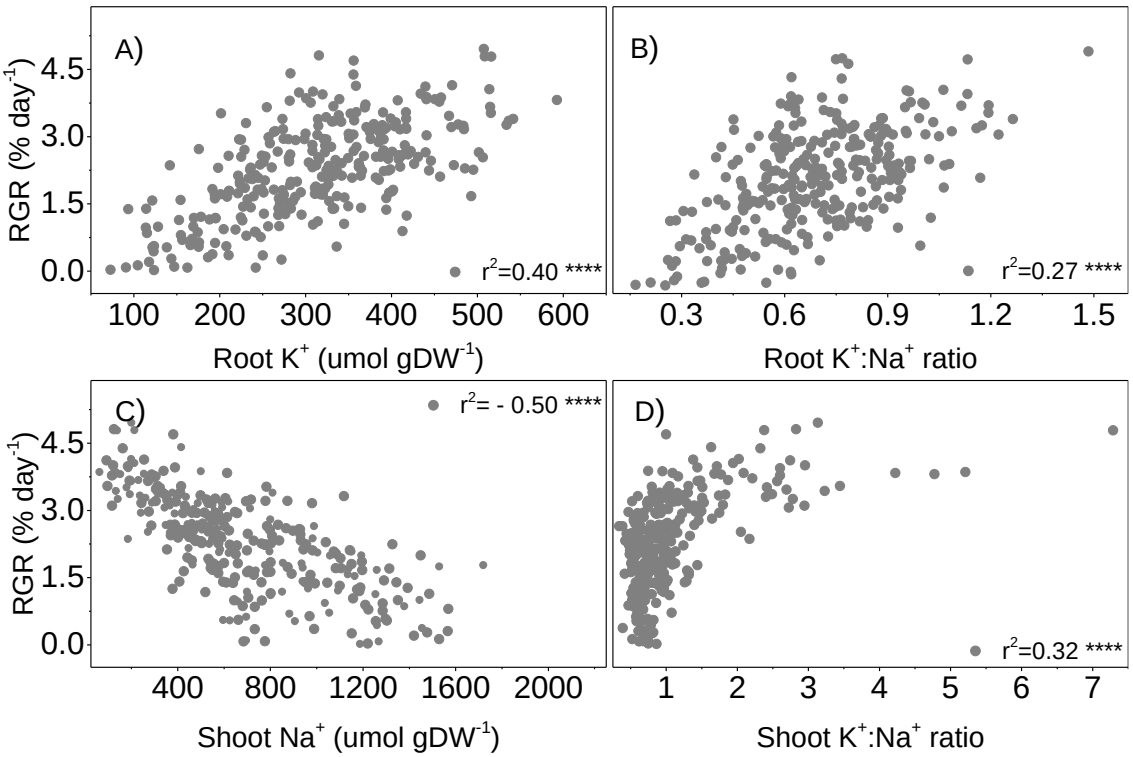


Figure 3.7. Correlations between the ion concentrations and RGR of accessions exposed to long term saline conditions. (**A**) Correlation between root K^+ and RGR. (**B**) Correlation between root K^+:Na^+ ratio and RGR. (**C**) Correlation between shoot Na^+ and RGR. (**D**) Correlation between shoot K^+:Na^+ ratio and RGR. ****Correlation is significant at the 0.0001 level (two-tailed).

3.3.6 Association mapping for RGR and RGR-reduction

The diversity panel used in this study largely consists of commercial cultivars that have been selected for their high yields. Nevertheless, both the growth (Supplementary data 3.6 and Supplementary data 3.9) and tissue cation concentration results (Table 3.2) show that there is considerable diversity between lines regarding tolerance, irrespective of whether it is based on RGR, RGR-reduction or when considering root K^+ and shoot Na^+, parameters that closely correlate with salt tolerance. In turn, these findings strongly suggest that considerable genetic diversity is present within the panel which may underpin the variation in these traits.

To test this notion, phenotypic and genotypic data were interrogated for the occurrence of significant associations. To avoid the impact of generic growth effects, growth data (RGR and RGR-reduction) on their own were not used as parameters for these analyses. Instead, data derived from tissue cation concentrations on their own or in combination with growth parameters (during saline

growth) were entered as traits and used to scrutinise the entire complement of SNPs. Using these conditions and a 5% false discovery rate threshold, association signals were obtained.

For the traits of rice accessions exposed to long-term-control conditions, no association signal was identified by GWAS (Supplementary data 3.19). By contrast, the GWAS revealed 3 major and minor association signals for the RGR but not for the RGR-reduction traits of the 306 accessions exposed to medium term saline conditions (Supplementary data 3.20). To identify candidate genes within the genomic regions of the major and minor association signals on chromosomes 4, 7 and 1, a significant SNP position was selected. The SNP positions shown were those which were part of the association signals after zooming-in. Thus, the most significant SNP positions identified 51 candidate genes for the RGR trait (Supplementary data 3.21 and 3.22). For plants that were exposed to long term saline conditions, the GWAS revealed minor and major association signals for the RGR and RGR-reduction traits. An important finding was the strong correlation between RGR and RGR-reduction association signals on chromosomes 2 and 8 (Figure 3.8). Further analysis allowed having a clearer view of the major associations on chromosome 8, and this association signal ranged from 17.70 to 18.15 Mb, which is a ~ 450 kb window (Figure 3.9).

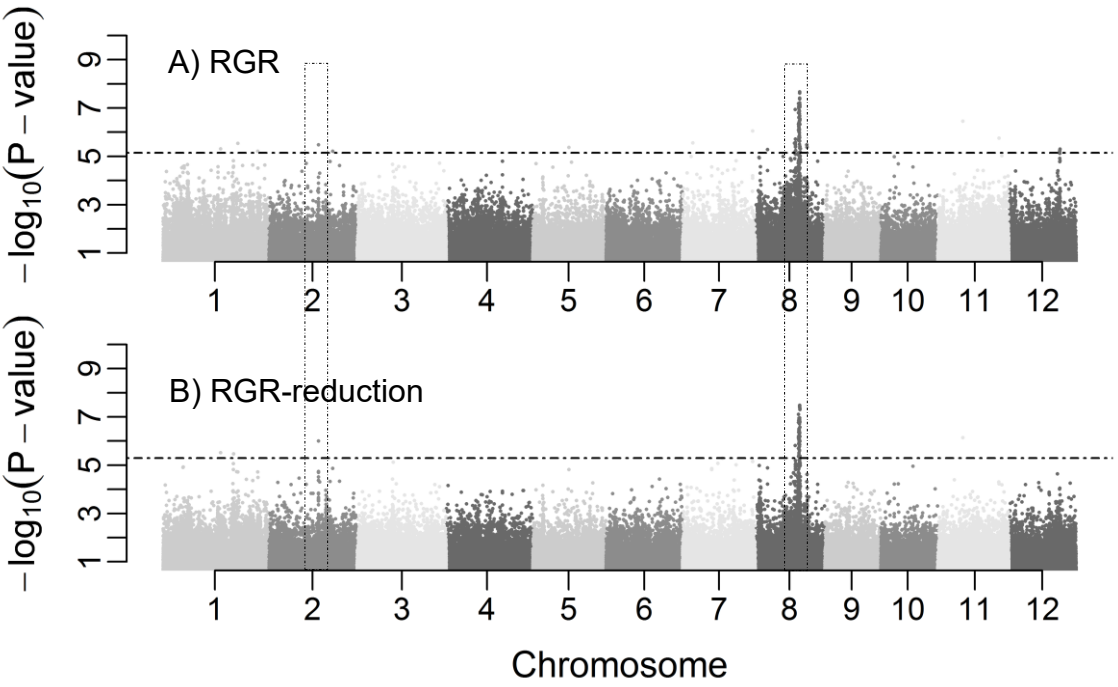


Figure 3.8. Manhattan plots showing the association signals. (**A**) GWAS analysis using RGR of the 306 accessions as phenotypic data. (**B**) GWAS analysis using RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold.

To identify candidate genes across the genomic regions where there were major association signals on chromosome 8, 3 peak SNPs were denoted (Figure 3.9). The number of significant SNPs for each of the peaks range from 10 to 20. The 3 most significant SNPs from each of the peaks identified 37 candidate genes for RGR and RGR-reduction (Table 3.2). Figure 3.10 shows a physical map of the candidate genes identified within a 250 kb window containing the most significant SNP position of the third peak. Besides these major association signals on chromosome 8, there were minor association signals on chromosomes 1, 7 and 8 when using either RGR or RGR-reduction traits. When using these traits as metrics, the GWAS revealed 11 minor and major association signals. The top significant SNP positions identified 146 candidate genes, some of which overlapped. Therefore, 98 candidate genes were selected when using RGR and RGR-reduction traits (Table 3.2 and Supplementary data 3.23).

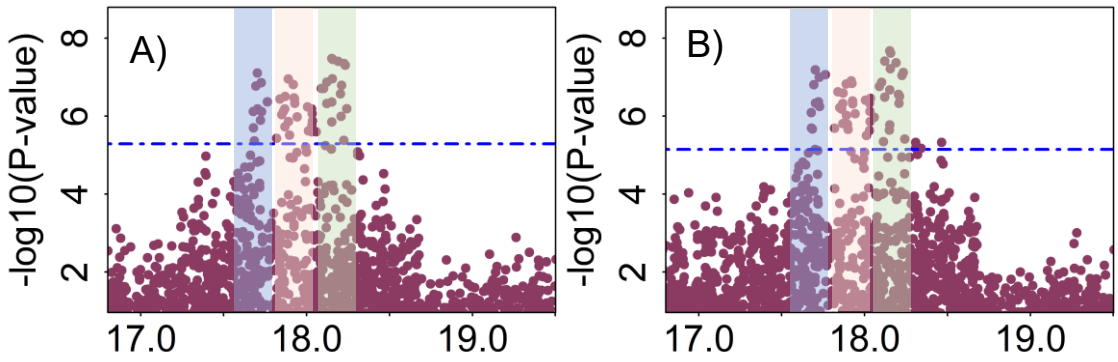


Figure 3.9. Zoom-in of regional association signals. (**A**) Zoom-in of regional association signals for the RGR trait. (**B**) Zoom-in of regional association signals for the RGR-reduction trait. Plants were exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions between 16.9 and 19.4 Mb of chromosome 8. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold. Bars coloured in blue, orange and green illustrate each of the peaks.

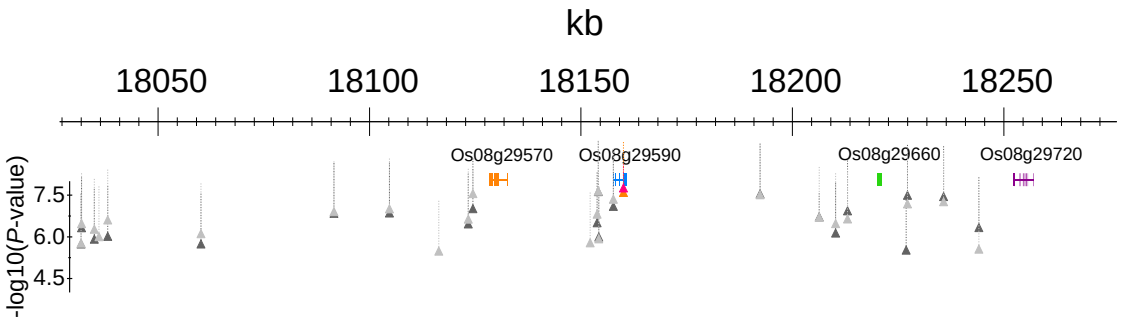


Figure 3.10. Candidate genes within a 250 kb rice genome browser window. Candidate genes were identified by GWAS using the RGR and RGR-reduction traits of the 306 accessions as phenotypic data. Plants were exposed to long term saline conditions (50 mM NaCl). The light and dark grey triangles with dashed lines representing significant SNP positions identified through GWAS for RGR and RGR-reduction traits. X-axis indicates the SNP positions across the zoom-in of rice genomic region. The y-axis shows the P-value for the association test at each locus on a log scale. Candidate genes are illustrated with different colours: ATP-binding cassette transporter OsABCG44 "orange"; zinc finger, C3HC4 type family protein "blue"; Transcription factor WRKY69 "green" and mitochondrial carrier "purple".

Table 3.2. GWAS summary of the RGR and RGR-reduction traits. Plants were exposed to long term saline conditions (50 mM NaCl). Table summarises the SNP positions by trait and chromosome.

Phenotype	Chr	Significant SNP positions			All genes	Candidate genes
		Peak 1	Peak 2	Peak 3		
RGR	2	20176969	-	-	24	11
	7	28623794	-	-	32	20
	8	17702303	17919735	18151724	67	37
	12	20907788	-	-	28	13
RGR-reduction	1	28743606	-	-	30	17
	2	20176969	-	-	24	11
	8	17702303	17919735	18151724	67	37
Overlapped genes						48
Candidate genes						98

NOTE: relative growth rate (RGR), relative growth rate reduction (RGR-reduction) traits. Chromosome (Chr), significant SNP position identified from association signals (Peak). Total number of genes were identified within a 250 kb genome browser window (All genes). Selected candidate genes (Candidate genes). Genes that were identified more than once using different traits (Overlapped genes).

3.3.7 Association mapping for K^+: control conditions

The GWAS approach did not reveal any association signal for the K^+ concentrations in roots and shoots of the 306 accessions exposed to long term control conditions (Supplementary data 3.24).

3.3.8 Association signals for K^+ and Na^+: short term saline conditions

To measure associations between genotypic and phenotypic data of accessions exposed to short term saline conditions, several GWAS analyses were performed using single and combined traits (Methods, section 3.2.5). Figure 3.11 shows a GWAS output which illustrates a major association signal on chromosome 10 and minor association signals on chromosomes 4, 11 and 12 for shoot Na^+. To identify candidate genes within genomic regions of the minor and major association signals revealed by GWAS, a significant SNP position was selected from which 126 candidate genes were identified. In summary, using single and combined traits, the GWAS revealed 36 minor and major association signals (Supplementary data 3.25-27), and the selected significant SNP positions identified 699 candidate genes of which some overlapped. Therefore, 522 candidate genes were selected across the rice genome (Table 3.3 and Supplementary data 3.28).

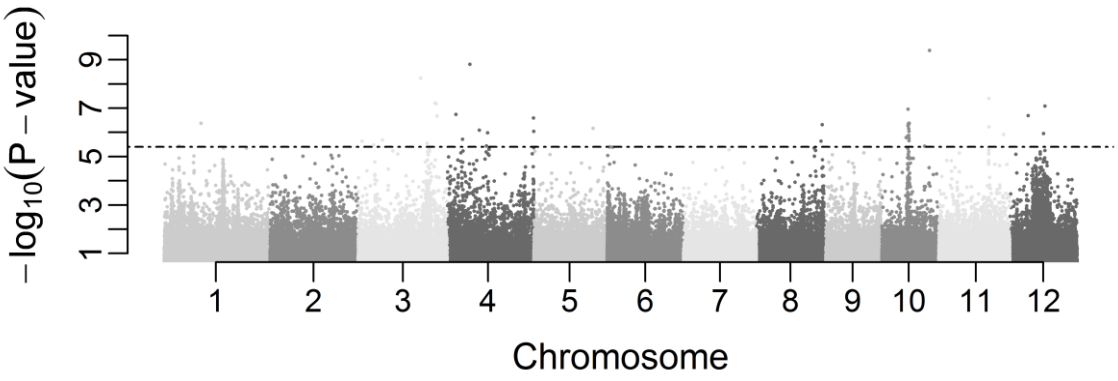


Figure 3.11. Manhattan plots showing the association signals. GWAS analysis using shoot Na^+ of the 306 accessions as phenotypic data of plants exposed to short term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold.

3.3.9 Association signals for K^+ and Na^+: medium term saline conditions

Regarding plants exposed to medium term saline conditions, the single and combined traits were submitted for GWAS analyses (Methods, section 3.2.5). Figure 3.12 shows a major association signal on chromosome 6 for shoot Na^+ and the top SNP position identified 32 candidate genes. Using single and combined traits, the GWAS revealed 35 minor and major association signals (Supplementary data 3.29-31), and the top significant SNP positions identified 613 candidate genes some of which overlapped. Therefore, 541 candidate genes were selected across the rice genome (Table 3.4 and Supplementary data 3.32).

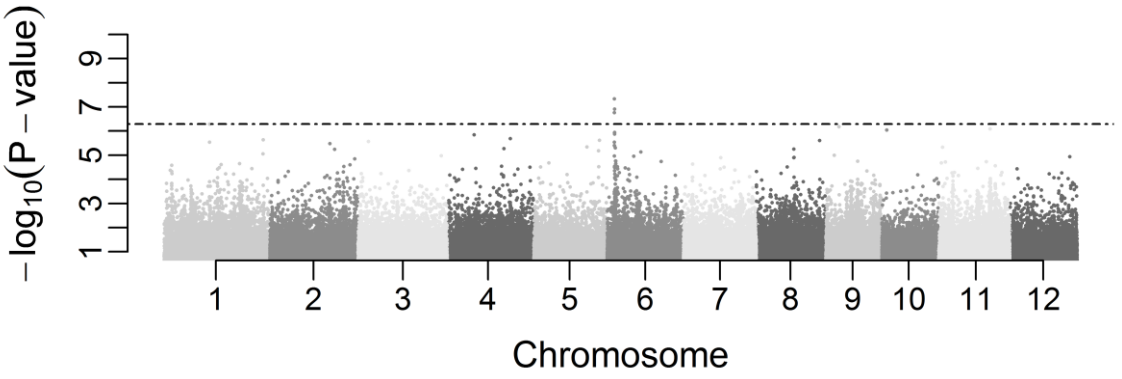


Figure 3.12. Manhattan plots showing the association signals. GWAS analysis using shoot Na^+ of the 306 accessions as phenotypic data of plants exposed to medium term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold.

Table 3.3. GWAS summary of the single and combined traits of plants exposed to short term saline conditions (50 mM NaCl). Table summarises the SNP positions by trait and chromosome.

Phenotype	Chr	Significant SNP positions			All genes	Candidate genes
		Peak 1	Peak 2	Peak 3		
Shoot Na^+	3	28239374	-	-	36	25
	4	35334586	-	-	35	26
	10	10597340	10956585	-	85	59
	11	20510138	-	-	32	16
Root Na^+/IPB	1	13810627	-	-	32	14
	9	22128231	-	-	45	32
Shoot Na^+/ LT-RGR-reduction	3	28239374	29601960	-	60	40
	9	11627293	-	-	26	13
Shoot Na^+/Root Na^+	8	24205168	-	-	40	26
Shoot Na^+/IPB	1	3245447	6061627	-	70	53
	3	29017303	-	-	29	16
	8	27215217	-	-	36	26
	10	10960138	-	-	60	46
Root K^+/MT-RGR-reduction	6	13043451	-	-	29	11
Root Na^+/MT-RGR-reduction	6	13043451	-	-	29	11
	11	26408294	27702229	-	59	38
Shoot K^+/MT-RGR-reduction	4	28120672	-	-	38	26
	6	13043451	17515773	-	52	23
	11	27696552	-	-	31	19
Root K^+/LT-RGR-reduction	2	20176969	30403984	-	67	41
	8	15664039	17659199	-	52	29
Root Na^+/LT-RGR-reduction	3	8419475	-	-	30	17
	8	15636920	17659199	-	51	28
	11	26145236	-	-	24	10
Shoot K^+/LT-RGR-reduction	2	20176969	-	-	24	11
	8	15636920	17659199	18207872	77	43
Overlapped candidate genes						177
Candidate genes						522

NOTE: shoot Na^+ (Shoot Na^+), ratio of the root Na^+ and initial plant biomass (Root Na^+/IPB), ratio of the shoot Na^+ and long term RGR-reduction (Shoot Na^+/ LT-RGR-reduction), ratio of the shoot Na^+ and root Na^+ (Shoot Na^+/Root Na^+), ratio of the shoot Na^+ and initial plant biomass (Shoot Na^+/IPB), ratio of the root K^+ and medium term RGR-reduction (Root K^+/MT-RGR-reduction), ratio of the root Na^+ and medium term RGR-reduction (Root Na^+/MT-RGR-reduction), ratio of the shoot K^+ and medium term RGR-reduction (Shoot K^+/MT-RGR-reduction), ratio of the root K^+ and long term RGR-reduction (Root K^+/LT-RGR-reduction, ratio of the root Na^+ and long term RGR-reduction (Root Na^+/LT-RGR-reduction) and ratio of the shoot K^+ and long term RGR-reduction (Shoot K^+/LT-RGR-reduction) traits. Chromosome (Chr), significant SNP position identified from association signals (Peak). Total number of genes identified within 250 kb genome browser window (All genes). Selected candidate genes (Candidate genes). Genes that were identified more than once using different traits (Overlapped genes).

Table 3.4. GWAS summary of the single and combined traits of plants exposed to medium term saline conditions (50 mM NaCl). Table summarises the SNP positions by trait and chromosome.

Phenotype	Chr	Significant SNP positions			All genes	Candidate genes
		Peak 1	Peak 2	Peak 3		
Shoot Na^+	6	2973544	-	-	43	32
LT-RGR/Root K^+	1	36214195	-	-	38	24
	2	517719	24625831	-	78	44
	8	15664039	17573123	17659199	59	33
	11	20771426	22711759	-	69	34
	12	2533494	-	-	36	23
MT-RGR/Root Na^+	6	1581066	-	-	37	30
	8	16308015	-	-	19	7
	11	20857639	-	-	26	10
MT-RGR-reduction/Root K^+	3	30854763	-	-	37	27
	9	13322676	-	-	17	3
	11	26975688	-	-	22	11
Shoot Na^+/Root K^+	2	29910163	-	-	41	32
	6	2973544	12853156	17434104	79	52
	8	15366952	-	-	21	9
	11	20811275	-	-	31	14
MT-RGR/Shoot K^+	1	19086884	-	-	28	10
Shoot Na^+/MT-RGR	6	2919672	-	-	39	28
	7	3510858	-	-	33	21
	8	15424481	-	-	23	9
	10	23112534	-	-	36	24
MT-RGR-reduction/Root Na^+	2	993907	-	-	41	34
Root K^+/MT-RGR-reduction	6	13043451	17653875	-	55	21
Shoot K^+/MT-RGR-reduction	3	25885262	-	-	29	13
	4	28567754	-	-	23	16
	6	13043451	17493338	-	51	23
	10	22086504	-	-	38	29
Overlapped candidate genes						72
Candidate genes						541

NOTE: shoot Na^+, ratio of the long term RGR and root K^+ (LT-RGR/Root K^+), ratio of the medium term RGR and root Na^+ (MT-RGR/Root Na^+), ratio of the medium term RGR-reduction and root K^+ (MT-RGR-reduction/Root K^+), ratio of the shoot Na^+ and root K^+ (Shoot Na^+/Root K^+), ratio of the medium term RGR and shoot K^+ (MT-RGR/Shoot K^+), ratio of the shoot Na^+ and medium term RGR (Shoot Na^+/MT-RGR), ratio of the medium term RGR-reduction and root Na^+ (MT-RGR-reduction/Root Na^+), ratio of the root K^+ and medium term RGR-reduction (Root K^+/MT-RGR-reduction) and ratio of the shoot K^+ and medium term RGR-reduction (Shoot K^+/MT-RGR-reduction) traits. Chromosome (Chr), significant SNP position identified from association signals (Peak). Total number of genes identified within 250 kb genome browser window (All genes). Selected candidate genes (Candidate genes). Genes that were identified more than once using different traits (Overlapped genes).

3.3.10 Association signals for K^+ and Na^+: long term saline conditions

To determine associations between genotypic and phenotypic data of the rice accessions exposed to long term saline conditions, several GWAS analyses were performed using single and combined traits. To illustrate one of the GWAS outputs, Figure 3.13 shows that the most prominent association signal is located on chromosome 8. The association signal ranged from 17.5 to 18.2 Mb, which is a ~ 650 kb window, and contained 74 significant SNPs (Figure 3.14). Apart from the major association signal, there were minor association signals on chromosomes 1, 3, 7, 11 and 12. Interestingly, there was a strong correlation between either the RGR or RGR-reduction and the shoot Na^+ in regards to the SNP positions of the 306 accessions exposed to long term saline conditions. In summary, when using single and combined traits (Methods, section 3.2.5), the GWAS revealed 35 minor and major association signals (Supplementary data 3.33-34) and the top significant SNP positions identified 639 candidate genes some of which overlapped. Therefore, 590 candidate genes were selected across the rice genome (Table 3.5 and Supplementary data 3.35).

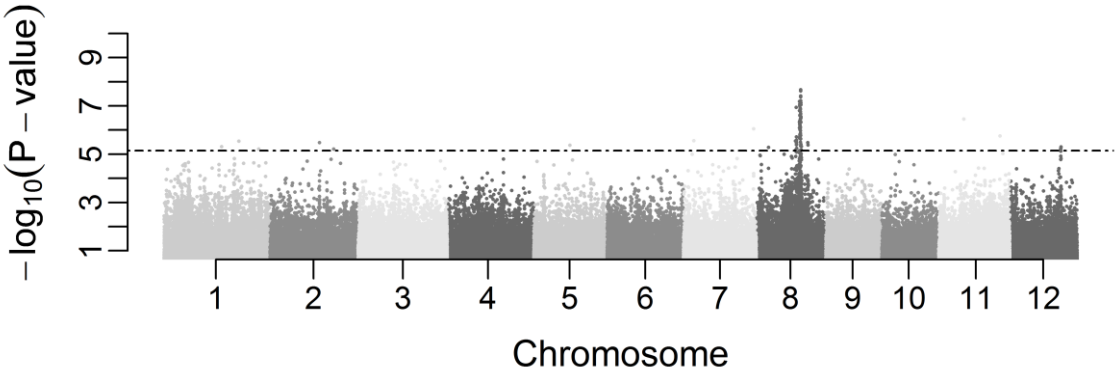


Figure 3.13. Manhattan plots showing the association signals. GWAS analysis using shoot Na^+ of the 306 accessions as phenotypic data of plants exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold.

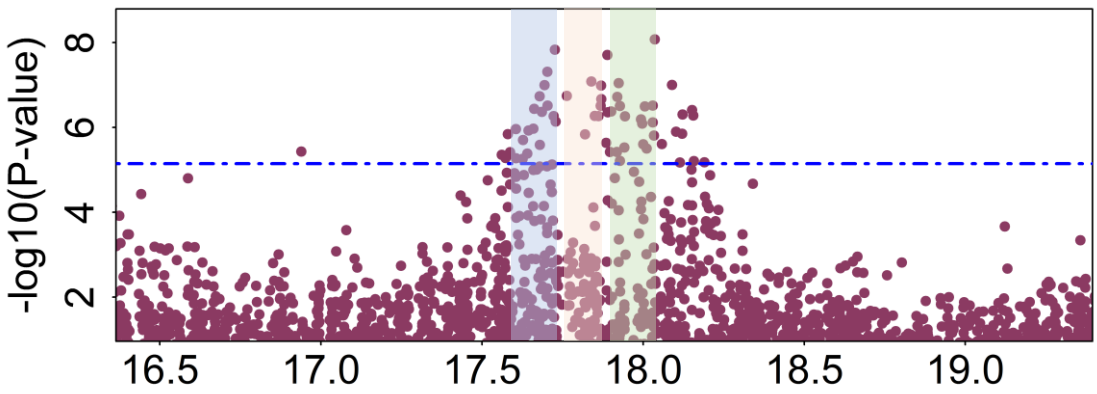


Figure 3.14. Zoom-in of regional association signals. Zoom-in of regional association signals for the shoot Na^+ trait of plants exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions between 17.5 and 18.2 Mb of chromosome 8. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold. Bars coloured in blue, orange and green illustrate each of the peaks.

Table 3.5. GWAS summary of the single and combined traits of plants exposed to long term saline conditions (50 mM NaCl). Table summarises the SNP positions by trait and chromosome.

Phenotype	Chr	Significant SNP positions			All genes	Candidate genes
		Peak 1	Peak 2	Peak 3		
Shoot Na^+	1	11019354	28030675	-	55	35
	3	3253521	-	-	30	19
	7	28623794	-	-	32	20
	8	4649422	8944201	17726170	108	59
				17889043		
				18032563		
	11	692873	-	-	49	29
	12	9861281	20730201	-	59	30
LT-RGR/Root K^+	5	23258024	-	-	31	18
LT-RGR/Root Na^+	1	31575018	-	-	35	20
	4	21295777	-	-	32	20
	6	1481649	17941895	-	70	48
	8	16308015	18151724	-	42	20
	12	1398148	2810394	-	69	43
LT-RGR/Shoot K^+	1	21128124	-	-	27	16
	2	32403445	-	-	34	28
	6	1581066	-	-	37	30
Shoot Na^+/LT-RGR-reduction	1	6059287	38260931	-	67	47
	2	4228071	25341378	-	73	46
	4	31177764	-	-	34	26
	8	10572853	-	-	22	8
	10	22507391	-	-	42	25
Root K^+/Shoot K^+	12	6954122	-	-	25	11
Shoot K^+/LT-RGR	8	18308455	-	-	23	12
Root K^+/LT-RGR-reduction	8	15664039	17659199	-	52	29
Overlapped candidate genes						49
Candidate genes						590

NOTE: shoot Na^+ (Shoot Na^+), ratio of long term RGR and root K^+ (LT-RGR/Root K^+), ratio of long term RGR and root Na^+ (LT-RGR/Root Na^+), ratio of long term RGR and shoot K^+ (LT-RGR/Shoot K^+), ratio of the shoot Na^+ and long term RGR-reduction (Shoot Na^+/LT-RGR-reduction), ratio of the root K^+ and shoot K^+ (Root K^+/Shoot K^+), ratio of the shoot K^+ and long term RGR (Shoot K^+/LT-RGR) and ratio of the root K^+ and long term RGR-reduction (Root K^+/LT-RGR-reduction) traits. Chromosome (Chr), significant SNP position identified from association signals (Peak). Total number of genes identified within 250 kb genome browser window (All genes). Selected candidate genes (Candidate genes). Genes that were identified more than once using different traits (Overlapped genes).

3.3.11 Identification of SNPs within coding regions of candidate genes

To gain insights into potential functional effects of the identified significant SNPs, all SNPs that exceeded the significance criterion were assessed for their location in the coding regions of genes and subsequently for their potential impact on protein composition. The coding sequences of all candidate genes were screened for the presence of non-synonymous SNPs (nsSNPs) to identify potential causative polymorphisms. As a result, 152 non-synonymous SNPs were identified using single and combined traits of rice accessions exposed to short, medium and long term saline conditions (Supplementary data 3.36). To illustrate the presence of non-synonymous SNPs, Figure 3.15 shows 3 SNPs that overlapped within the first exon of a zinc-finger, CH3C4 type family protein. The figure also shows the changes of the amino acids for the nsSNP.

Furthermore, in case relevant nsSNPs were found, allele frequencies were calculated and compared between a set of salt sensitive and salt tolerant accessions (60 in total) to determine correlations between phenotype and genotype. The 60 rice accessions were selected based on RGR-reduction traits of the plants exposed to long term saline conditions. The results of the correlational analysis are summarised in Table 3.6, and 18 potential non-synonymous SNPs with high correlation with the salt tolerance of rice accessions were identified from among them.

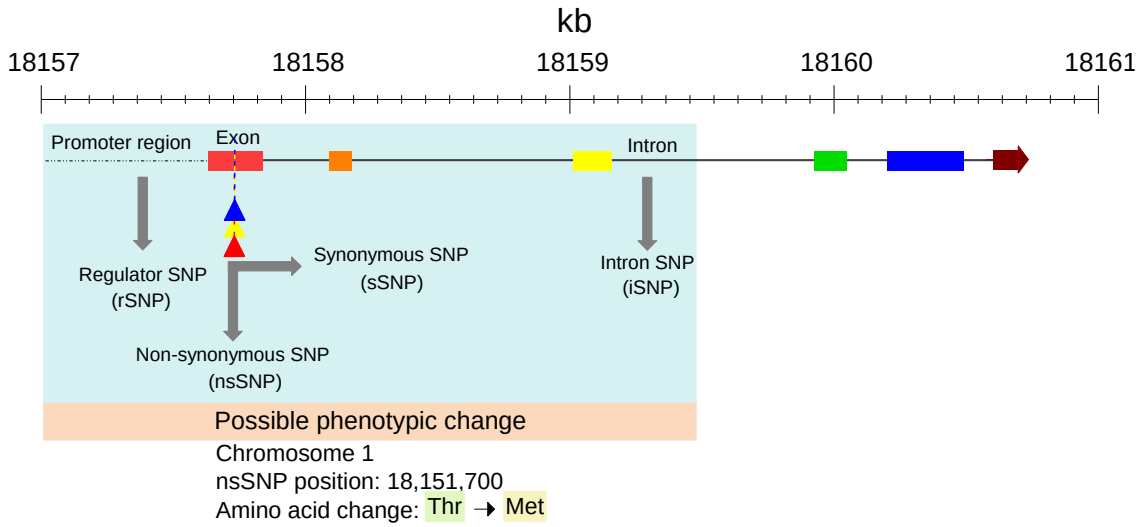


Figure 3.15. Single nucleotide polymorphism types within the genomic region of a candidate gene. The zinc finger, C3HC4 type family protein candidate gene was identified by GWAS using the RGR of the 306 accessions as phenotypic data of plants exposed to long term saline conditions (50 mM NaCl). Grey arrows illustrate the regulatory, intron, synonymous and non-synonymous SNPs across the genomic region of the candidate gene. The blue, yellow and red triangles with dashed lines represent the significant SNPs identified by GWAS.

Table 3.6. Correlations between non-synonymous SNPs and salt tolerance. Table summarises the gene locus, SNP position, amino acid changes and the ratios of alleles of the 30 salt tolerant and 30 sensitive accessions. The salt tolerant and sensitive accessions were selected based on RGR-reduction traits of plants exposed to long term saline conditions.

Locus	SNP	Amino acid changes	Reference allele	Alternate allele	[1]Unknown allele	Salt tolerant	[2]Unknown allele	Salt sensitive
LOC_Os06g22070	12803227	Ala → Val	C	T	-	27:3	-	30:0
	12803590	Ile → Met	C	G	-	27:3	1	29:0
LOC_Os06g29844	17197652	Met → Ile	G	A	2	28:0	4	26:0
	17200175	Cys → Ser	T	A	1	29:0	2	23:5
LOC_Os06g30310	17491528	Phe → Leu	C	A	-	27:3	-	30:0
	17493338	Arg → Gly	A	G	-	27:3	-	30:0
	17499757	Asn → Asp	A	G	-	27:3	-	30:0
LOC_Os06g30320	17507658	Ser → Gly	A	G	1	26:3	-	30:0
	17509098	Thr → Arg	C	G	1	26:3	3	27:0
	17511406	Glu → Gln	G	C	-	27:3	1	29:0
LOC_Os08g28780	17604953	Val → Ile	G	A	-	30:0	1	21:8
	17605046	Val → Met	G	A	-	30:0	1	23:6
LOC_Os08g28940	17701381	Arg → Trp	C	T	-	30:0	-	22:8
	17703384	Ala → Thr	G	A	-	30:0	-	21:9
	17702303	Leu → Ser	T	C	-	30:0	-	21:9
	17703384	Ala → Thr	G	A	-	30:0	-	21:9
LOC_Os08g38200	24205168	Lys → Ile	T	A	-	30:0	1	28:1
	24205696	Arg → Lys	C	T	-	30:0	-	29:1

NOTE: SNP position identified by GWAS (SNP), changes of amino acids in non-synonymous SNPs (Amino acid changes), reference and alternate alleles identified in allele finder website (reference and alternate alleles), no data available for alleles (Unknown alleles) and ratios of reference allele and alternate allele (Salt tolerant and salt sensitive).

3.3.12 Correlation between published major QTLs and SNPs

Although both QTL mapping and GWAS analyses can reveal association signals between phenotype and genotype, the GWAS has the advantage in that it generally reveals a specific position of genes and polymorphisms within a genomic region. To test how findings from the different techniques compare, the position of published QTLs was compared to that of SNP positions reported in this study. For accessions exposed to saline conditions, correlations were found between several minor and major QTLs and SNP positions identified through GWAS. Figure 3.16 shows maps of outcomes which agree in many positions across the rice genome. From correlation analysis, 23 SNPs were identified positioned near and three overlapping with major QTLs previously identified. The distances between the major QTLs and the SNP positions ranged from 0.02 to 4.65 Mb (Table 3.7).

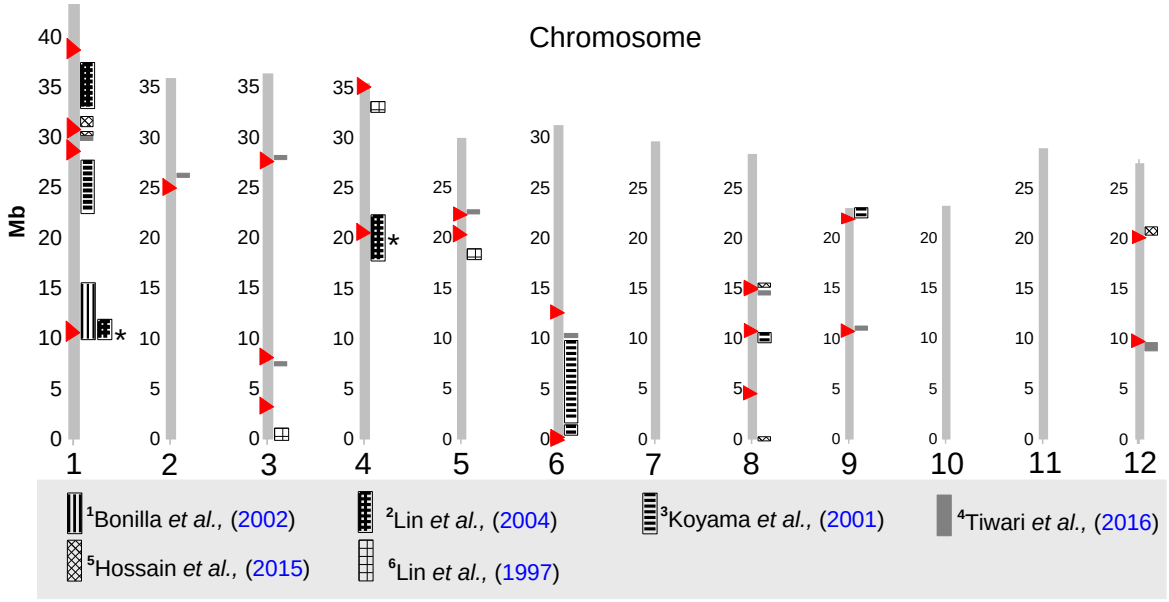


Figure 3.16. Adapted QTL cartographer for major QTLs identified in rice exposed to saline conditions. The graph shows the correlations between the QTLs identified by several research studies and the SNP positions identified through GWAS (red triangles). * indicates overlapped between QTL and GWAS SNP position.

Table 3.7. Overlap between the known QTLs for salt tolerance and SNP positions identified by GWAS in this study.

Phenotype	Chr	Symbol	QTL coordinate	GWAS Position	Distance (Mb)
Salt tolerance	1	[1]*qSaltol*	10800000-16400000	11019354	*
Shoot K^+	1	[2]*qSKC-1*	9820009-11232822	11019354	*
Na^+ uptake	1	[3]*qNAUP*	23971321-26193239	28030675	2.95
Shoot Na^+:K^+	1	[3]*qNAKRO*	23971321-26193239	28030675	2.95
Salt tolerance	1	[4]*qSSIGY1.1*	30100000	31575018	1.48
Na^+/K^+ ratio	1	[5]*qNaKR1.8*	31060000	31575018	0.52
Na^+ concentration	1	[5]*qNa1.6*	31060000	31575018	0.52
Panicle length	1	[5]*qPL1.2*	31600000	31575018	0.02
Pollen fertility	1	[5]*qPF1.4*	31060000	31575018	0.52
Plant height	1	[5]*qPH1.1*	32670000	31575018	1.09
Salt sensitivity	1	[2]*SALTSN*	33956950-37713775	38260931	2.43
Salt sensitivity	1	[2]*qRNTQ-1*	33956950-37713775	38260931	2.43
Salt tolerance	2	[4]*qSSIGY2.3*	26800000	25341378	1.46

Salt sensitivity	3	[6]*SALTSN*	484860-485333	3253521	2.77
Salt tolerance	3	[4]*qSSIGY3.2*	7300000	8419475	1.12
Salt tolerance	3	[4]*qSSIGY3.7*	28600000	28239374	0.36
Root K^+	4	[2]*qRKC-4*	19928370-22355854	21295777	*
Salt tolerance	4	[6]*qSD*	33663984-33664487	35334586	1.67
Salt sensitivity	4	[6]*SALTSN*	33663984-33664487	35334586	1.67
Salt sensitivity	5	[6]*SALTSN*	18874932-18875558	21132047	2.26
Salt tolerance	5	[4]*qSSIGY5.4*	23600000	23258024	0.34
K^+ uptake	6	[3]*qKUP*	1764586-1764760	1481649	0.28
K^+ uptake	6	[3]*qKUP*	1764586-9284228	1481649	4.04
Shoot Na^+	6	[3]*qNACONC*	1764586-9284228	1581066	3.94
Salt tolerance	6	[4]*qSSIGY6.3*	10800000	13043451	2.24
Tiller	8	[5]*qTN8.1*	0	4649422	4.65
Shoot K^+	8	[3]*qClLV-8.1a*	10293816-10293912	10572853	0.28
Salt tolerance	8	[4]*qSSIGY8.3*	14800000	15636920	0.84
Salt tolerance	8	[4]*qSSIGY8.3*	14800000	15664039	0.86
Grain yield	8	[5]*qBM8.2*	15710000	15664039	0.05
Salt tolerance	9	[4]*qSSIGY9.1*	11900000	11627293	0.27
K^+ uptake	9	[3]*qKUP*	22720624-22720860	22128231	0.59
Salt tolerance	12	[4]*qSSIGY12.1*	9400000	9861281	0.46
Grain yield	12	[5]*qGY12.1*	21200000	20907788	0.29

NOTE: *Overlapped between QTL and GWAS SNP position. References: [1]Bonilla *et al.* (2002); [2]Lin *et al.* (2004); [3]Koyama *et al.* (2001); [4]Tiwari *et al.* (2016); [5]Hossain *et al.* (2015); [6]Lin *et al.* (1997). The distance (Mb) between GWAS SNP positions and QTL positions was calculated using the average of QTL coordinates.

3.4 Discussion

Rice is a much-demanded food source in many countries yet it is highly sensitive to saline conditions. This leads to a concern for future rice production due to the increasing salinisation of agricultural land areas. Therefore, it is crucial to identify novel genes and alleles associated with salinity tolerance in diverse accessions to build a foundation of knowledge that could safeguard the production of rice. Several studies have used conventional and high-throughput phenotyping to elucidate dynamic responses of accessions associated with growth and morphology in response to saline conditions (Gaikwad *et al.*, 2014; Kavitha *et al.*, 2012; Lin *et al.*, 2004). The study here has

demonstrated the effectiveness of phenotyping for dissecting the genetic architecture of complex traits such as RGR, RGR-reduction and K^+ and Na^+ in roots and shoots. To assess plant growth and cation in roots and shoots in saline conditions, several statistical approaches were performed on the different data sets.

3.4.1 Salinity effects on growth

Rice has shown substantial variation in tolerance for different abiotic conditions (Frei, 2015; Lin et al., 2004) including osmotic and ionic stress. For salt tolerance, most of the research has been focused on morphological and physiological parameters (Platten et al., 2013; Mitchell et al., 1998). In this study, when screening a population of 306 accessions from 79 different countries for salinity tolerance, it was shown that rice plants have a wide spectrum of phenotypes based on growth as well as K^+ and Na^+ concentrations in roots and shoots.

As expected, the IPB of plants was significantly different between rice accessions but not between treatments. Furthermore, the IPB correlated with the FPB of plants exposed to the control, medium and long term saline conditions. However, there were no correlations between the IPB and RGR values of the plants exposed to these treatments (Supplementary data 3.37). This difference is because the FPB is the absolute growth values of plants for 60 days and the RGR is purely the effects of treatments during a period of 30 days. These results are consistent with data obtained using either a single replicate or mean values of three plants. Moreover, the RGR of the plants in control conditions was higher compared to the RGR of the salinity treated plants. The RGR of the plants was not significantly different between medium and long term saline conditions but there was a significant difference for the RGR-reduction traits. For plants exposed to medium and long term saline conditions, the growth rate of plants was similar. However, there were accessions that reduced their growth rates that led to high RGR-reduction of plants in long term saline conditions.

The data recording growth showed two important characteristics: firstly, growth variability increases drastically when plants become salt stressed (from around 2.5-fold to around 25-fold variability between the lowest and highest rates). Secondly, the lack of difference between medium and long term exposure times suggests that in both non-saline and saline conditions, the growth of rice stabilises within ~7 days of treatment to a level that subsequently changes little thereafter, at least up to 30 days. RGRs provide a good indicator of vigour, and correlation between control-RGR and salt-RGR values were carried out to test whether this property was consistent across accessions. No significant correlations were detected indicating that RGR values from control and saline treatments are not related (Supplementary data 3.8). This clearly suggests that growth characteristics of

accessions are greatly affected by salinity, and performance in control conditions is not a good predictor of potential tolerance.

To establish whether growth patterns had any subpopulation related characteristics, RGRs and RGR-reductions were grouped into the two major subpopulations of *indica* and *japonica* varieties and were separately analysed. Average RGR and RGR-reduction in all growth conditions were not significantly different between these subpopulations (Supplementary data 3.38), irrespective of whether plants underwent medium term or long term salinity exposure.

3.4.2 Salinity effects on K^+ and Na^+ in roots and shoots

Plants have complex mechanisms to balance ion composition when coping with saline conditions. After the onset of salinity, a plant's growth stops and there is a high rate of Na^+ and low rate of K^+ uptake through the plant's roots (Apse et al., 1999; Tester and Davenport, 2003). After hours or days, plants adapt to the osmotic and ionic effects of saline conditions but the growth rate is not completely recovered and there is a continuous accumulation of Na^+ in tissues (Chen *et al.*, 2005; Le Rudulier *et al.*, 1984). These events were similarly observed during this study when 306 rice accessions were exposed to short, medium and long term salt stress conditions.

Much study has shown that plants can exclude Na^+ from tissues which generally leads to high accumulation of Na^+ in roots to prevent high accumulation of this ion in photosynthetic tissues (Møller *et al.*, 2009). After 6 hours of treatment, the root Na^+ drastically increased while during the same period only a very small proportion of Na^+ was translocated to the shoot. After 7 days of saline treatment, root tissue Na^+ accumulation significantly increased while it still remained relatively low in shoots. It has been argued that uptake of inorganics like Na^+, Cl^- and to a lesser extent K^+, is a relatively energy efficient manner of osmotic adjustment to saline conditions (Cramer, 2002; Yeo *et al.*, 1991). Influx of Na^+ and Cl^- may be beneficial in the early stages of stress but a risky strategy in the longer term due to the build-up of ion toxicity. High root Na^+ and low shoot Na^+ levels of plants remained after 30 days of saline conditions. Interestingly, even after this prolonged exposure, around 10 accessions managed to limit shoot Na^+ to values below 100 umol gDW-1 (Supplementary data 3.39).

For K^+ concentrations, the shoot K^+ is generally increased to some extent by salinisation although the root K^+ in the long term treatment dropped from ~440 to ~314 umol gDW-1. It was evident that variation in the shoot K^+ values between accessions remained fairly constant and is, at most, approximately a 5-fold difference between the lowest and highest values. In contrast, values of

the shoot Na^+ fluctuated by a factor of almost 100-fold suggesting a far larger genetic diversity for this trait compared to the shoot K^+ concentrations.

To assess how K^+ and Na^+ are associated with salt tolerance for the accessions, several correlation analyses were performed on the different data sets using either absolute plant biomass or relative growth values and the ion concentrations. The strongest results were obtained from long term salinisation treatment, where the root K^+ and shoot Na^+ positively and negatively correlated with the absolute and relative growth values, respectively (Figure 3.7 and Supplementary data 3.40). These findings support the idea that low Na^+ concentration in shoots and high K^+ in roots can be considered as indicators of rice salinity tolerance (Kavitha et al., 2012; Wang et al., 2012). These results are consistent with previous research in Arabidopsis (Guan et al., 2013), wheat (Guo et al., 2015), barley (Adem et al., 2014) and other rice populations (Platten et al., 2013; Lin et al., 2004) exposed to saline treatments. Meanwhile, the shoot K^+ negatively correlated with the absolute and relative growth values (Supplementary data 3.18). This result is somewhat contradictory to the reported concept that high shoot K^+ is associated with salt tolerance (Wei *et al.*, 2013; Lin *et al.*, 2004; Chen *et al.*, 2007). However, ion regulation is acknowledged to be a complex trait, and Platten *et al.* (2013) likewise did not find correlations between the shoot K^+ and salinity tolerance of ~100 accessions exposed to high saline conditions in their work. A possible explanation for these results might be that accessions showing high Na^+ may also have K^+ toxicity.

It is worth noting the IPB of accessions was sometimes highly variable between plants, which led to high standard errors of the absolute and relative growth rates as well as the K^+ concentrations in roots and shoots, and therefore some accessions with low IPB might have had low salt tolerance. To support some results of this study it would be worth reassessing accessions that had high standard errors in their values, using a greater number of plants to reduce standard error and confirm their tolerance phenotype.

3.4.3 Physical mapping: overlapped genes in three terms of salinisation

Salinity is a complex trait that may implicate thousands of genes during a plant's life cycle. For this reason, the main aims of this study were (a) to identify well-known genes and (b) to identify novel determinant candidate genes for salinity tolerance using GWAS. To determine the number of single candidate genes by terms of salinisation, overlapped candidate genes were removed from the lists of genes identified by GWAS using different traits (Tables 3.2-3.5 and Supplementary data 3.21). As a result, 522, 541 and 623 single candidate genes were identified using GWAS for short, medium and long term saline conditions, respectively. Further analysis showed that 29 candidate genes were

identified into the three terms of salinisation using single and combined traits (Supplementary data 3.41).

To gain insights into the putative function of the candidate genes, bioinformatics and literature were used. Using an integrative bioinformatics framework website (http://bis.zju.edu.cn/ricenetdb/), these genes can be localised in chloroplast, cytosol, mitochondria, nucleus and plasma membrane (Liu *et al.*, 2013). One interesting finding is the identification of histone deacetylase (*OsHDA705*), which plays roles in gene regulation by acetylation and de-acetylation and contains two nsSNPs (Ruijter *et al.*, 2003; McCouch *et al.*, 2016), and microarray experiments have shown that *OsHDA705* (LOC_Os08g25570) is downregulated in saline conditions (Luu TN *et al.*, 2012). Another important finding was the identification of *OsFBX289* (LOC_Os08g28940), which is an F-box domain containing protein that is involved in protein degradation via the ubiquitination complex (Qiao *et al.*, 2009). *OsFBX289* is located on chromosome 8, ~17.4 Mb and contains nine nsSNPs, four of which were identified by GWAS. From this group of candidate genes, there might be potential determinant genes that play roles for salinity tolerance. For example, specific protein kinases identified here by GWAS have been shown to have roles in sensing external stimuli (Passricha *et al.*, 2016; Le Gall *et al.*, 2015) and in the process of gene phosphorylation (Halfter *et al.*, 2000). Furthermore, GWAS identified proto-oncogene protein (MYB) transcription factors, a member of which family has been shown to play roles in the regulation of *OsHKT1;1* gene expression (Wang *et al.*, 2015).

3.4.4 Physical mapping: relatively well-known genes and novel determinant genes

To assess the effectiveness of GWAS approach in the identification of relatively well-known determinant genes for saline conditions, identified candidate genes were categorised based on their putative functions and published data. However, it is worth noting that the number of well-known genes could vary according to knowledge of a reader. As expected, GWAS identified relatively well-known determinant genes for saline conditions that have already been partially characterised. The GWAS approach identified sodium, calcium and potassium channels. In addition, GWAS identified potassium and sodium transporters, and cation/H^+ exchangers. Furthermore, this approach identified determinant genes that play roles in sensing external stimuli, osmoprotectants, plant stress signalling, plant senescence, ROS scavenging through enzymes and transcription factors that regulate gene expression using different domains (Figure 3.17).

To summarise the potential functions of the relatively well-known genes, candidate genes were reviewed for their putative functions.

One interesting finding is that high-affinity Na^+ transporter (OsHKT1;3) was identified by GWAS and its location is on chromosome 2, ~ 4.2 Mb, contains six nsSNPs. This gene has been reported to mediate Na^+ transport and is highly expressed in root cortex and vascular tissues (Rosas-Santiago *et al.*, 2015; Singh *et al.*, 2016a). Moreover, *OsCNGC9* is one of the members of cyclic nucleotide gated channels which may mediate transport of ions such as K^+, Na^+ and Ca^{2+} (Nawaz et al., 2014). Another important finding is the identification of non-selective cation channel *OsTPC1*, which is localised in tonoplast. Although, the electrophysiological properties of TPC1 channels prevent a role in Na^+ transport, recent literature suggests that TPC1 has a role in salt induced long distance Ca^{2+} signalling (Peiter *et al.*, 2005).

Regarding K^+ carriers, this approach identified of the shaker-type K^+ channel (*OsKAT1*), high-affinity K^+ transporters (*OsHAK9*, *OsHAK11*, *OsHAK25*) and inward-rectifying potassium channel (*OsAKT2/3*), which have been shown to mediate K^+ transport across cell membranes and enhance salt tolerance (Obata *et al.*, 2007; Qi *et al.*, 2008; Bañuelos *et al.*, 2002; Hosoo *et al.*, 2014).

It is likely that the maintenance of high K^+/Na^+ ratio in the cytosol is one of the key determinants for rice tolerance (Maathuis and Amtmann, 1999; Lin et al., 2004). The best known Na^+ compartmentation and efflux proteins are NHXs and SOS1 (Bassil *et al.*, 2011; Olías *et al.*, 2009), but there are many ion exchanger family genes such as *OsCAX1a*, *OsCHX15* and *OsEFCAX1* that might have roles in ion homeostasis in plant cells in saline conditions (Singh *et al.*, 2015a; Doroshenk *et al.*, 2010; Singh *et al.*, 2014). These ion exchangers were identified using combined traits i.e. ratios of growth and ions concentrations in roots and shoots.

Genes were identified for sensing external stimuli, osmoprotectants, plant stress signalling, plant senescence, ROS scavenging through enzymes and transcription factors that regulate gene expression. Notable examples for sensing external stimulus are cell wall-associated kinases (WAKs), leucine-rich repeat protein kinases (RPKs) and stress activated protein kinases (SAPKs). These candidate genes have been shown to express in biotic and abiotic stress conditions, demonstrating a possible role in decoding Ca^{2+} signals (Xu *et al.*, 2013; Kohorn and Kohorn, 2012a; de Oliveira *et al.*, 2014). Senescence associated genes (SAGs) are genes related to natural senescence in different conditions and their expressions can be induced by ABA and ROS (Lee *et al.*, 2001). It is well known that high production of ROS is related to oxidative stress. Plants have mechanisms for scavenging ROS through cytosolic ascorbate peroxidases (APXs), chloroplastic and cytosolic glutathione

reductases (GRs), superoxide dismutases (SODs), and cytochrome P450 reductase enzymes (Rosenvasser *et al.*, 2006; Liang *et al.*, 2014). The GWAS approach not only identified genes related to senescence (*OsSAG20*) but also genes for ROS scavenging (Cytochrome P450 and Glutathione), which may be interesting for further characterisation. Furthermore, the GWAS approach identified several family genes related to plant hormones and transcription factors. Much study has shown that plant hormones play essential roles in the process of plant senescence during biotic and abiotic stress conditions, while TFs play important roles in the regulation of gene activities via secondary messengers and plant hormone signalling pathways (Kohli et al., 2013; Sewelam et al., 2013).

So far, GWAS has partially shown its effectiveness to identify genes that were previously shown to play a role in salinity. These include transporters such as *OsHKT1;1*, *OsHKT1;4*, *OsHKT1;5*, *OsNHX1* and *OsSOS1*. To test if any significant SNPs from this study are in the genomic vicinity of these genes, their genomic distances were calculated. Table 3.8 shows that in all cases, the distance was more than 400 kb whereas it was over 1 Mb in the case of *OsSOS1*. A possible explanation for this might be a low inherent genetic variability within these genes or the low number of SNPs of the genotyped accessions. Another possible explanation for this is that this experiment may have needed higher number of accessions, replication (plants) and measurement of other traits.

Table 3.8. Summary of well-known gene positions for saline conditions and GWAS SNP positions.

Locus	Gene	Coordinates	GWAS position	Distance (kb)
LOC_Os01g19290	*OsHKT1;5*	11458955-11463442	11019354	442
LOC_Os04g52260	*OsHKT1;1*	30724244-30727084	31177764	452
LOC_Os04g52260	*OsHKT1;4*	30734183-30739334	31177764	441
LOC_Os07g47710	*OsNHX1*	28164412-28169335	28623794	457

NOTE: the distance (kb) between GWAS SNP positions and gene positions was calculated using the average of gene coordinates.

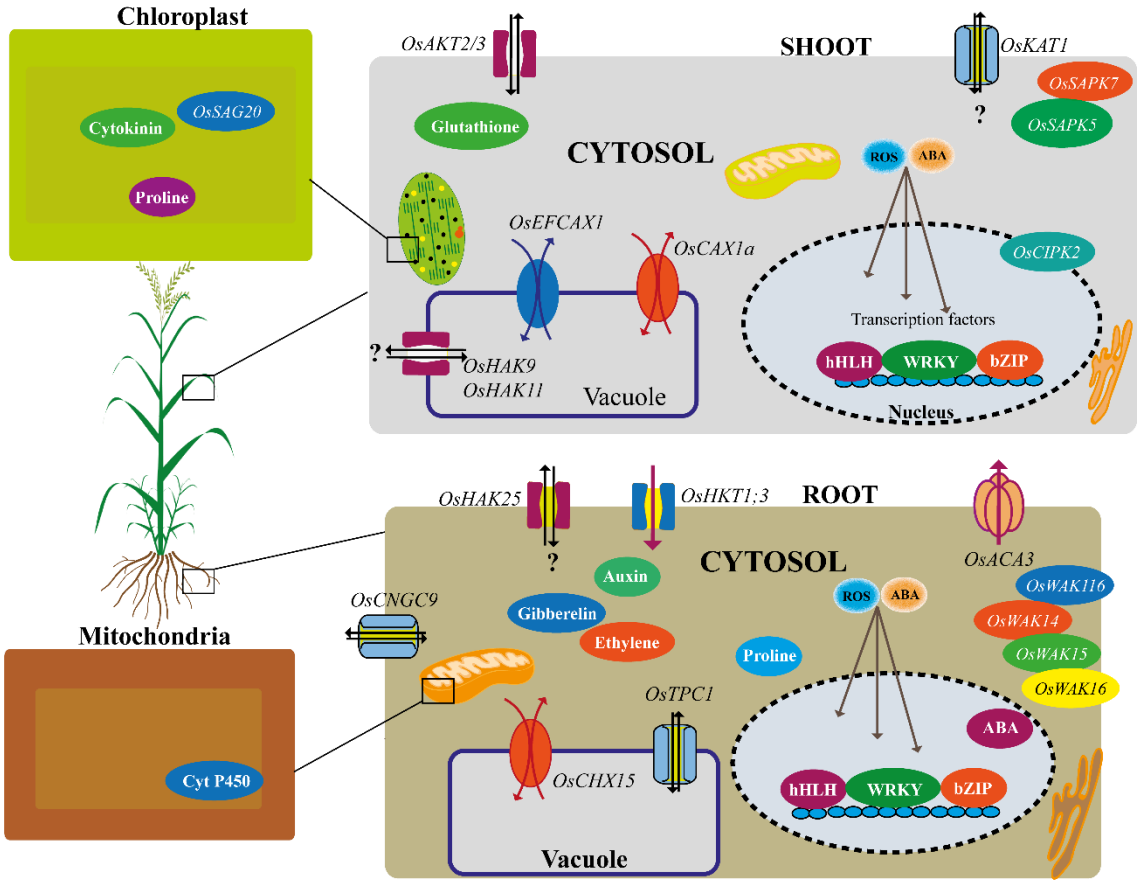


Figure 3.17. Schematic of plant root and shoot cells and candidate genes identified by GWAS. The diagram shows the candidate genes localisations in different plant cell organelles. Gene localisation was performed using published data and an integrative bioinformatics framework website (http://bis.zju.edu.cn/ricenetdb/). Candidate gene annotations: cyclic nucleotide gated channel (OsCNGC9), inward-rectifying potassium channel (OsAKT2/3), inward-rectifying potassium channel (OsKAT1), non-selective cation channel (OsTPC1), calcium-transporting ATPase 9 (OsACA9), high-affinity K^+ transporters (OsHAK9, OsHAK11, OsHAK25), high-affinity K^+ transporter (OsHKT1;3), sodium/calcium exchangers (OsCAX1a, OsCHX15, OsEFCAX1). Regarding protein kinases, CBL-interacting protein kinase 2 (OsCIPK2), Serine/threonine-protein kinases (OsSAPK5, OsSAPK7), wall-associated kinases (OsWAK116, OsWAK14, OsWAK15, OsWAK16, OsWAK2). For ROS detoxification and osmoprotectants, cytochrome P450 enzyme (Cyt P450), glutathione S-transferase (Glutathione). For transcription factors, basic helix-loop-helix domain (bHLH), basic leucine zipper (bZIP) and WRKY transcription factors.

3.4.5 Physical mapping: novel determinant genes

This study found a large group of loci for transporters, protein kinases, transcription factors and other proteins that contribute to uptake and distribution on various cations. According to this data and many publications, it can be inferred that many hundreds or ever thousands of transcripts are either up- or downregulated under saline conditions. Further research should be undertaken to investigate the potential functions of the novel candidate genes (Supplementary data 22, 23, 28, 32 and 35).

It is worth mentioning that GWAS analysis was performed using mean values of three plants. To assess whether GWAS reveal the same association signals and number of significant SNPs when using single replicates (plants), further analyses are required. However, previous studies have shown that using between two and five plants, the GWAS approach has identified association signals with high resolution and identified well-known candidate genes (Pantalião *et al.*, 2016; Huang *et al.*, 2010). Nonetheless, the number of replicates that is required may vary among traits and a high number of replicates may increase the robustness of the analysis.

3.4.6 GWAS efficiency and correlation between genetic and physical mapping

When comparing previous GWAS outcomes of rice accessions under saline conditions, Kumar *et al.* (2015) identified 20 association signals across the rice genome and 44 significant SNPs. However, this widely differed from the findings presented here except two SNP positions that showed vicinity with the published qSaltol QTL (Bonilla *et al.*, 2002). There may be several reasons for this discrepancy such as the use of different growth conditions, germplasm, fertilisation and traits. Moreover, they used lower number of SNPs and accessions, but higher number of replicates (plants). Furthermore, plants were exposed to saline conditions from 56-day old plants to the end of the plant's life cycle.

To evaluate correlations between genetic and physical mapping approaches, published QTLs and GWAS SNP positions were plotted (Figure 3.16). These results confirm the association between genomic and physical mapping. As shown in Figure 3.16, the general position of 34 QTLs correlated well with 23 SNPs, and three of the SNPs overlapped with the QTLs. Furthermore, the interval with distances between QTLs and SNPs ranged from 0.02 to 4.65 Mb (Table 3.7). These differences might be due to variation in terms of genomic and physical mapping scales and the different estimation procedure of probabilities for associations between phenotypic and genotypic data. In addition, there was a variation in the experimental conditions in terms of number of markers, rice population, and phenotypic data, which may have a direct effect on the findings. Therefore, it is concluded that the

findings of the present study effectively complement previous studies undertaken which allow us to use genomic and physical mapping techniques to explore and understand the multiple and complicated mechanisms of rice plants which are induced under salt stress.

3.5 Conclusions

The present study was designed to identify relatively well-known and novel genes that determine the variation of growth and ion concentrations under saline conditions. This study has shown that rice plants have a wide spectrum of phenotypes based on growth as well as K^+ and Na^+ concentrations in roots and shoots. Statistical analysis supported that there was a strong relationship between the RGR and RGR-reduction of accessions exposed to long term saline conditions. Regarding ion concentrations, experiments confirmed that high K^+ and low Na^+ as well as high K^+:Na^+ ratio in rice plant tissues are related to salinity tolerance.

Furthermore, this study has shown that GWAS identified association signals for accessions exposed to short, medium and long term saline conditions that were not revealed before using genetic mapping. It also identified specific relatively well-characterised genes that mediated K^+ and Na^+ transport.

These findings provide support and complement previous outcomes in the identification of association signals through genetic mapping. Moreover, this research may provide useful information for future studies to enhance our understanding of the multiple, dynamic and complicated mechanism of plants under saline conditions. Future research studies might explore in depth the association signals, the role of nonsynonymous SNPs and candidate genes through mutation and gene editing techniques.

Chapter 4

A Forward Genetic Screen to Identify Novel Determinant Genes for Salt Tolerance

4.1 Introduction

Forward genetic screening approaches have been applied to find and explore novel determinant genes in abiotic stress. The approach consists of finding mutants that show different phenotypes compared to wild-type plants in a certain condition (Stamatiou *et al.*, 2013). Many forward genetic screenings have been used for several model plant species including Arabidopsis (An *et al.*, 2005; Dobritsa *et al.*, 2011; Himelblau *et al.*, 2009). For example, Xiong *et al.* (2006) identified drought inhibition of lateral root growth (dig) mutants and further characterisation of these mutants revealed that they exhibited drought stress adaptation. Other examples are salt overly sensitive (sos) mutants which were identified as salt sensitive genotypes. Further studies confirmed that overexpression of SOS enhances salt tolerance by the extrusion of Na^+ from the cytoplasm to the apoplast where it is less toxic (Yang *et al.*, 2009; Halfter *et al.*, 2000). Despite its importance in the identification of genes, genetic screening might not be able to detect small genes due to the low effectiveness for mutagenesis. Also, genes with pleiotropic functions might not be identified, especially if a phenotype is the result of more than one gene (Wang and Sherwood, 2011; Vidaurre and Bonetta, 2012).

Over the past few decades, significant advances in our understanding of the molecular and cellular responses to salt stress have been made using forward genetic screening in Arabidopsis mutants (Tol *et al.*, 2016; Pandey *et al.*, 2004; Allu *et al.*, 2014). These studies have shown that salinity stress is a complex trait that involves diverse morphological, physiological and molecular responses and the contribution of hundreds if not thousands of genes.

To obtain further insights into genes that are potentially important in the response to salinity, a large collection (~7000) of homozygous Arabidopsis T-DNA mutants was screened by growing plants in saline soil conditions. The aim of this study was to identify novel genes that have putative functions in salt tolerance.

4.2 General Methods

Homozygous T-DNA Arabidopsis mutants were obtained from the European Arabidopsis Stock Centre NASC and exposed to saline conditions. To identify candidate genes using this approach, the research was conducted in three main experiments. The first and second experiments consisted of the exposure of wild-type *Arabidopsis thaliana* (L.) ecotype Columbia (Col-0) and either 6868 or 41 Arabidopsis genotypes to saline soil conditions for 3 weeks. The third experiment consisted of the exposure of 11 genotypes to hydroponic salt stress conditions for 6 days.

4.2.1 Screening experiments

For the first and second experiments, seeds of wild-type Col-0 and either 6868 mutants or 41 were sown in F2+sand substrate, respectively (Levington, UK) soil in P60 growing trays and stratified at 4ºC for 3 days. The 41 mutants were selected from the outcomes of the first experiment. Three seeds were sown per line and the screening was carried out in 7 batches. Trays containing stratified seeds were transferred to a glasshouse with 16 h light/ 8 h dark conditions and night-day temperatures of 20-23ºC for 28 days. Before initial treatment, superfluous seedlings were thinned out to leave one plant per pot. Subsequently, plants were exposed for 3 weeks to 100 mM NaCl by filling trays with 2L of salinised tap water. To monitor growth and morphological changes, trays were photographed weekly.

For the third experiment, wild-type Col-0 and mutant genotypes were sown in F2+sand, stratified for 3 days and grown for 15 days in growth room conditions with a day-time temperature of 21ºC and night-time temperature of 18ºC and 16 hours of photoperiod. Plants were transferred to 1-litre hydroponic boxes containing hydroponic medium and allowed to acclimatise for 4 days before salt treatment. The treatment consisted of the exposure of genotypes either to Arteca & Arteca control hydroponic medium or hydroponic medium plus 75 mM NaCl (Podar, 2013; Arteca and Arteca, 2000).

The protocols described in the "General Methods" section of Chapter 2 were followed for measurements of relative growth rate (RGR) and relative growth rate reduction (RGR-reduction) as well as K^+ and Na^+ concentrations in root and shoot tissues.

4.2.2 Statistical analysis

To analyse phenotypic data of Arabidopsis genotypes, all collected data was analysed using Statistical Analysis System version 9.3. Analysis of variance (ANOVA) and pair sample T-test analysis were conducted in SAS 9.3 and R software packages (SAS, 1999; R Development Core

Team, 2016). Tukey's honest significant test (Tukey HSD; P<0.05) was used to test the differences between the means of the different data sets, where significant differences were detected by ANOVA.

4.3 Results

4.3.1 Effects of salinity on growth and ion concentrations

For the first experiment, the 6868 Arabidopsis T-DNA mutants showed a varying degree of salinity tolerance. By comparing wild-type and mutants, 171 and 487 genotypes were identified which showed relative tolerance and sensitivity, respectively. Based on bioinformatics, this collection was narrowed down to 41 genotypes which were sown, grown and exposed to salinity again to test for phenotype consistency (Second experiment). After the second round of screening 11 genotypes were found to consistently show altered response to salinity (Supplementary data 4.1).

In a third experiment, the RGR and tissue ion concentrations of the 11 genotypes were determined using hydroponics conditions. In control conditions, the RGR and root K^+ of the wild-type and 11 genotypes did not show significant difference between genotypes (Supplementary data 4.2 and 4.3). In contrast, when genotypes were exposed to saline conditions, the RGR and RGR-reduction were significantly different between mutants (Supplementary data 4.4). After 6 days of saline conditions, the root K^+ and shoot K^+ values were similar across all genotypes (Supplementary data 4.5). However, the root Na^+ and shoot Na^+ were significantly different between lines (Supplementary data 4.6).

4.3.2 Effects of salinity on growth rates

The two genotypes that showed significant difference for relative growth values, K^+ and Na^+ concentrations in roots and shoots were taken forward for further characterisation. These mutants carry an insertion in either *AtCYP97B3* (showing increased tolerance) and *AtSAG18* (showing increased sensitivity). CYP97B3 is a Cytochrome P450, a protein that is generally determinant in oxidative stress responses, while SAG18 encodes a senescence associated gene which is induced by ozone but its endogenous function is poorly characterised.

Further statistical analyses were required to assess differences between the wild-type Col-0 and mutants. In the control conditions, the RGR was not significantly different between these three genotypes (Figure 4.1). In contrast, when genotypes were exposed to saline conditions, the tolerant genotype showed higher RGR, $t(10) = 2.49$, $p < 0.05$, and lower RGR-reduction, $t(10) = -3.40$, $p < 0.01$, compared to the wild-type. Further analysis showed that the sensitive genotype showed lower RGR, $t(10) = -2.67$, $p < 0.05$, and higher RGR-reduction, $t(10) = 2.75$, $p < 0.05$, in comparison to the

wild-type (Figure 4.2). Figure 4.3 shows a representative example of how these genotypes responded when grown in saline soil, showing that the phenotypes are consistent between various growth methods.

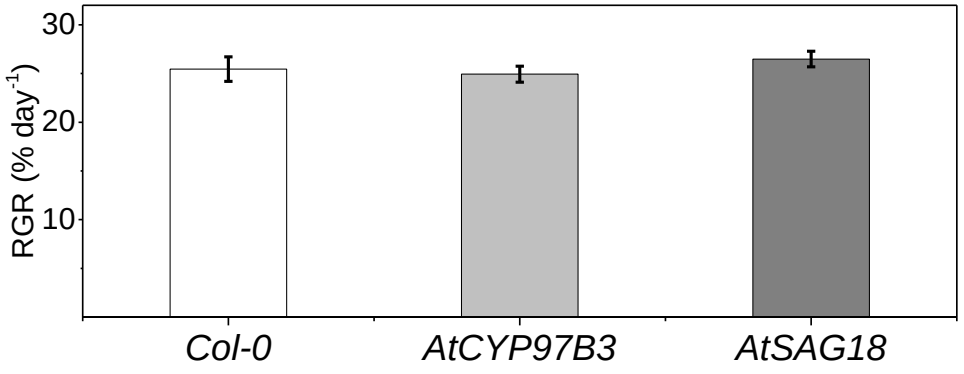


Figure 4.1. Relative growth rates of Arabidopsis genotypes exposed to control conditions. RGR of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. Bars represent the mean ±SE of six plants.

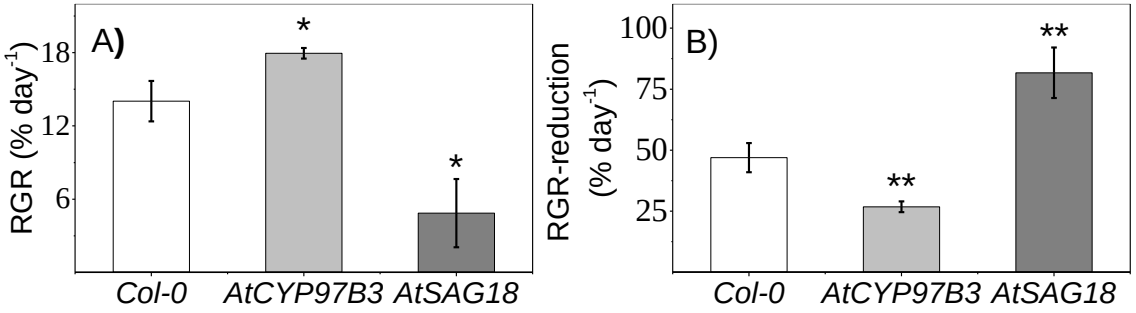


Figure 4.2. Relative growth rates of Arabidopsis genotypes exposed to saline conditions. (**A**) RGR of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. (**B**) RGR-reduction of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. Means followed by asterisk (*) are significantly different between the Col-0 and T-DNA mutants. *T-test is significant at (P≤ 0.05; paired-test). **T-test is significant at (P≤ 0.01; paired-test). Bars represent the mean ±SE of six plants.

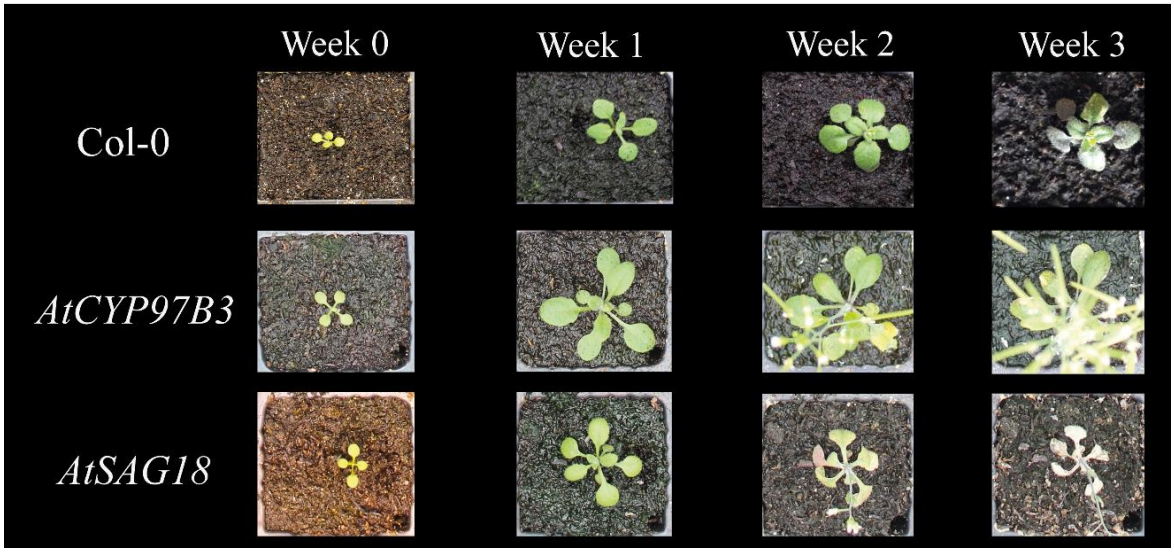


Figure 4.3. Salt tolerance phenotypes of the wild-type Col-0, AtCYP97B3 (tolerant) and AtSAG18 (sensitive) genotypes.

In addition to growth, the 3 genotypes were tested for tissue K^+ and Na^+ concentrations. When plants were exposed to control hydroponic conditions, the statistical tests revealed that neither the root K^+ nor shoot K^+ was significantly different between the wild-type and the tolerant or sensitive genotype (Figure 4.4). Interestingly, for K^+ concentrations of plants exposed to saline conditions, the tolerant genotype showed higher root K^+, $t(10) = 2.68$, $p < 0.05$, and lower shoot K^+, $t(10) = -2.80$, $p < 0.05$, in comparison to the wild-type (Figure 4.5).

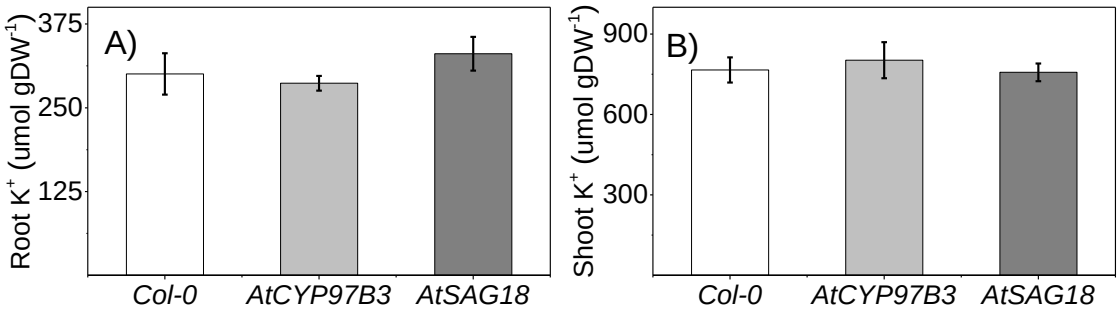


Figure 4.4. K^+ concentrations in roots and shoots of Arabidopsis genotypes exposed to control conditions. (**A**) Root K^+ of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. (**B**) Shoot K^+ of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. Bars represent the mean ±SE of six plants.

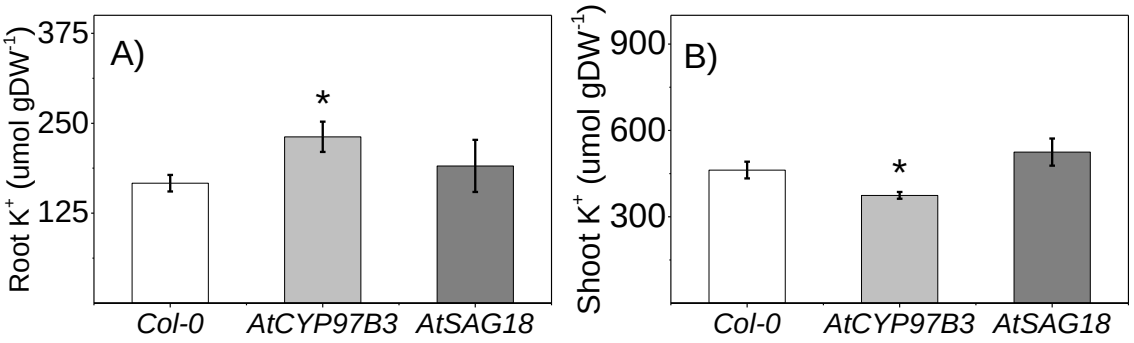


Figure 4.5. K^+ concentrations in roots and shoots of Arabidopsis genotypes exposed to saline conditions. (**A**) Root K^+ of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. (**B**) Shoot K^+ of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. Means followed by asterisk (*) are significantly different between the Col-0 and T-DNA mutants. *T-test is significant at ($P \leq 0.05$; paired-test). Bars represent the mean ±SE of six plants.

When plants were grown in saline conditions, the tolerant genotype showed higher root Na^+, $t(10) = 3.21$, $p < 0.01$, and lower shoot Na^+, $t(10) = -2.65$, $p < 0.05$, compared to the wild-type. However, there was no significant difference between the wild-type and sensitive genotype for either root Na^+ or shoot Na^+ (Figure 4.6).

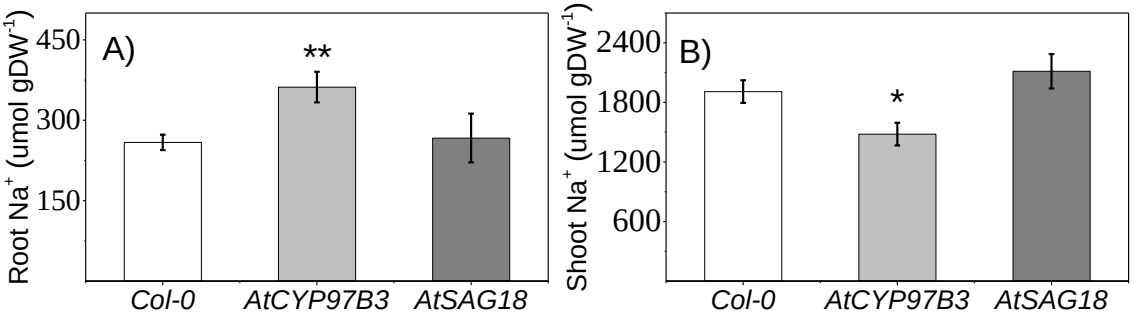


Figure 4.6. Na^+ concentrations in roots and shoots of Arabidopsis genotypes exposed to saline conditions. (**A**) Root Na^+ of the wild-type Col-0, salt tolerant (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. (**B**) Shoot Na^+ of the wild-type Col-0, salt sensitive (AtCYP97B3) and salt sensitive (AtSAG18) genotypes. Means followed by asterisk (*) are significantly different between the Col-0 and T-DNA mutants. *T-test is significant at ($P \leq 0.05$; paired-test). **T-test is significant at ($P \leq 0.01$; paired-test). Bars represent the mean ±SE of six plants.

4.4 Discussion

The aim of this research was to identify novel genes related to salt tolerance through forward genetic screening. This approach proved to be successful because one salt tolerant and one salt sensitive mutant were found which showed growth phenotypes with relative growth rates, K^+ and Na^+ concentrations that were significantly different from the wild-type plants. However, these are preliminary results and further genetic proofs by assessing the salt phenotype of second alleles are required for the two candidates.

Although one outcome of these experiments had a large degree of false positive, the findings do suggest that forward genetic screening is able to identify new genes that might be determinants in saline conditions. Nonetheless, a phenotype might be related to more than one gene or there may not be an obvious relationship between the genes (Wang and Sherwood, 2011; Vidaurre and Bonetta, 2012). The genes identified in this work are annotated as being related to oxidative stress detoxification and senescence which both have been shown to be related to salinity stress (Hanukoglu, 2006; Lee *et al.*, 2001).

Another important finding was that the tolerant genotype may achieve salt tolerance by retaining K^+ and Na^+ in roots rather than either increasing K^+ or decreasing Na^+ in shoots. These results are likely to be related to the reduction of Na^+ through loading and unloading of this ion in xylem sap mediated by certain members of HKT transporters (Rodríguez-Navarro and Rubio, 2006). These results agree with what has been previously published for Arabidopsis where salt tolerance correlated with high retention or K^+ and Na^+ in root tissues (Yang *et al.*, 2013; Farquharson, 2009).

Salt tolerance in plants involves diverse morphological, physiological and molecular responses and the contribution of hundreds if not thousands of genes. The forward screen approach has identified *AtCYP97B3* and *AtSAG18*, which have yet to be characterised in more depth. Previous studies have reported that some Cytochrome P450s have a potential role in reactive oxygen species (ROS) signalling during salinity stress (Mao *et al.*, 2013; Apel and Hirt, 2004). Concerning the sensitive genotype, the *AtSAG18* gene is related to senescence which may be related to phenotype (Zhou *et al.*, 2011; Wang *et al.*, 2011). There are still many unanswered questions about the gene functions and therefore further research is required.

4.5 Conclusion

The aim of the present study was to identify novel genes through forward genetic screening. This approach was able to identify two novel genes that might be determinants in the tolerance to high saline conditions. The current data highlights the importance of finding more genes due to the complex mechanism of plants in saline conditions. Furthermore, the present outcomes provide additional evidence that salt tolerance of Arabidopsis is related to high growth, high K^+ and low Na^+ concentrations in plant tissues. Based on the present outcomes, further research might explore potential functions of the candidate genes to enhance our understanding of the multiple, dynamic and complicated mechanism of plants under saline conditions.

Chapter 5

Final Conclusions

5.1 Impact of Salinity on Rice and Identification of Determinant Genes

Salinity has been shown to have a great impact on agricultural lands and its impact will increase in the coming years because of global warming and climate change. To supply rice demand, it is necessary to improve this cereal for different biotic and abiotic conditions through a combination of genetic engineering and conventional breeding programmes. To achieve salinity tolerance in rice, much study has shown that it is a complex trait and many genes play essential roles at different plant stages. During the last decades, different approaches have been used to unravel the mechanisms of glycophytes and halophytes under saline conditions and to characterise determinant genes that can be introgressed to agricultural crops. So far, there are thousands of published candidate genes identified by DNA microarray, genetic and physical mapping, and forward and reverse genetic screening approaches. However, there are only a few genes that have been introgressed to elite rice cultivars and assessed for their tolerances under saline field conditions.

5.2 Aims of the study

For chapter two, the objectives were (a) to assess a correlation between osmotic and salinity tolerance in rice cultivars and (b) to identify common physiological responses of rice cultivars to osmotic and saline conditions. For chapter three, the objectives were to identify relatively well-known and novel determinant genes for osmotic and ionic components of salinity through GWAS. This study also assessed the efficiency of GWAS to get insights into amino acid changes of SNP positions, and evaluated correlations between genetic and physical mapping approaches. For chapter four, the objective was to identify novel determinant genes for salt tolerance using a forward genetic screen.

5.3 Research outcomes

Chapter two showed that there was a strong positive correlation between osmotic-tolerance and salinity-tolerance of rice cultivars based on absolute and relative growth values. Even though osmotic and salinity are different conditions, this research has shown that growth reduction of rice plants overlapped (Singh *et al.*, 2016b; Wu *et al.*, 2015). This study has shown that the tolerance, based on absolute and relative growth values, of 12 rice cultivars exposed to PEG and NaCl were comparable to the published drought tolerance. In addition, rice plants have shown overlapped

reduction of K^+ in roots and shoots (Fuchs *et al.*, 2005), high production of reactive oxygen species (Miller *et al.*, 2010) and reduction of water loss by transpiration (Hsiao *et al.*, 1976). However, this is a relative comparison due to different experimental conditions such as plant age, the severity of drought conditions and different measured traits.

For chapter three, the results showed that there was a considerable diversity between rice accessions regarding salt tolerance based on absolute and relative growth values, root K^+ and shoot Na^+. The Na^+ uptake may have contributed to osmotic adjustment of rice plants exposed to short term saline conditions (Yeo *et al.*, 1991; Cramer, 2002). These results support the idea that Na^+ can partially replace K^+ (Huyen *et al.*, 2013), however, plants may have no efficient mechanism to control the transport and distribution of Na^+ (Anil *et al.*, 2005). When comparing K^+ and Na^+ in plant tissues, there was evidence that the variation in tissue K^+ values between accessions remains fairly constant and is at most approximately 5-fold. In contrast, values of tissue Na^+ fluctuate by a factor of almost 100 suggesting a far larger genetic diversity for this trait compared to tissue K^+ contents.

Where physical mapping is concerned, the results of this investigation not only complemented those of earlier findings but also GWAS revealed new association signals that were not revealed before using either QTL or GWAS mapping. The GWAS approach identified 120 association signals and ~1500 candidate genes across the rice genome, 29 of which overlapped in the terms of salinisation and covered 32 well-known genes. Regarding novel association signals, this approach identified major association signals on chromosomes 3, 6, 8, 9, and 10. Moreover, 17 significant SNPs revealed by GWAS correlated with 22 published QTLs.

The GWAS data identified *OsHKT1;3* which is a Na^+ selective transporter that is highly expressed in root cortex and vascular tissues, and which contains six non-synonymous SNPs (Jabnoune *et al.*, 2009; Rosas-Santiago *et al.*, 2015). This candidate gene was identified using combined traits of rice accessions exposed to long term saline conditions. Furthermore, by calculating the distance between significant SNPs revealed by GWAS and the position of HKT genes, *OsHKT1;1*, *OsHKT1;4* and *OsHKT1;5* were identified. A recent study has shown that *OsHKT1;1* is a determinant gene in the regulation of Na^+ via xylem sap and in limiting Na^+ accumulation in leaves (Wang *et al.*, 2015). The *OsHKT1;4* has been partially shown to function in xylem Na^+ unloading but does not have a profound influence on the growth and ion accumulation (Suzuki *et al.*, 2016), thus further research is required to determine whether the *OsHKT1;4* is related to salt tolerance. Regarding *OsHKT1;5*, this ion carrier has been shown to mediate Na^+ transport and it is hypothesised to have function in the recirculation of Na^+ via xylem (Ren *et al.*, 2005).

The identification of non-synonymous SNPs provided insights into the potential functional changes of amino acids and their associations with salt tolerance. The GWAS approach has identified 152 ns-SNPs, 18 of which showed high correlation with the salt tolerance of rice accessions exposed to saline conditions. Further analysis showed that the number ns-SNPs of the overlapped candidate genes in the three terms of salinisation ranged from 1 to 15, but none of the amino acid changes correlated with the salt tolerance. Moreover, the ns-SNPs of the *OsHKT1;3*, *OsHKT1;5* and *OsHKT1;4* did not correlate with salt tolerance of accessions. These results may need further analysis using different traits for salinity tolerance and to explore the potential of ns-SNPs by association signals.

Where the fourth chapter was concerned, forward genetic screening successfully identified one salt tolerant (*AtCYP97B3*) and one salt sensitive (*AtSAG18*) mutant. Even though these are preliminary results, published data mentions that certain members of Cytochrome P450s confer detoxification of reactive oxygen species (Hanukoglu, 2006) and specifically the *AtCYP97B3* has been shown to be a determinant in the biosynthesis of carotenoid (Ruiz-Sola and Rodríguez-Concepción, 2012). Where senescence associated genes (*SAGs*) are concerned, much study has shown that SAGs are related to natural senescence of plants (Lee *et al.,* 2001). Particularly, the *AtSAG18* is a determinant gene for plant senescence under ozone conditions (Miller *et al.,* 1999). Despite these promising results, questions remain to be explored about the specific roles of these candidate genes. Nonetheless, the assessment of the second alleles of these candidate genes is required before going further.

Securing genetic diversity and selecting efficient progeny are the most important factors in plant breeding. To identify genes related to a trait, there are several approaches. Using microarrays, Jangam *et al.* (2016) have identified ~1500 candidate genes for abiotic conditions and 63 may have roles in post-transcriptional regulation of genes. Even though these genes did not overlap with the candidate genes identified by the current GWAS approach, there were seven genes that showed similar molecular functions. Furthermore, Hossain *et al.* (2016) identified over 300 candidate genes with molecular functions overlapping with candidate genes identified by GWAS.

The QTL mapping has also identified hundreds of genes, many of which have yet to be characterised for saline conditions. One of the remarkable outcomes is identification of *OsHKT1;5* which was identified within the genomic region of the *qSaltol* QTL (Bonilla *et al.*, 2002) and was shown to be in the vicinity of SNP positions identified in this study and by Kumar *et al.* (2015). Recent findings showed that the *qSaltol* has been successfully introgressed into several elite rice varieties (Vu *et al.,* 2012; Luu TN *et al.,* 2012; Singh *et al.,* 2011; Usatov *et al.,* 2015) and assessed

for their salt tolerance in field conditions (Hoque *et al.*, 2015; Huyen *et al.*, 2013; Linh *et al.*, 2012). Babu *et al.* (2017) introgressed *qSaltol* in Pusa Basmati 112 rice cultivar, assessed its tolerance during three consecutive years and found significant improvements in agronomical traits. Therefore, it would be worth analysing published determinant candidate genes identified by different approaches and narrowing-down a subset of genes that show overlapping molecular functions.

5.4 Validity and Reliability of Research Outcomes

To assess the genetic variation the rice in control conditions, this study has found that rice did not show significant differences between rice cultivars for the RGR and K^+ concentrations in roots and shoots. However, using over 300 rice accessions, these traits were significantly different between accessions because of genetic diversity. On the other hand, the literature mentions that relative growth rate values are the most suitable parameter to express salinity tolerance (Negrão *et al.*, 2016). The RGR and RGR-reduction traits correlated with the root K^+ and shoot Na^+ of rice accessions, and the strongest correlations were observed in long term saline conditions.

In reviewing the literature, the terms of salinisation are widely variable between experiments. Exposing plants from few minutes to 24 hours is considered short term salinisation, and from few days to whole plant life cycle is considered long term salinisation (Yeo *et al.*, 1991; Al-Tamimi *et al.*, 2016; Kumar *et al.*, 2015). In this study, rice plants were exposed during 30 days for long term, between 6 and 7 days for medium term, and between 6 and 24 hours for short term salinisation. However, Arabidopsis genotypes were exposed during three weeks to salinised soil and 6 days in salinised hydroponic conditions as long terms. This variation is due to salt treatment concentrations, weather conditions and plant life cycle.

On the other hand, much study has argued that *indica* rice population has higher salt tolerance in comparison to *japonica* (Platten *et al.*, 2013; Lee *et al.*, 2003). This study did not find significant differences between the main rice populations based on relative growth values. This controversy could be due to the number of accessions, experimental conditions and the measured traits. However, when using a smaller number of rice cultivars and absolute growth values, osmotic and salinity tolerance of plants were significantly different between populations. The Nerica lines (crosses cross between O. sativa *indica* and O. *glaberrima*) showed higher tolerances to osmotic and saline conditions compared to *indica* and *japonica* populations. Based on this data, it is inferred that rice cultivars from different genetic backgrounds may have contained salt tolerant and sensitive lines. For further screening to different conditions, it is recommended to include well-known positive and

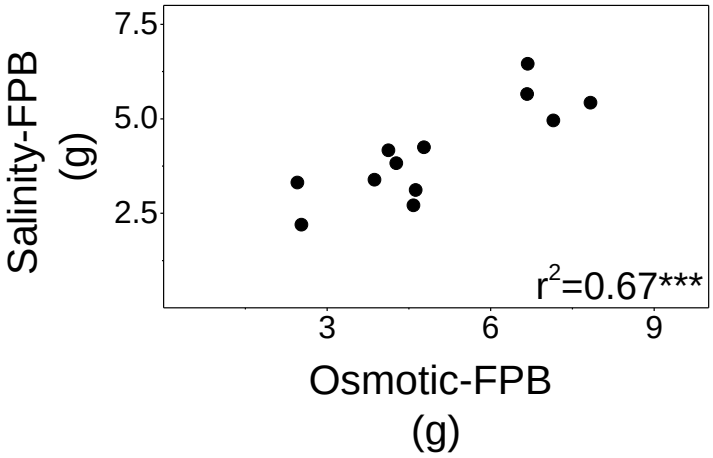
negative rice cultivar controls, similar number of accessions by populations and more than three plant-replicates.

5.5 Concluding Remarks and Future Work

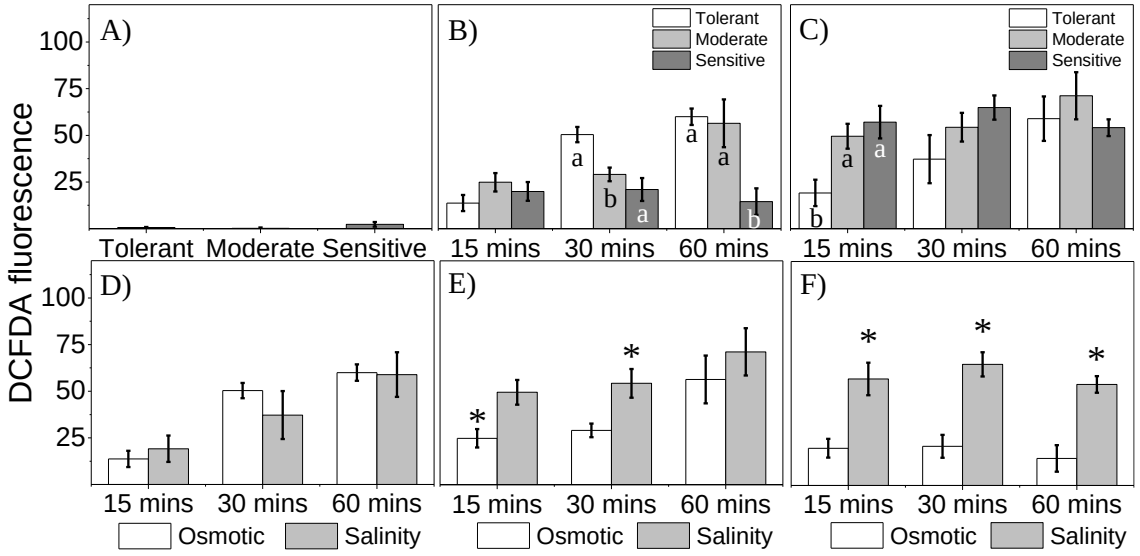
Salinity will continue to put a major constraint on agriculture worldwide. The identification, characterisation and introgression of genes encoding salinity tolerance to vegetable and cereal crops may accelerate the process of plant adaptation to saline conditions, increasing the ability of glycophytes to tolerate saline conditions in the way that halophytes do. Progress in this respect has been slow for many reasons but mainly because of the multigenic nature of salt tolerance. This is reflected in the wide dispersion of tolerance traits across the genome, which in turn necessitates the introgression of many QTLs and genes to achieve a high yielding and tolerant variety.

This study has identified novel salt tolerant lines that can be exploited in breeding programs, and the GWAS and forward genetic screening approaches have identified association signals and novel potential candidate genes that can be useful for genetic transformation. On the other hand, manipulation of single and double genes has shown promising results in controlled conditions but applications in actual agricultural contexts have been limited so far. Furthermore, the combination of genetic engineering and conventional breeding programmes allows useful traits to be introgressed into commercial crops in a smaller amount of time although improved varieties will still need to go through extensive testing programmes.

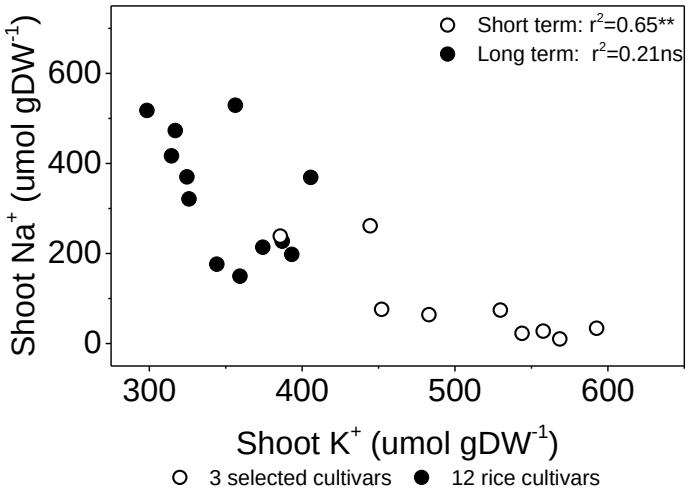
Appendix



Supplementary data 2.1. Correlation between osmotic and salinity inducers on absolute growth values of rice cultivars. ***Correlation is significant at the 0.01 level (two-tailed).



Supplementary data 2.2. Reactive oxygen species quantificated in root sections of the selected rice cultivars. (**A**) ROS levels in root sections of rice plants exposed to control conditions. (**B**) ROS levels in root sections of rice plants exposed to osmotic conditions. (**C**) ROS levels in root sections of rice plants exposed to saline conditions. Significance was calculated by one-way ANOVA. Means followed by different letters show significant difference between cultivars (Tukey's honest significant test HSD, P<0.05). (**D**) ROS levels of tolerant cultivar. (**E**) ROS levels of moderate cultivar. (**F**) ROS levels of sensitive cultivar. Means followed by asterisk (*) are significantly different between osmotic-induced and salt-induced conditions. Significance was identified by paired t-test (P<0.05). Bars show the mean ±SE of six sections of roots from different plants.

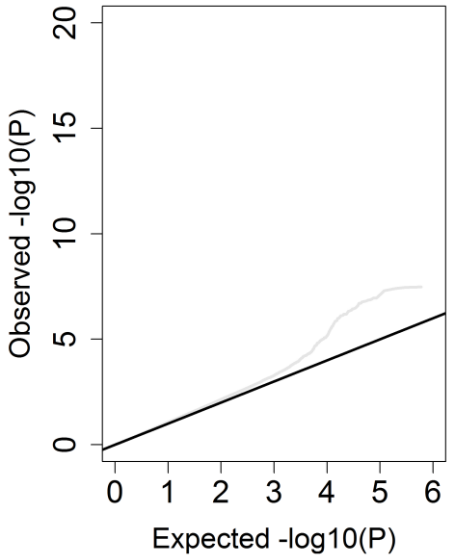


Supplementary data 2.3. Correlation between shoot K^+ and shoot Na^+ of rice cultivars. Rice plants were exposed to short (1,3 and 6 days) and long (30 days) term saline conditions. ^{ns}indicates the correlation is not significant at the 0.05 level (two-tailed). **indicates the correlation is significant at the 0.01 level (two-tailed).

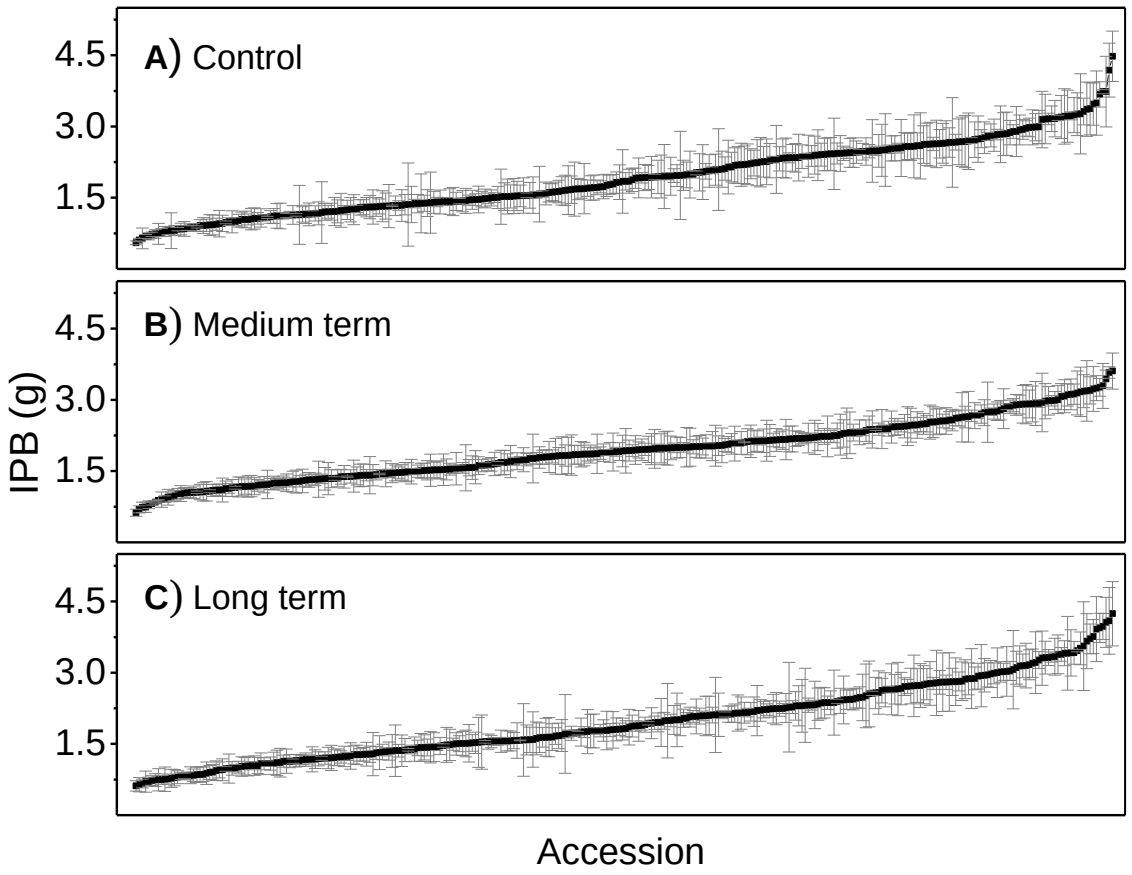
Supplementary data 3.1. Single and combined metrics used for GWAS. 52 single and combined traits were obtained and submitted to GWAS. A set of 52 metrics were obtained for the short, medium and long term saline conditions.

Traits			
Root K^+	IPB/Root Na^+	Shoot K^+/MT.RGR	LT.RGR/Shoot Na^+
Root Na^+	IPB/Shoot K^+	Shoot Na^+/MT.RGR	Root K^+/LT.RGR
Shoot K^+	IPB/Shoot Na^+	MT.RGR-reduction/Root K^+	Root Na^+/LT.RGR
Shoot Na	Root K^+/IPB	MT.RGR-reduction/Root Na^+	Shoot K^+/LT.RGR
Root K^+/Na^+	Root Na^+/IPB	MT.RGR-reduction/Shoot K^+	Shoot Na^+/LT.RGR
Shoot K^+/Na^+	Shoot K^+/IPB	MT.RGR-reduction/Shoot Na^+	LT.RGR-reduction/Root K^+
Root Na^+/K^+	Shoot Na^+/IPB	Root K^+/MT.RGR-reduction	LT.RGR-reduction/Root Na^+
Shoot Na^+/K^+	MT.RGR/Root K^+	Root Na^+/MT.RGR-reduction	LT.RGR-reduction/Shoot K^+
Root K^+/Shoot K^+	MT.RGR/Root Na^+	Shoot K^+/MT.RGR-reduction	LT.RGR-reduction/Shoot Na^+
Shoot K^+/Root K^+	MT.RGR/Shoot K^+	Shoot Na^+/MT.RGR-reduction	Root K^+/LT.RGR-reduction
Root Na^+/Shoot Na^+	MT.RGR/Shoot Na^+	LT.RGR/Root K^+	Root Na^+/LT.RGR-reduction
Shoot Na^+/Root Na^+	Root K^+/MT.RGR	LT.RGR/Root Na^+	Shoot K^+/LT.RGR-reduction
IPB/Root K^+	Root Na^+/MT.RGR	LT.RGR/Shoot K^+	Shoot Na^+/LT.RGR-reduction

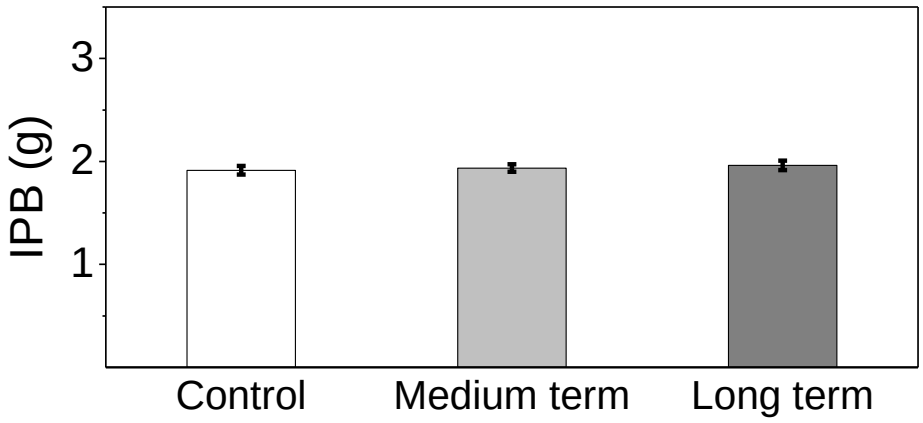
NOTE: initial plant biomass (IPB), relative growth rate traits of medium term saline conditions (MT.RGR), relative growth rate reduction traits of medium term saline conditions (MT.RGR-reduction).



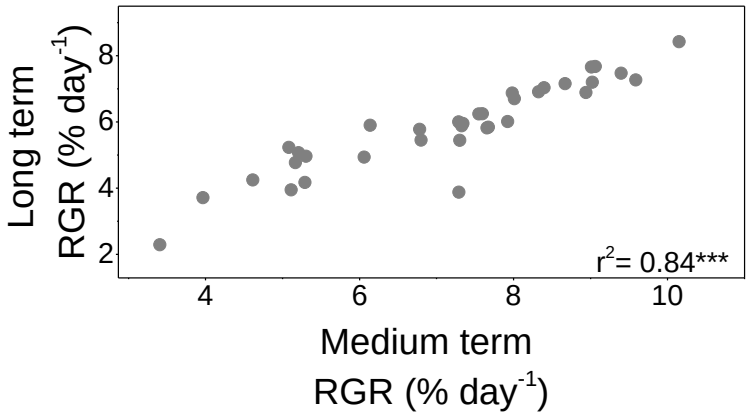
Supplementary data 3.2. Quantile-quantile (QQ) plot. The QQ plot shows the distribution of expected P-values against their observed values.



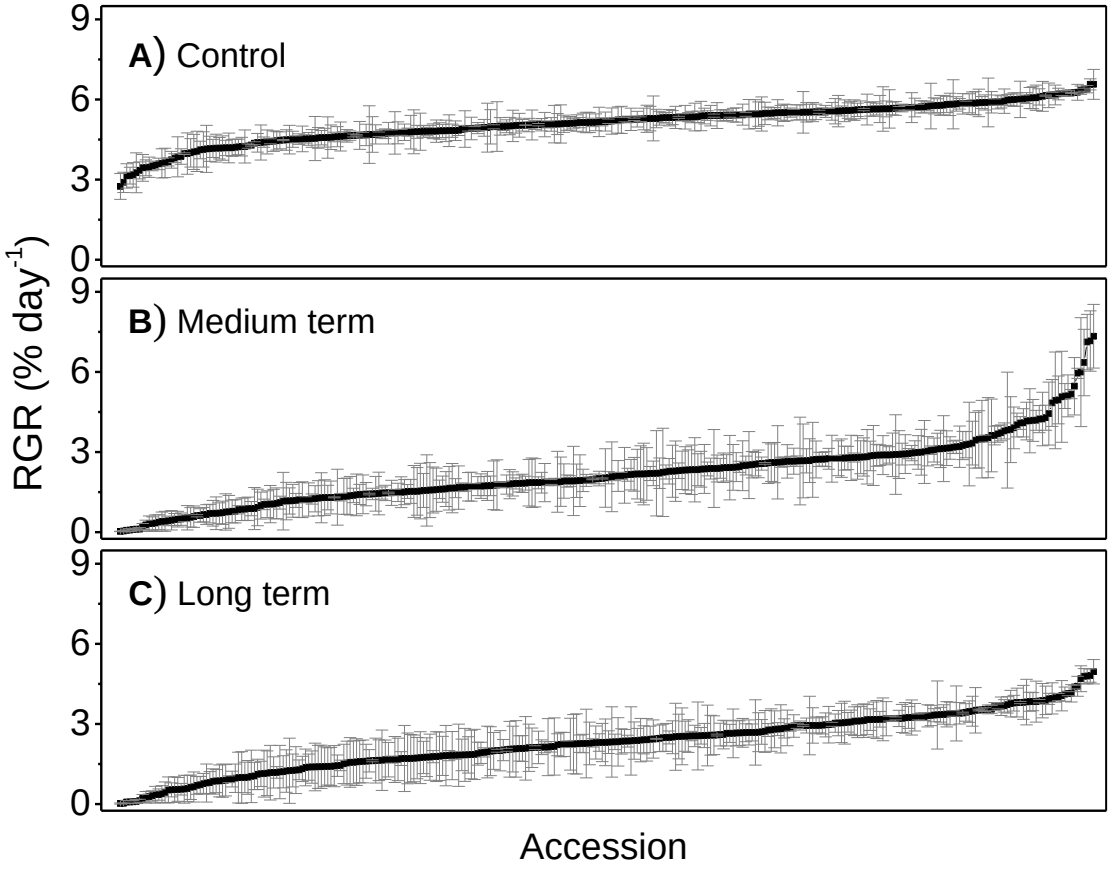
Supplementary data 3.3. Initial plant biomass of rice accessions. (**A**) Plants exposed to control hydroponic medium for 30 days. (**B**) Plants exposed to 50 mM NaCl for 7 days. (**C**) Plants exposed to 50 mM NaCl for 30 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



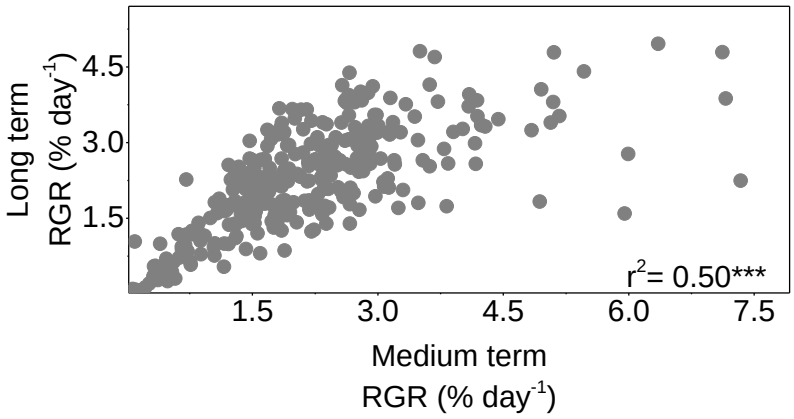
Supplementary data 3.4. Initial plant biomass of rice accessions exposed to control conditions, medium and long term saline conditions. Average IPB of 306 accessions at 30-day old. Bars show the mean ±SE of 918 plants.



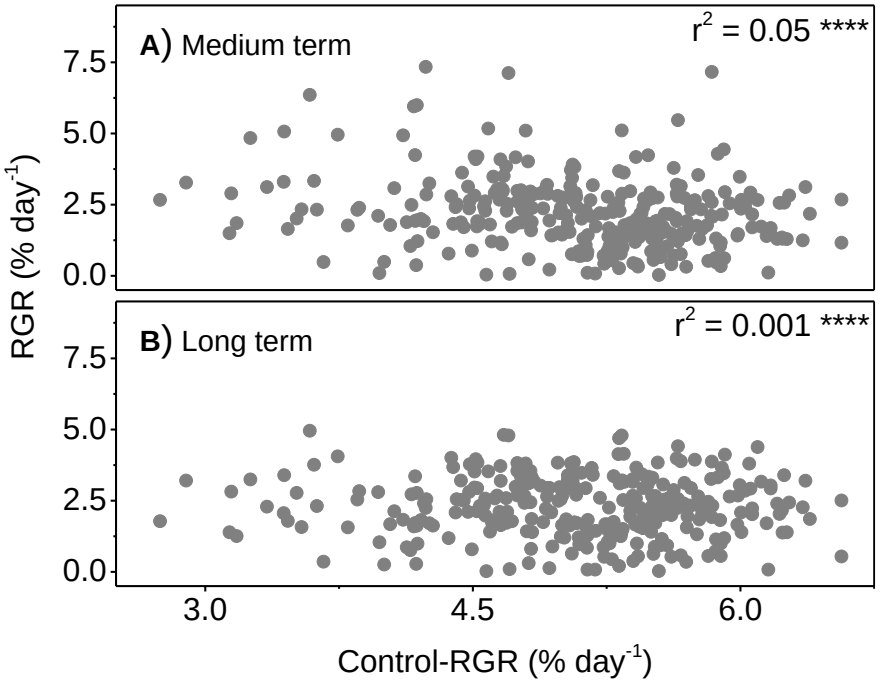
Supplementary data 3.5. Correlation between medium-term-RGR and long-term-RGR of rice cultivars grown in control conditions. ***Correlation is significant at the 0.001 level (two-tailed).



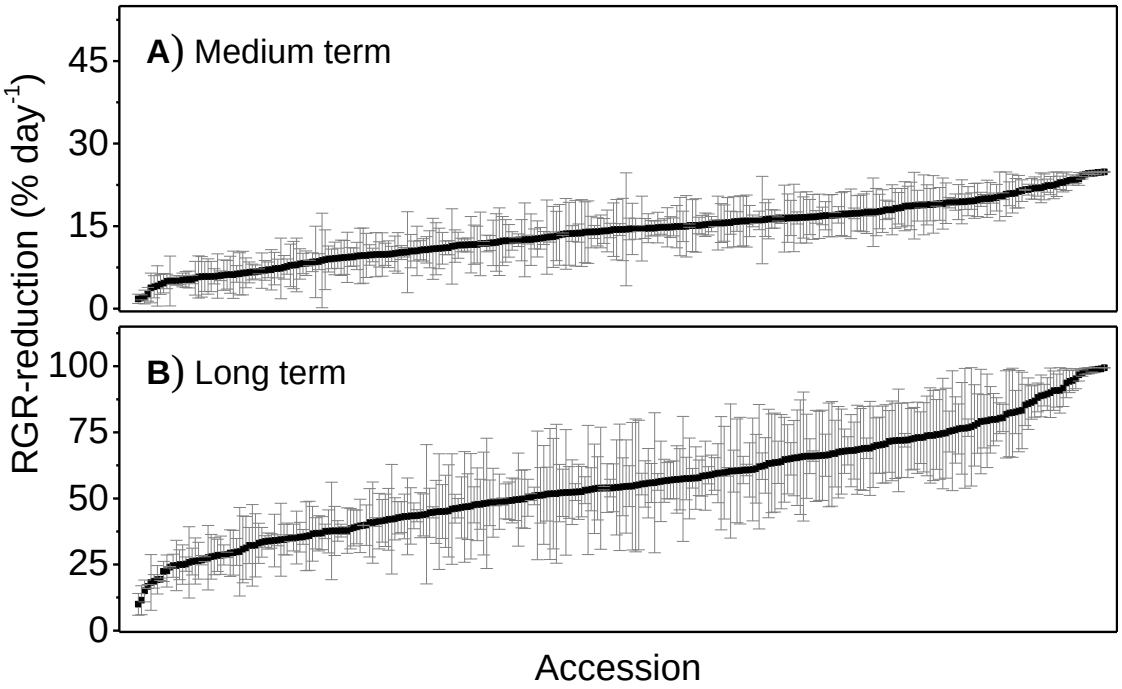
Supplementary data 3.6. Relative growth rate of rice accessions. (**A**) Plants exposed to control hydroponic medium for 30 days. (**B**) Plants exposed to 50 mM NaCl for 7 days. (**C**) Plants exposed to 50 mM NaCl for 30 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



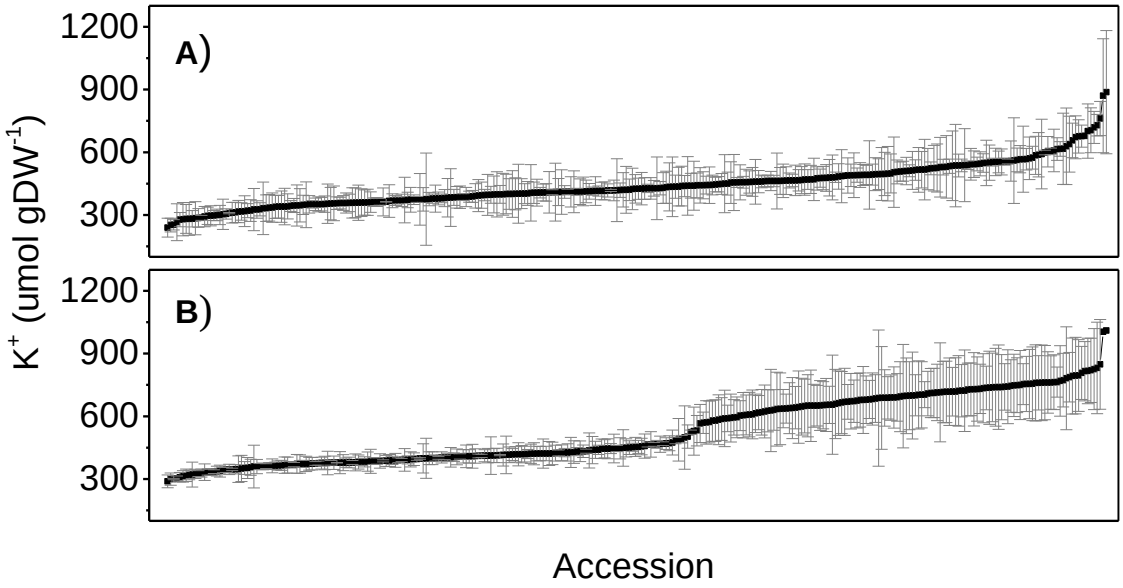
Supplementary data 3.7. Correlation between medium-term-RGR and long-term-RGR of 306 accessions exposed saline conditions. ***Correlation is significant at the 0.001 level (two-tailed).



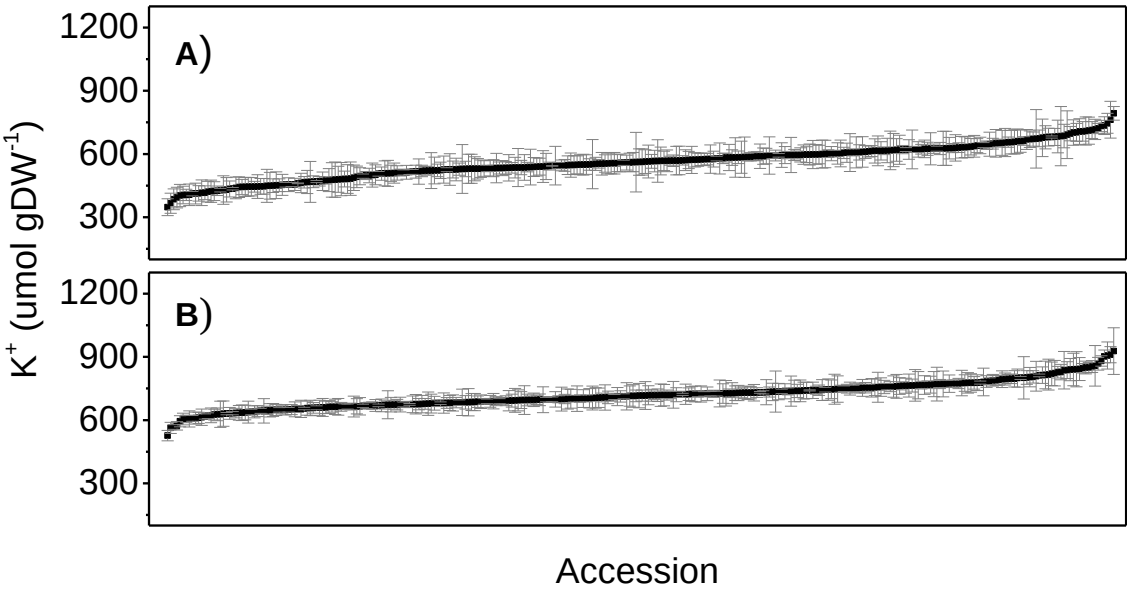
Supplementary data 3.8. Correlations between control-RGR and salt-RGR of accessions. (**A**) Correlation between control-RGR and medium term salt-RGR. (**B**) Correlation between control-RGR and long term salt-RGR. Control-RGR and salt-RGR data were from medium and long term trials. ****Correlation is significant at the 0.0001 level (two-tailed).



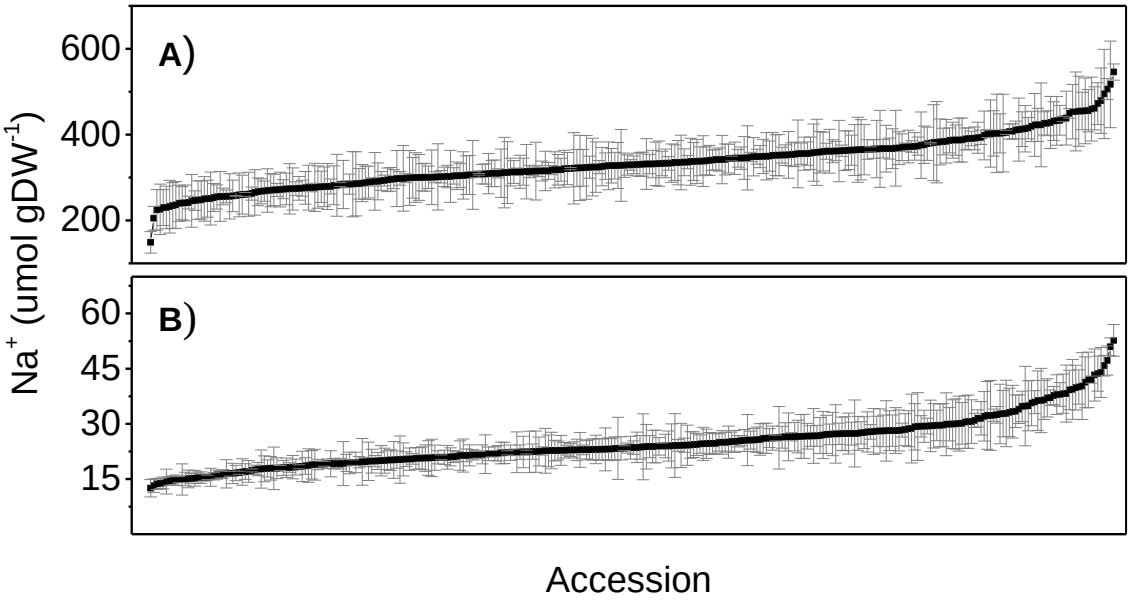
Supplementary data 3.9. Relative growth rate reduction of rice accessions. (**A**) Plants exposed to 50 mM NaCl for 7 days. (**B**) Plants exposed to 50 mM NaCl for 30 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



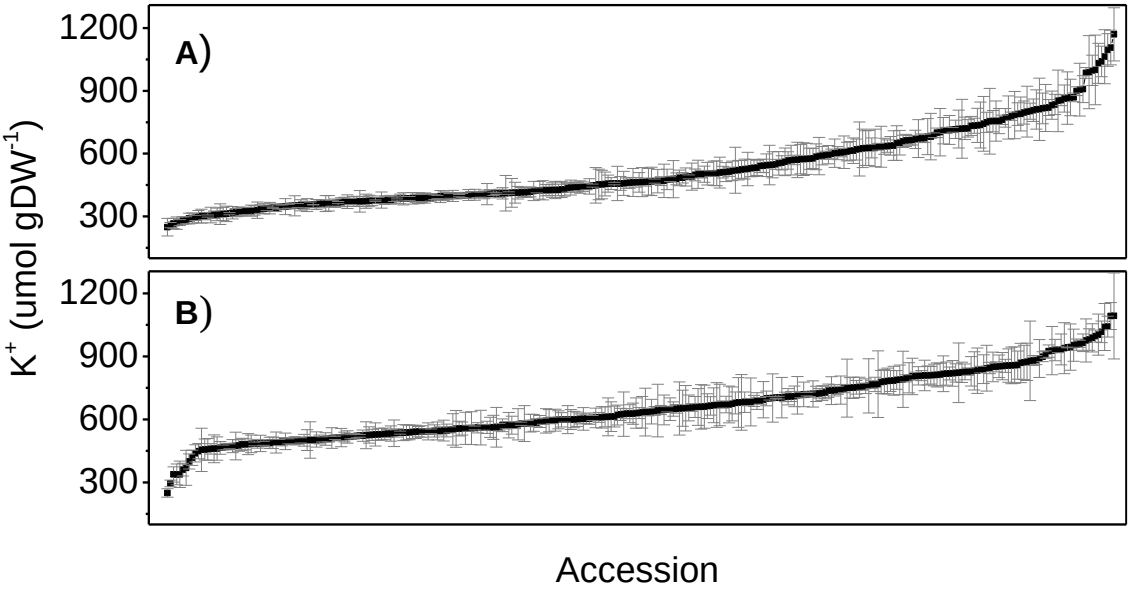
Supplementary data 3.10. K^+ concentrations in roots and shoots of rice accessions. (**A**) Root K^+ of plants exposed to control hydroponic medium for 30 days. (**B**) Shoot K^+ of plants exposed to control hydroponic medium for 30 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



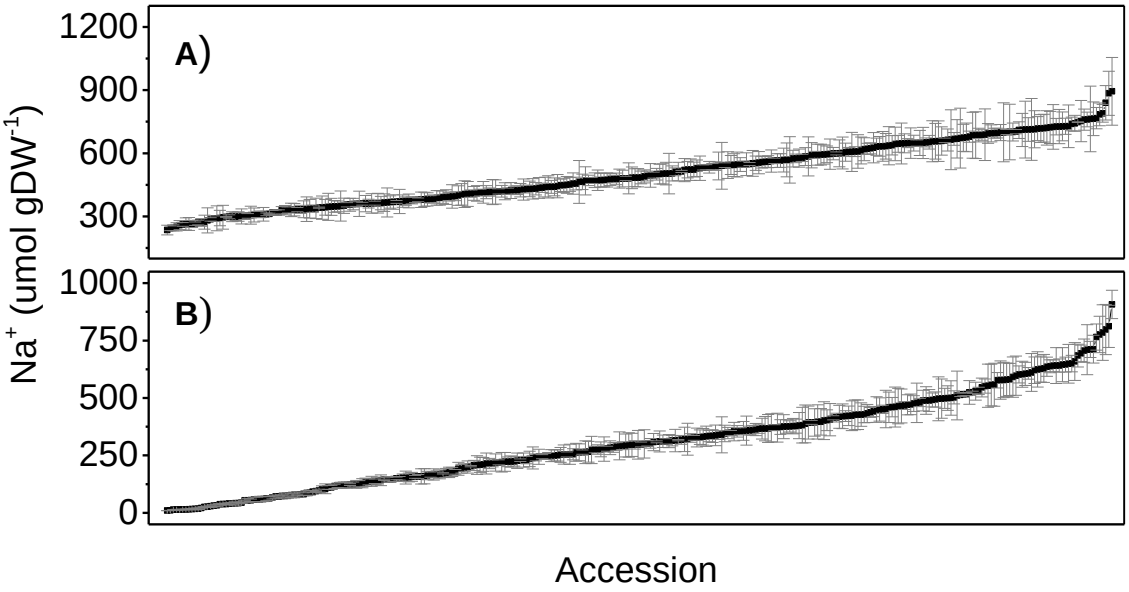
Supplementary data 3.11. K^+ concentrations in roots and shoots of rice accessions. (**A**) Root K^+ of plants exposed to 50 mM NaCl for 6 hours. (**B**) Shoot K^+ of plants exposed to 50 mM NaCl for 6 hours. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



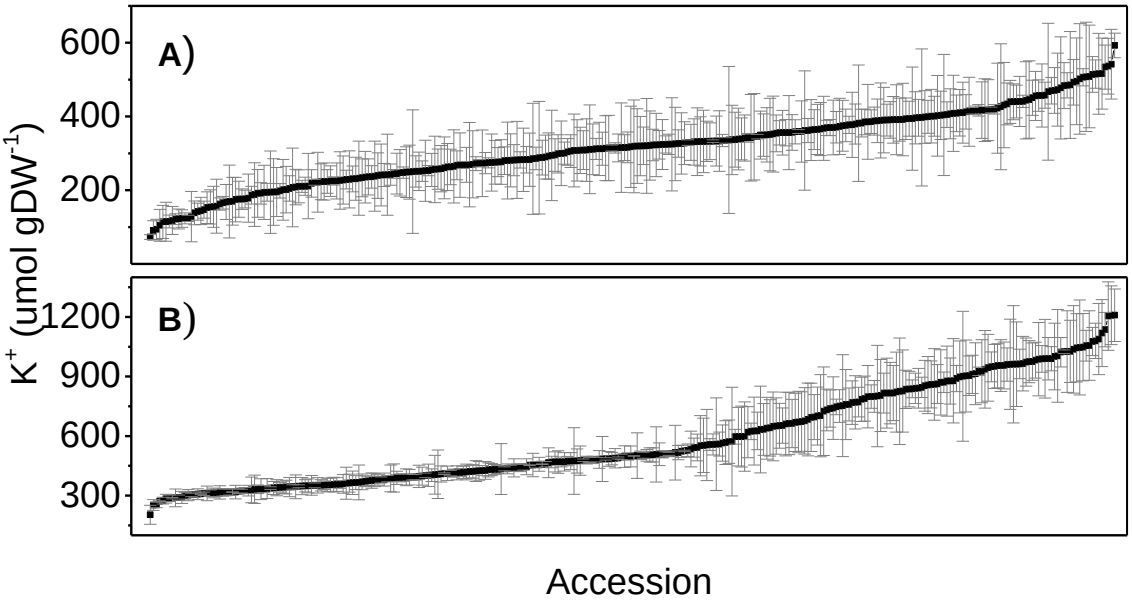
Supplementary data 3.12. Na^+ concentrations in roots and shoots of rice accessions. (**A**) Root Na^+ of plants exposed to 50 mM NaCl for 6 hours. (**B**) Shoot Na^+ of plants exposed to 50 mM NaCl for 6 hours. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



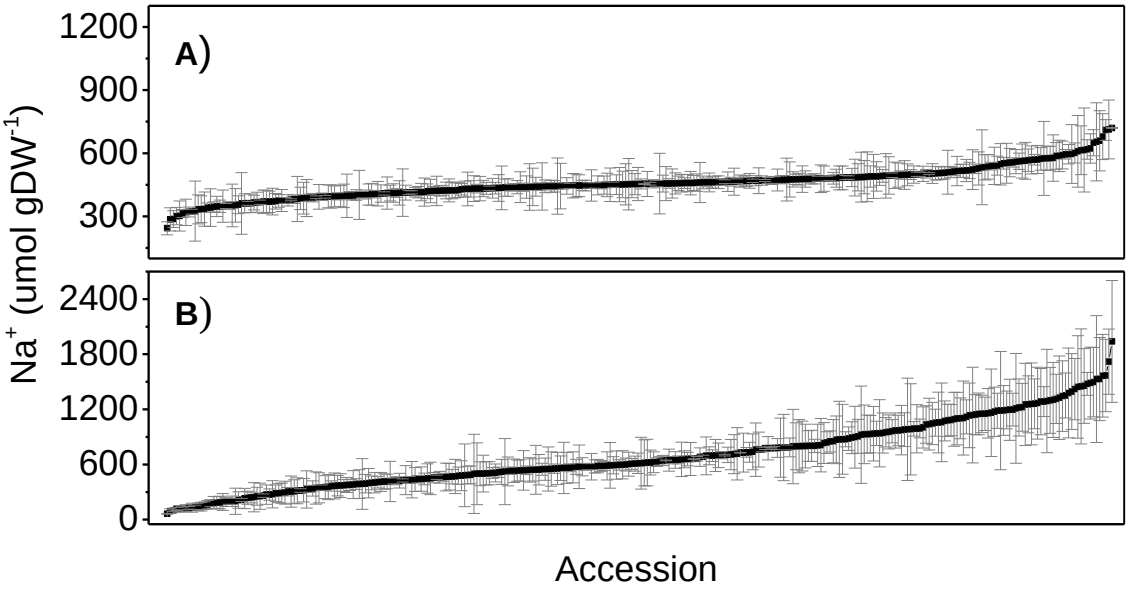
Supplementary data 3.13. K^+ concentrations in roots and shoots of rice accessions. (**A**) Root K^+ of plants exposed to 50 mM NaCl for 7 days. (**B**) Shoot K^+ of plants exposed to 50 mM NaCl for 7 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



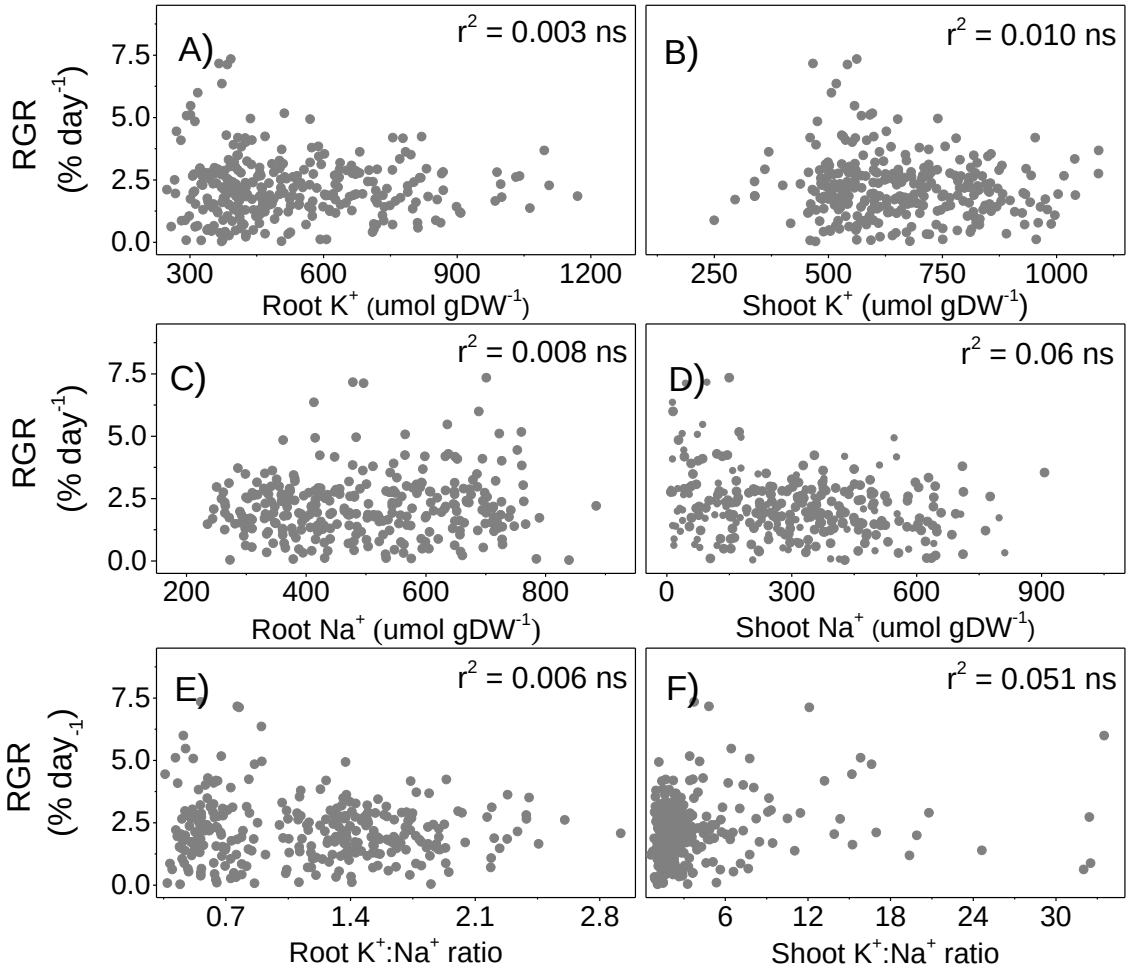
Supplementary data 3.14. Na^+ concentrations in roots and shoots of rice accessions. (**A**) Root Na^+ of plants exposed to 50 mM NaCl for 7 days. (**B**) Shoot Na^+ of plants exposed to 50 mM NaCl for 7 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



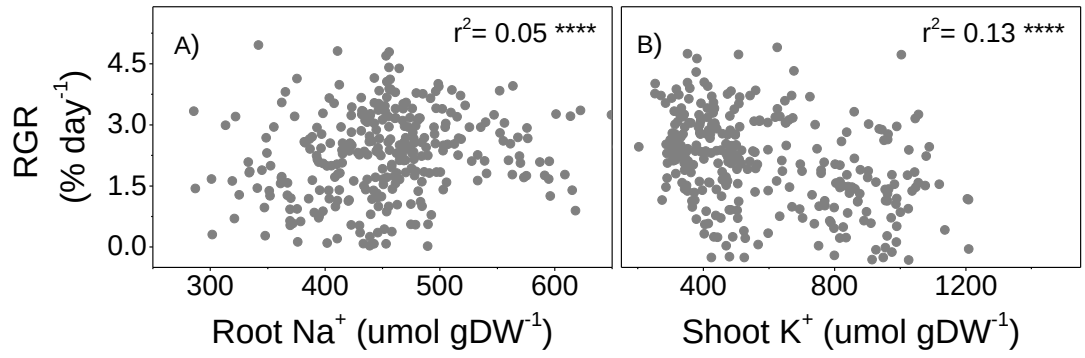
Supplementary data 3.15. K^+ concentrations in roots and shoots of rice accessions. (**A**) Root K^+ of plants exposed to 50 mM NaCl for 30 days. (**B**) Shoot K^+ of plants exposed to 50 mM NaCl for 30 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



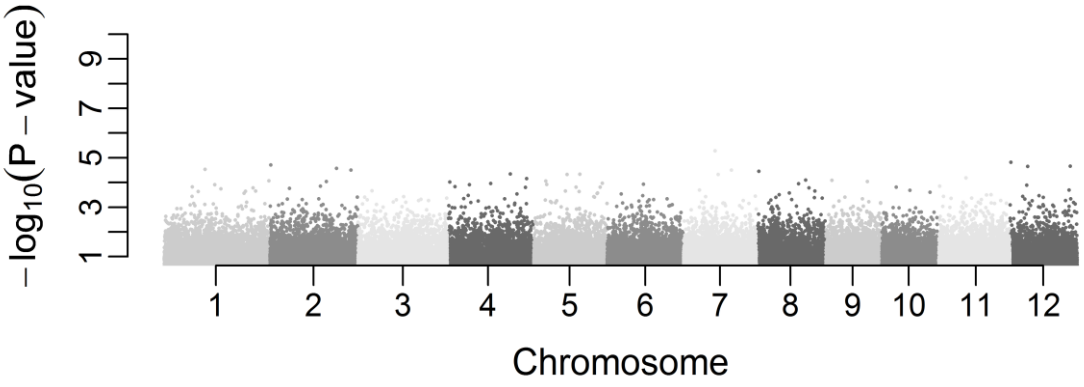
Supplementary data 3.16. Na^+ concentrations in roots and shoots of rice accessions. (**A**) Root Na^+ of plants exposed to 50 mM NaCl for 30 days. (**B**) Shoot Na^+ of plants exposed to 50 mM NaCl for 30 days. Significance was calculated by one-way ANOVA ($P<0.05$). Means are significantly different between accessions (Tukey's honest significant test HSD, $P<0.05$). Graphs show the means ±SE of three plants.



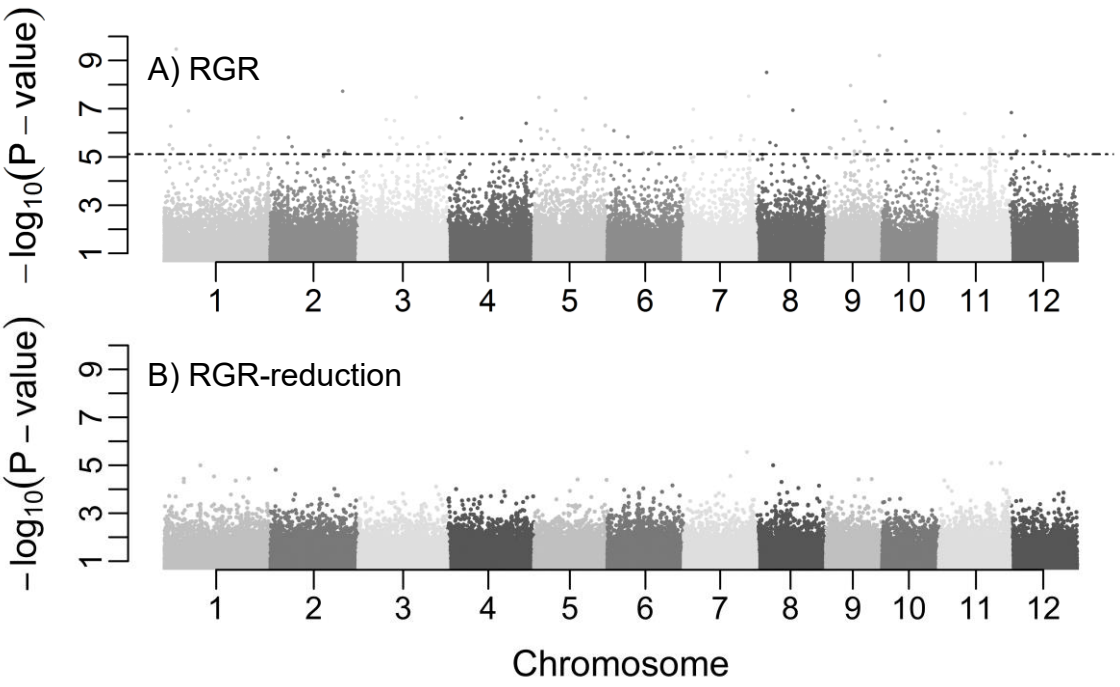
Supplementary data 3.17. Correlations between ion concentrations and RGR of accessions exposed to medium term saline conditions. (**A**) Correlation between root K^+ and RGR. (**B**) Correlation between shoot K^+ and RGR. (**C**) Correlation between root Na^+ and RGR. (**D**) Correlation between shoot Na^+ and RGR. (**E**) Correlation between ratio of root K^+:Na^+ and RGR. **F**) Correlation between ratio of shoot K^+:Na^+ and RGR. ^{ns}indicates the correlation is not significant at the 0.05 level (two-tailed).



Supplementary data 3.18. Correlations between ion concentrations and RGR of accessions exposed to long term saline conditions. (**A**) Correlation between root Na^+ and RGR. (**B**) Correlation between shoot K^+ and RGR. ****Correlation is significant at the 0.0001 level (two-tailed).



Supplementary data 3.19. Manhattan plot showing the association signals. GWAS analysis using RGR of the 306 accessions as phenotypic data of plants exposed to long term control conditions. The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.20. Manhattan plots showing the association signals. (**A**) GWAS analysis using RGR of the 306 accessions as phenotypic data. (**B**) GWAS analysis using RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to medium term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale. The horizontal dashed line represents the significance threshold.

Supplementary data 3.21. GWAS summary of the RGR trait. Plants were exposed to medium term saline conditions (50 mM NaCl). Table summarises the SNP positions by trait and chromosome.

Phenotype	Chr	Significant SNP positions			All genes	Candidate genes
		Peak 1	Peak 2	Peak 3		
RGR	5	21132047	-	-	38	21
	7	28699934	-	-	29	20
	11	20857639	-	-	26	10
Summary					93	51

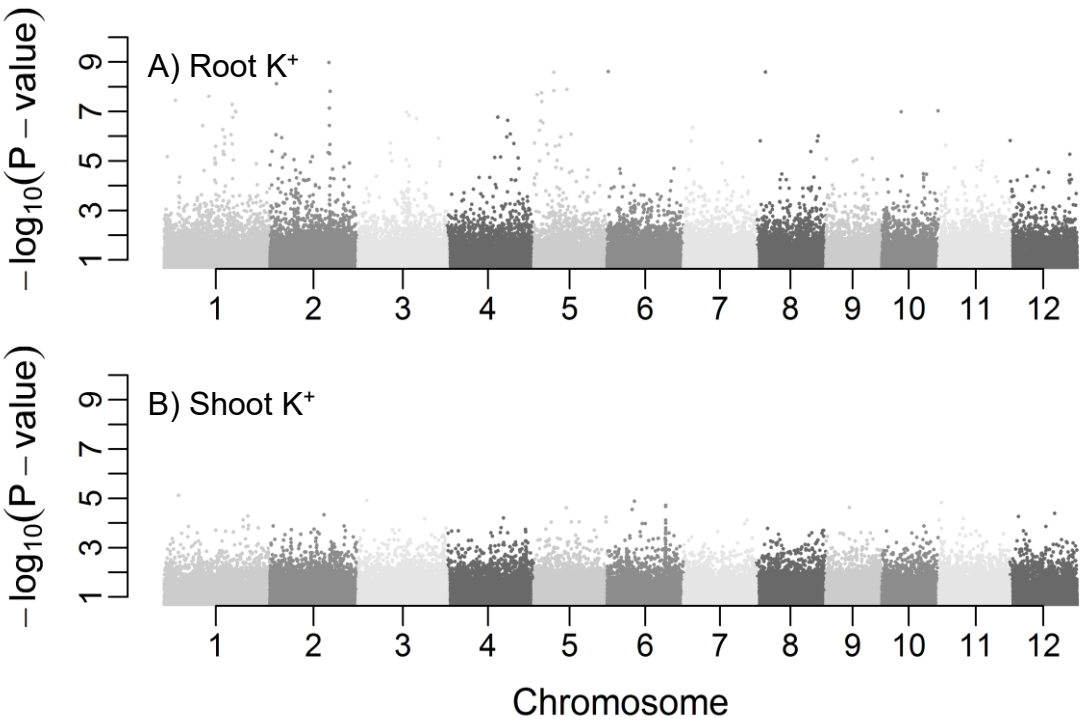
NOTE: relative growth rate (RGR) trait, chromosome (Chr), significant SNP position identified from association signals (Peak). Total number of genes were identified within 250 kb genome browser window (All genes). Selected candidate genes (Candidate genes).

Supplementary data 3.22. Summary of candidate genes identified by GWAS. Significant SNP positions were identified by GWAS using the RGR trait. Traits were obtained from plants exposed to medium term saline conditions (50 mM NaCl). Table summarises the gene locus of candidate genes.

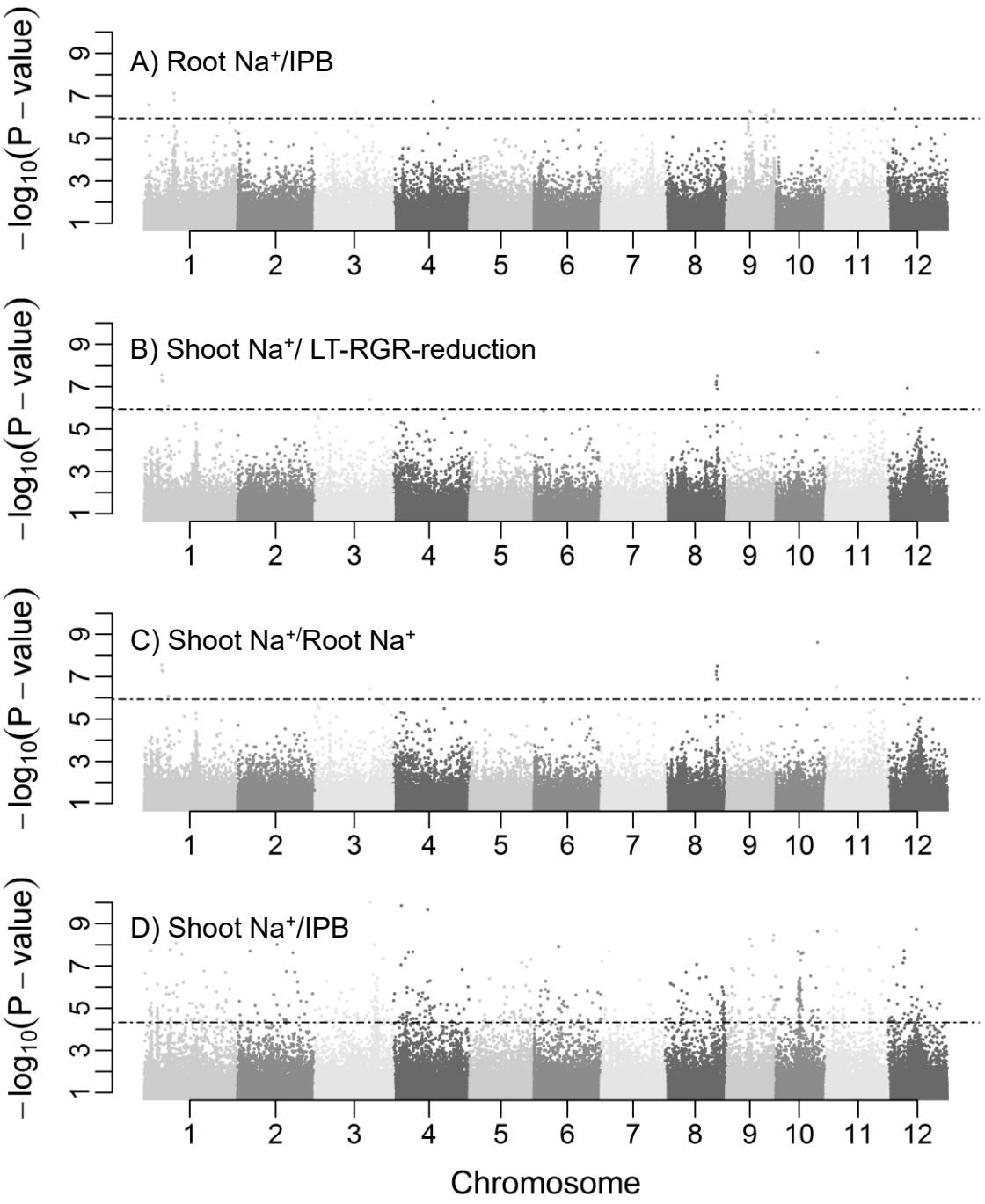
LOC_Os05g35380	LOC_Os05g35400	LOC_Os05g35410	LOC_Os05g35440
LOC_Os05g35460	LOC_Os05g35470	LOC_Os05g35480	LOC_Os05g35500
LOC_Os05g35520	LOC_Os05g35540	LOC_Os05g35570	LOC_Os05g35580
LOC_Os05g35594	LOC_Os05g35650	LOC_Os05g35690	LOC_Os05g35710
LOC_Os05g35720	LOC_Os05g35730	LOC_Os05g35740	LOC_Os05g35760
LOC_Os05g35770	LOC_Os07g47860	LOC_Os07g47960	LOC_Os07g47990
LOC_Os07g48010	LOC_Os07g48020	LOC_Os07g48030	LOC_Os07g48040
LOC_Os07g48050	LOC_Os07g48060	LOC_Os07g48090	LOC_Os07g48100
LOC_Os07g48130	LOC_Os07g48140	LOC_Os07g48150	LOC_Os07g48160
LOC_Os07g48170	LOC_Os07g48180	LOC_Os07g48200	LOC_Os07g48229
LOC_Os07g48244	LOC_Os11g35390	LOC_Os11g35400	LOC_Os11g35430
LOC_Os11g35450	LOC_Os11g35490	LOC_Os11g35500	LOC_Os11g35550
LOC_Os11g35580	LOC_Os11g35660	LOC_Os11g35710	

Supplementary data 3.23. Summary of candidate genes identified by GWAS. Significant SNP positions were identified by GWAS using the RGR and RGR-reduction traits. Traits were obtained from plants exposed to long term saline conditions (50 mM NaCl). Table summarises the gene locus of candidate genes.

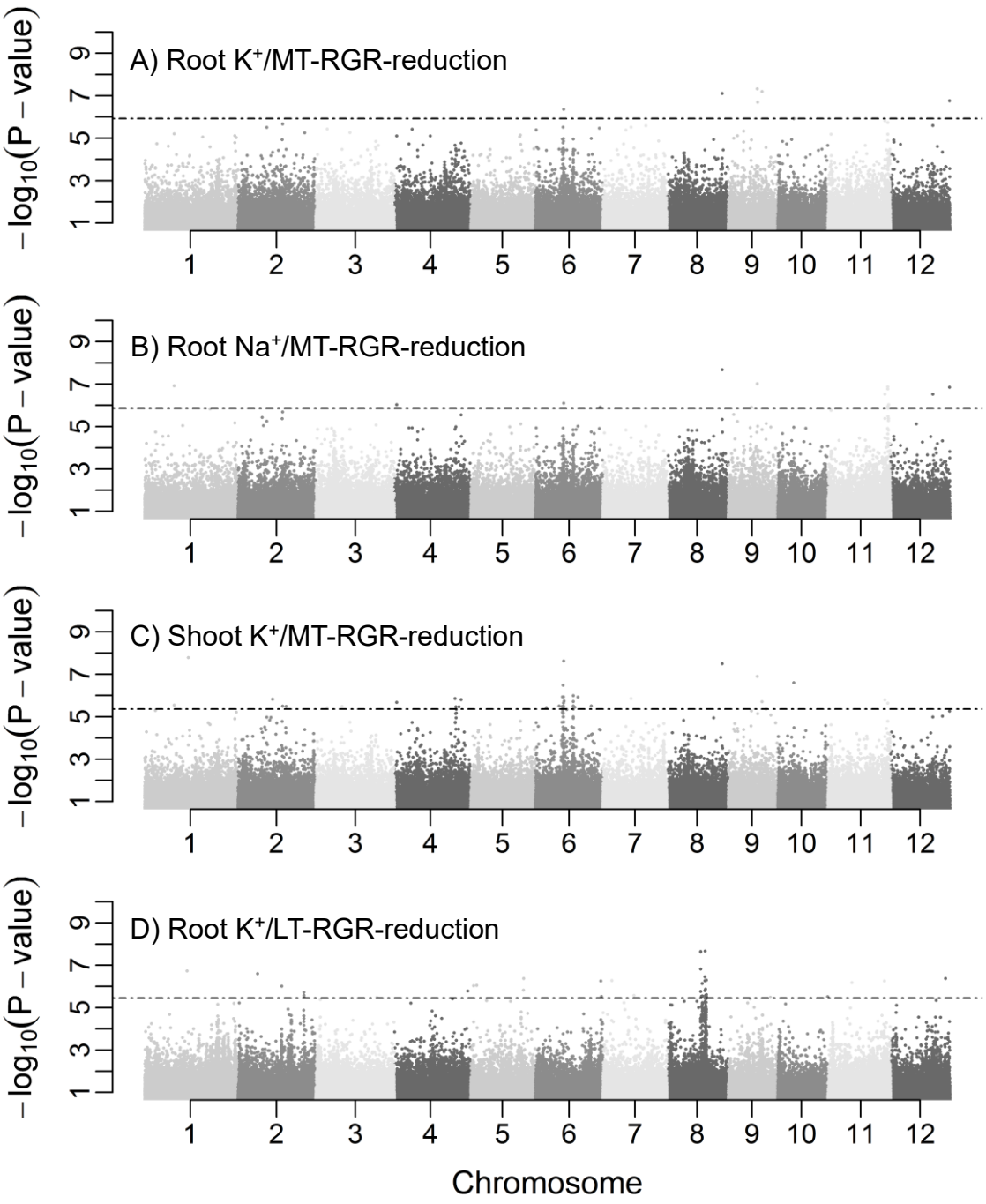
LOC_Os01g49830	LOC_Os01g49890	LOC_Os01g49900	LOC_Os01g49920
LOC_Os01g49940	LOC_Os01g50010	LOC_Os01g50030	LOC_Os01g50040
LOC_Os01g50050	LOC_Os01g50060	LOC_Os01g50070	LOC_Os01g50080
LOC_Os01g50100	LOC_Os01g50110	LOC_Os01g50160	LOC_Os01g50170
LOC_Os01g50200	LOC_Os02g33680	LOC_Os02g33710	LOC_Os02g33730
LOC_Os02g33770	LOC_Os02g33820	LOC_Os02g33850	LOC_Os02g33720
LOC_Os02g33740	LOC_Os02g33780	LOC_Os02g33840	LOC_Os02g33860
LOC_Os07g47710	LOC_Os07g47720	LOC_Os07g47750	LOC_Os07g47760
LOC_Os07g47780	LOC_Os07g47790	LOC_Os07g47820	LOC_Os07g47830
LOC_Os07g47860	LOC_Os07g47960	LOC_Os07g47990	LOC_Os07g48010
LOC_Os07g48020	LOC_Os07g48030	LOC_Os07g48040	LOC_Os07g48050
LOC_Os07g48060	LOC_Os07g48090	LOC_Os07g48100	LOC_Os07g48130
LOC_Os08g28780	LOC_Os08g28800	LOC_Os08g28830	LOC_Os08g28860
LOC_Os08g28880	LOC_Os08g28900	LOC_Os08g28950	LOC_Os08g29020
LOC_Os08g29100	LOC_Os08g28790	LOC_Os08g28820	LOC_Os08g28840
LOC_Os08g28870	LOC_Os08g28890	LOC_Os08g28940	LOC_Os08g28980
LOC_Os08g29040	LOC_Os08g29110	LOC_Os08g29150	LOC_Os08g29170
LOC_Os08g29340	LOC_Os08g29160	LOC_Os08g29200	LOC_Os08g29370
LOC_Os08g29400	LOC_Os08g29520	LOC_Os08g29570	LOC_Os08g29600
LOC_Os08g29660	LOC_Os08g29710	LOC_Os08g29730	LOC_Os08g29500
LOC_Os08g29530	LOC_Os08g29590	LOC_Os08g29650	LOC_Os08g29669
LOC_Os08g29720	LOC_Os12g34290	LOC_Os12g34300	LOC_Os12g34310
LOC_Os12g34320	LOC_Os12g34330	LOC_Os12g34340	LOC_Os12g34370
LOC_Os12g34380	LOC_Os12g34450	LOC_Os12g34460	LOC_Os12g34510
LOC_Os12g34524	LOC_Os12g34550		



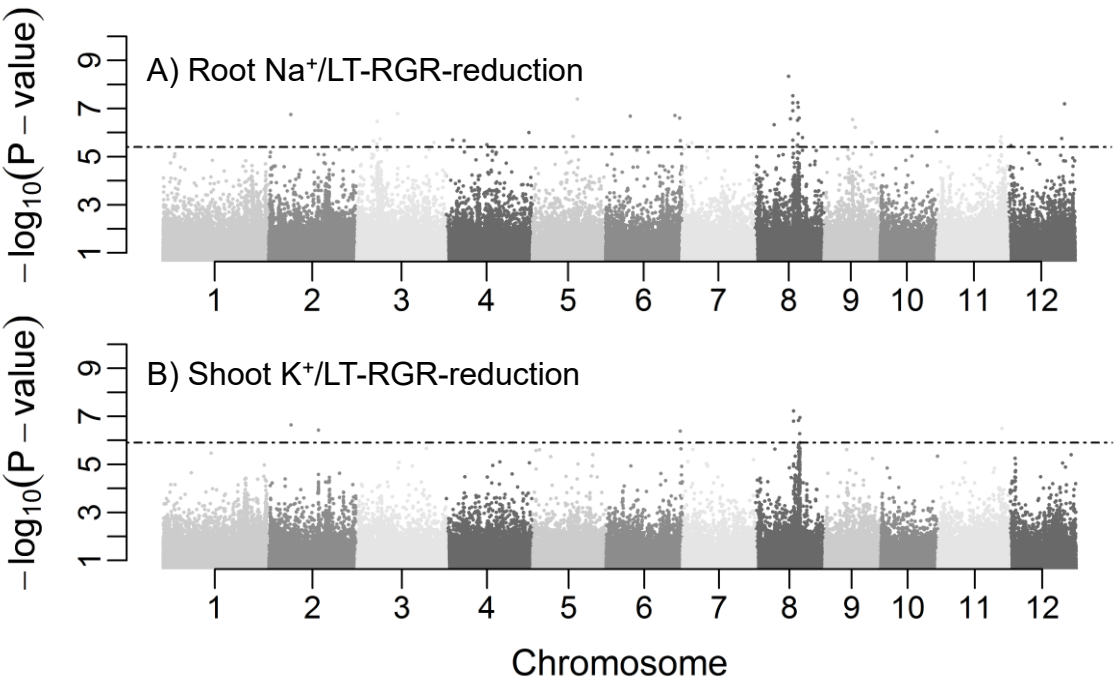
Supplementary data 3.24. Manhattan plots showing the association signals. (**A**) GWAS analysis using root K^+ of the 306 accessions as phenotypic data. (**B**) GWAS analysis using shoot K^+ of the 306 accessions as phenotypic data. Plants were exposed to long term control conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.25. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of the root Na^+ and initial plant biomass of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of the shoot Na^+ and long term RGR-reduction of the 306 accessions as phenotypic data. (**C**) GWAS analysis using ratio of the shoot Na^+ and root Na^+ of the 306 accessions as phenotypic data. (**D**) GWAS analysis using ratio of the shoot Na^+ and initial plant biomass of the 306 accessions as phenotypic data. Plants were exposed to short term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.26. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of the root K^+ and medium term RGR-reduction of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of the root Na^+ and medium term RGR-reduction of the 306 accessions as phenotypic data. (**C**) GWAS analysis using ratio of the shoot K^+ and medium term RGR-reduction of the 306 accessions as phenotypic data. (**D**) GWAS analysis using ratio of the root K^+ and long term RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to short term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.27. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of the root Na^+ and long term RGR-reduction of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of the shoot K^+ and long term RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to short term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.

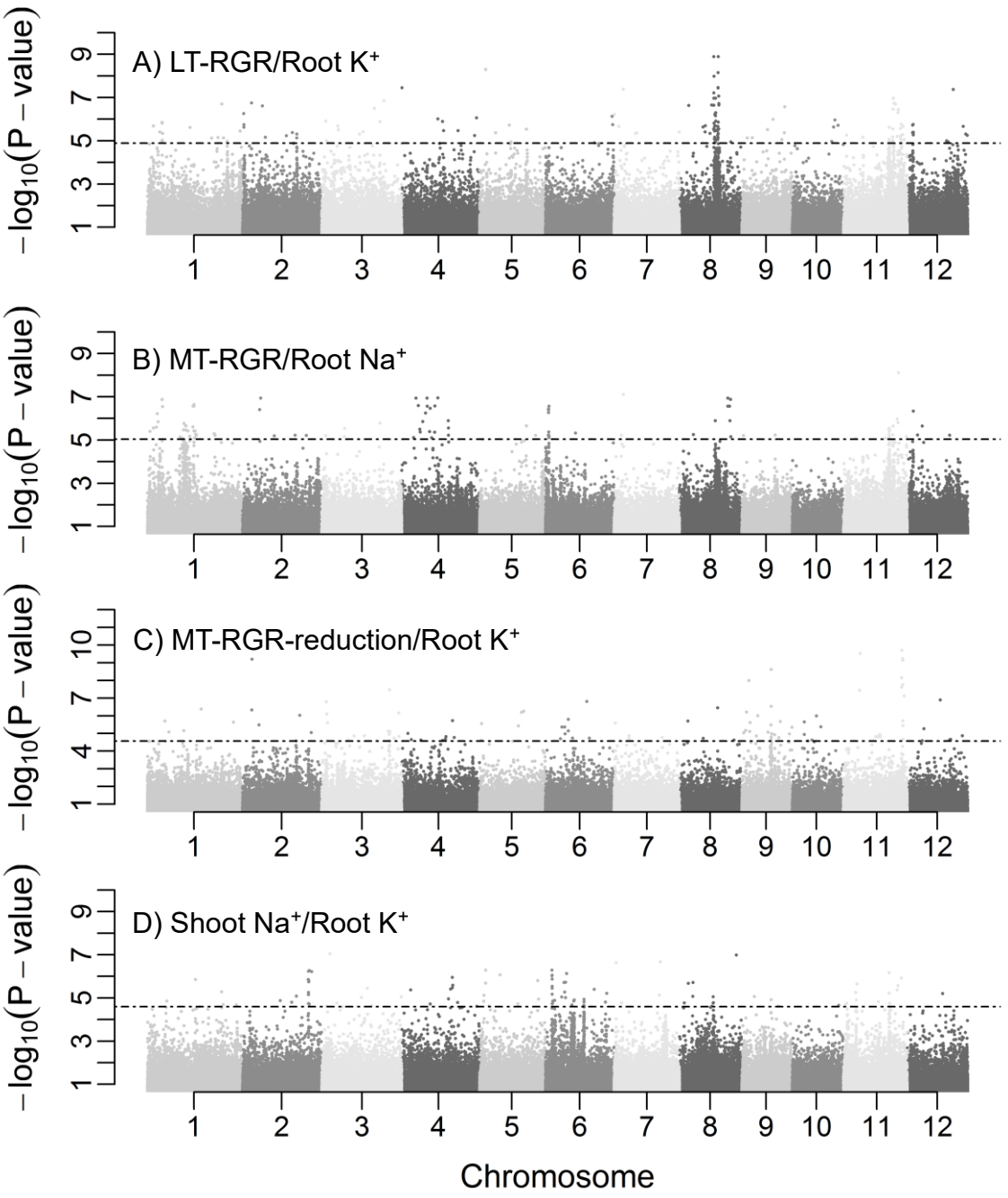
Supplementary data 3.28. Summary of candidate genes identified by GWAS. Significant SNP positions were identified by GWAS using the single and combined traits. Traits were obtained from plants exposed to short term saline conditions (50 mM NaCl). Table summarises the gene locus of candidate genes.

LOC_Os01g06630	LOC_Os03g49500	LOC_Os08g25710	LOC_Os09g38640
LOC_Os01g06640	LOC_Os03g49510	LOC_Os08g25734	LOC_Os09g38670
LOC_Os01g06660	LOC_Os03g49520	LOC_Os08g25820	LOC_Os09g38690
LOC_Os01g06670	LOC_Os03g49524	LOC_Os08g25890	LOC_Os09g38700
LOC_Os01g06720	LOC_Os03g49550	LOC_Os08g25900	LOC_Os09g38710
LOC_Os01g06730	LOC_Os03g49570	LOC_Os08g28700	LOC_Os10g20710
LOC_Os01g06740	LOC_Os03g49580	LOC_Os08g28710	LOC_Os10g20830
LOC_Os01g06750	LOC_Os03g49600	LOC_Os08g28730	LOC_Os10g20840
LOC_Os01g06760	LOC_Os03g49610	LOC_Os08g28780	LOC_Os10g20860
LOC_Os01g06790	LOC_Os03g49620	LOC_Os08g28790	LOC_Os10g20880
LOC_Os01g06820	LOC_Os03g49640	LOC_Os08g28800	LOC_Os10g20890
LOC_Os01g06836	LOC_Os03g49700	LOC_Os08g28820	LOC_Os10g20910
LOC_Os01g06870	LOC_Os03g49710	LOC_Os08g28830	LOC_Os10g20990
LOC_Os01g06876	LOC_Os03g49720	LOC_Os08g28840	LOC_Os10g21000
LOC_Os01g06890	LOC_Os03g49730	LOC_Os08g28860	LOC_Os10g21050
LOC_Os01g06900	LOC_Os03g49750	LOC_Os08g28870	LOC_Os10g21060
LOC_Os01g06920	LOC_Os03g49770	LOC_Os08g28880	LOC_Os10g21090
LOC_Os01g06940	LOC_Os03g49790	LOC_Os08g28890	LOC_Os10g21100
LOC_Os01g06960	LOC_Os03g49800	LOC_Os08g28900	LOC_Os10g21234
LOC_Os01g06980	LOC_Os03g50620	LOC_Os08g28940	LOC_Os10g21238
LOC_Os01g06990	LOC_Os03g50730	LOC_Os08g28950	LOC_Os10g21244
LOC_Os01g07000	LOC_Os03g50760	LOC_Os08g28980	LOC_Os10g21250
LOC_Os01g07010	LOC_Os03g50780	LOC_Os08g29020	LOC_Os10g21254
LOC_Os01g07020	LOC_Os03g50800	LOC_Os08g29040	LOC_Os10g21258
LOC_Os01g07030	LOC_Os03g50810	LOC_Os08g29500	LOC_Os10g21264
LOC_Os01g07040	LOC_Os03g50820	LOC_Os08g29520	LOC_Os10g21268
LOC_Os01g07050	LOC_Os03g50850	LOC_Os08g29530	LOC_Os10g21290
LOC_Os01g07060	LOC_Os03g50860	LOC_Os08g29570	LOC_Os10g21298
LOC_Os01g07080	LOC_Os03g50880	LOC_Os08g29590	LOC_Os10g21300
LOC_Os01g07090	LOC_Os03g50885	LOC_Os08g29600	LOC_Os10g21308
LOC_Os01g07110	LOC_Os03g50920	LOC_Os08g29650	LOC_Os10g21310
LOC_Os01g07120	LOC_Os03g50940	LOC_Os08g29660	LOC_Os10g21312
LOC_Os01g07140	LOC_Os03g50960	LOC_Os08g29669	LOC_Os10g21314
LOC_Os01g11120	LOC_Os03g50980	LOC_Os08g29710	LOC_Os10g21322
LOC_Os01g11130	LOC_Os03g51000	LOC_Os08g29720	LOC_Os10g21324

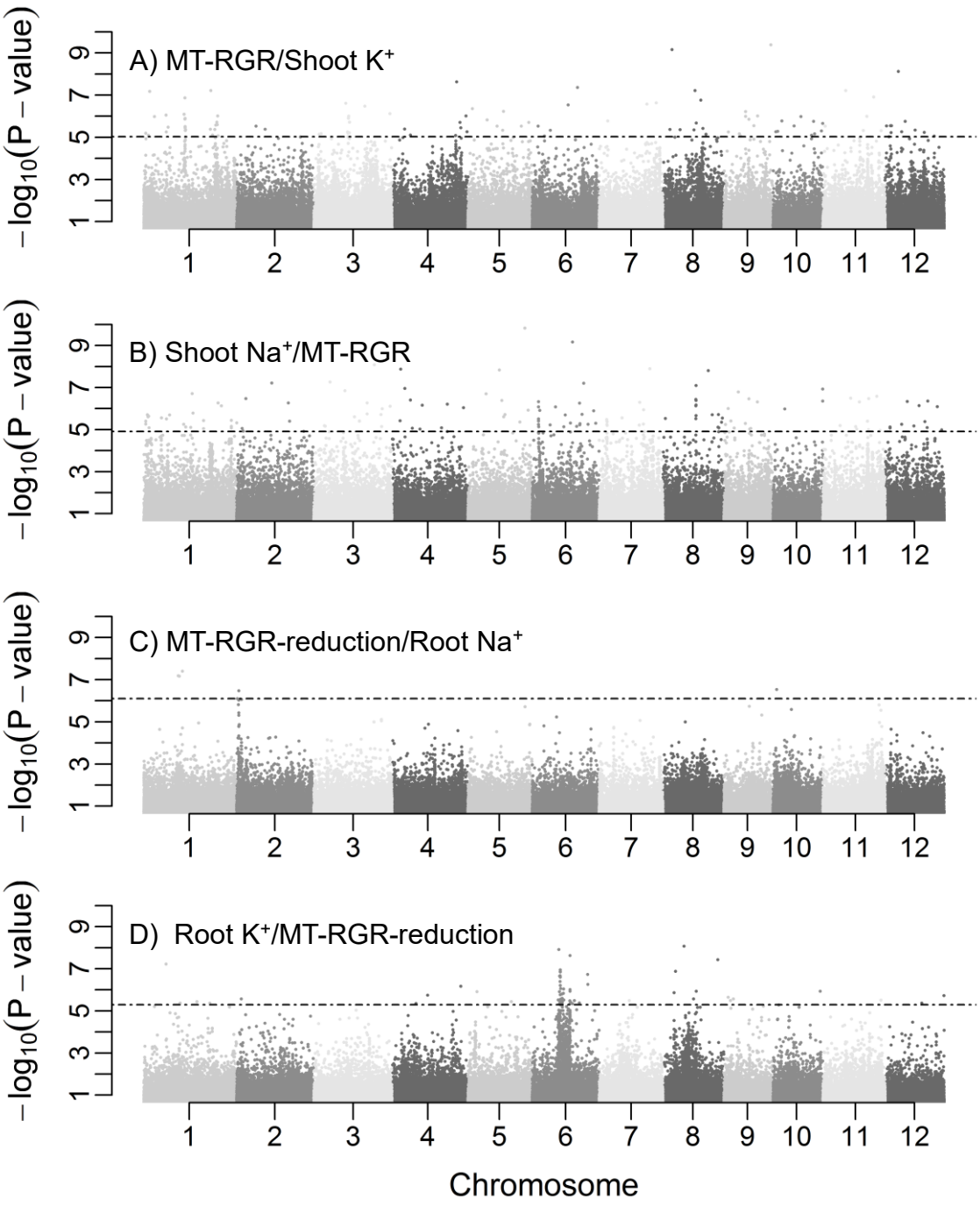
LOC_Os01g11150	LOC_Os03g51520	LOC_Os08g29730	LOC_Os10g21326
LOC_Os01g11160	LOC_Os03g51550	LOC_Os08g29760	LOC_Os10g21328
LOC_Os01g11200	LOC_Os03g51580	LOC_Os08g29770	LOC_Os10g21332
LOC_Os01g11220	LOC_Os03g51600	LOC_Os08g29809	LOC_Os10g21342
LOC_Os01g11230	LOC_Os03g51610	LOC_Os08g38020	LOC_Os10g21344
LOC_Os01g11240	LOC_Os03g51620	LOC_Os08g38060	LOC_Os10g21346
LOC_Os01g11250	LOC_Os03g51650	LOC_Os08g38080	LOC_Os10g21352
LOC_Os01g11260	LOC_Os03g51670	LOC_Os08g38086	LOC_Os10g21356
LOC_Os01g11270	LOC_Os03g51680	LOC_Os08g38092	LOC_Os10g21358
LOC_Os01g11280	LOC_Os03g51690	LOC_Os08g38100	LOC_Os10g21360
LOC_Os01g11300	LOC_Os03g51710	LOC_Os08g38110	LOC_Os10g21366
LOC_Os01g11340	LOC_Os03g51740	LOC_Os08g38130	LOC_Os10g21372
LOC_Os01g11350	LOC_Os03g51760	LOC_Os08g38160	LOC_Os10g21396
LOC_Os01g11370	LOC_Os03g51790	LOC_Os08g38170	LOC_Os10g21400
LOC_Os01g11414	LOC_Os03g51830	LOC_Os08g38200	LOC_Os10g21404
LOC_Os01g11460	LOC_Os04g47150	LOC_Os08g38210	LOC_Os10g21418
LOC_Os01g11480	LOC_Os04g47160	LOC_Os08g38220	LOC_Os10g21540
LOC_Os01g11490	LOC_Os04g47170	LOC_Os08g38250	LOC_Os10g21590
LOC_Os01g24440	LOC_Os04g47190	LOC_Os08g38270	LOC_Os10g21230
LOC_Os01g24460	LOC_Os04g47220	LOC_Os08g38280	LOC_Os10g21236
LOC_Os01g24470	LOC_Os04g47240	LOC_Os08g38300	LOC_Os10g21240
LOC_Os01g24480	LOC_Os04g47250	LOC_Os08g38320	LOC_Os10g21248
LOC_Os01g24550	LOC_Os04g47270	LOC_Os08g38330	LOC_Os10g21266
LOC_Os01g24560	LOC_Os04g47280	LOC_Os08g38340	LOC_Os10g21394
LOC_Os01g24570	LOC_Os04g47290	LOC_Os08g38360	LOC_Os10g21398
LOC_Os01g24590	LOC_Os04g47300	LOC_Os08g38370	LOC_Os10g21402
LOC_Os01g24600	LOC_Os04g47320	LOC_Os08g38380	LOC_Os10g21406
LOC_Os01g24680	LOC_Os04g47330	LOC_Os08g38400	LOC_Os10g21470
LOC_Os01g24690	LOC_Os04g47360	LOC_Os08g38410	LOC_Os10g21560
LOC_Os01g24710	LOC_Os04g47370	LOC_Os08g38440	LOC_Os11g34820
LOC_Os01g24740	LOC_Os04g47380	LOC_Os08g42850	LOC_Os11g34830
LOC_Os01g24750	LOC_Os04g47390	LOC_Os08g42890	LOC_Os11g34840
LOC_Os02g33680	LOC_Os04g47400	LOC_Os08g42910	LOC_Os11g34850
LOC_Os02g33710	LOC_Os04g47410	LOC_Os08g42920	LOC_Os11g34860
LOC_Os02g33720	LOC_Os04g47420	LOC_Os08g42930	LOC_Os11g34880
LOC_Os02g33730	LOC_Os04g47480	LOC_Os08g42950	LOC_Os11g34920
LOC_Os02g33740	LOC_Os04g47520	LOC_Os08g42960	LOC_Os11g34940
LOC_Os02g33770	LOC_Os04g47530	LOC_Os08g42980	LOC_Os11g34970
LOC_Os02g33780	LOC_Os04g47570	LOC_Os08g43000	LOC_Os11g34990
LOC_Os02g33820	LOC_Os04g47580	LOC_Os08g43010	LOC_Os11g35020

LOC_Os02g33840	LOC_Os04g47590	LOC_Os08g43020	LOC_Os11g35030
LOC_Os02g33850	LOC_Os04g59200	LOC_Os08g43040	LOC_Os11g35040
LOC_Os02g33860	LOC_Os04g59210	LOC_Os08g43050	LOC_Os11g35060
LOC_Os02g49530	LOC_Os04g59260	LOC_Os08g43070	LOC_Os11g35090
LOC_Os02g49540	LOC_Os04g59300	LOC_Os08g43080	LOC_Os11g35120
LOC_Os02g49550	LOC_Os04g59310	LOC_Os08g43090	LOC_Os11g43200
LOC_Os02g49560	LOC_Os04g59320	LOC_Os08g43120	LOC_Os11g43250
LOC_Os02g49570	LOC_Os04g59340	LOC_Os08g43130	LOC_Os11g43320
LOC_Os02g49590	LOC_Os04g59360	LOC_Os08g43160	LOC_Os11g43360
LOC_Os02g49600	LOC_Os04g59370	LOC_Os08g43170	LOC_Os11g43410
LOC_Os02g49610	LOC_Os04g59380	LOC_Os08g43190	LOC_Os11g43420
LOC_Os02g49630	LOC_Os04g59430	LOC_Os08g43200	LOC_Os11g43480
LOC_Os02g49650	LOC_Os04g59440	LOC_Os08g43210	LOC_Os11g43500
LOC_Os02g49670	LOC_Os04g59450	LOC_Os08g43220	LOC_Os11g43510
LOC_Os02g49680	LOC_Os04g59470	LOC_Os08g43230	LOC_Os11g43520
LOC_Os02g49690	LOC_Os04g59480	LOC_Os08g43240	LOC_Os11g43550
LOC_Os02g49700	LOC_Os04g59494	LOC_Os09g19229	LOC_Os11g43580
LOC_Os02g49710	LOC_Os04g59510	LOC_Os09g19330	LOC_Os11g43590
LOC_Os02g49720	LOC_Os04g59520	LOC_Os09g19360	LOC_Os11g43600
LOC_Os02g49730	LOC_Os04g59540	LOC_Os09g19380	LOC_Os11g43610
LOC_Os02g49760	LOC_Os04g59550	LOC_Os09g19390	LOC_Os11g43620
LOC_Os02g49770	LOC_Os04g59560	LOC_Os09g19400	LOC_Os11g43650
LOC_Os02g49790	LOC_Os04g59580	LOC_Os09g19500	LOC_Os11g43700
LOC_Os02g49800	LOC_Os04g59590	LOC_Os09g19510	LOC_Os11g43740
LOC_Os02g49820	LOC_Os04g59600	LOC_Os09g19550	LOC_Os11g43750
LOC_Os02g49830	LOC_Os04g59620	LOC_Os09g19560	LOC_Os11g43760
LOC_Os02g49840	LOC_Os04g59624	LOC_Os09g19570	LOC_Os11g43770
LOC_Os02g49850	LOC_Os06g22290	LOC_Os09g19640	LOC_Os11g43790
LOC_Os02g49860	LOC_Os06g22340	LOC_Os09g19650	LOC_Os11g43800
LOC_Os02g49880	LOC_Os06g22420	LOC_Os09g38210	LOC_Os11g43820
LOC_Os02g49920	LOC_Os06g22440	LOC_Os09g38239	LOC_Os11g43830
LOC_Os02g49950	LOC_Os06g22460	LOC_Os09g38268	LOC_Os11g43860
LOC_Os02g49970	LOC_Os06g22490	LOC_Os09g38300	LOC_Os11g43890
LOC_Os03g15180	LOC_Os06g22550	LOC_Os09g38310	LOC_Os11g43900
LOC_Os03g15210	LOC_Os06g22560	LOC_Os09g38320	LOC_Os11g43934
LOC_Os03g15220	LOC_Os06g22600	LOC_Os09g38330	LOC_Os11g45540
LOC_Os03g15230	LOC_Os06g22610	LOC_Os09g38340	LOC_Os11g45580
LOC_Os03g15250	LOC_Os06g22650	LOC_Os09g38350	LOC_Os11g45710
LOC_Os03g15270	LOC_Os06g30179	LOC_Os09g38370	LOC_Os11g45730
LOC_Os03g15290	LOC_Os06g30310	LOC_Os09g38380	LOC_Os11g45750

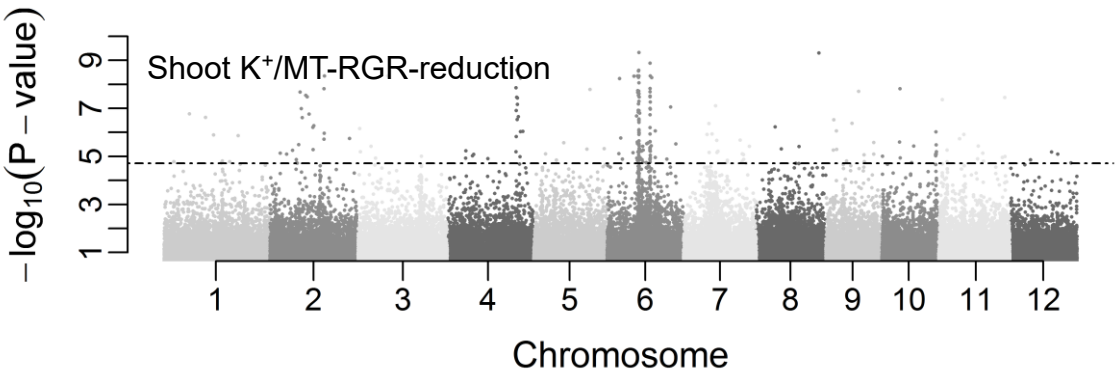
LOC_Os03g15320	LOC_Os06g30320	LOC_Os09g38390	LOC_Os11g45790
LOC_Os03g15340	LOC_Os06g30330	LOC_Os09g38400	LOC_Os11g45920
LOC_Os03g15350	LOC_Os06g30370	LOC_Os09g38410	LOC_Os11g45930
LOC_Os03g15360	LOC_Os06g30380	LOC_Os09g38420	LOC_Os11g45970
LOC_Os03g15370	LOC_Os06g30390	LOC_Os09g38440	LOC_Os11g45560
LOC_Os03g15390	LOC_Os06g30420	LOC_Os09g38500	LOC_Os11g45600
LOC_Os03g15420	LOC_Os06g30430	LOC_Os09g38510	LOC_Os11g45620
LOC_Os03g15430	LOC_Os06g30440	LOC_Os09g38520	LOC_Os11g45720
LOC_Os03g15440	LOC_Os06g30460	LOC_Os09g38530	LOC_Os11g45740
LOC_Os03g15480	LOC_Os06g30500	LOC_Os09g38540	LOC_Os11g45780
LOC_Os03g49400	LOC_Os08g25490	LOC_Os09g38550	LOC_Os11g45850
LOC_Os03g49420	LOC_Os08g25570	LOC_Os09g38560	LOC_Os11g45924
LOC_Os03g49430	LOC_Os08g25624	LOC_Os09g38570	LOC_Os11g45950
LOC_Os03g49440	LOC_Os08g25720	LOC_Os09g38580	LOC_Os11g45980
LOC_Os03g49464	LOC_Os08g25799	LOC_Os09g38610	
LOC_Os03g49480	LOC_Os08g25590	LOC_Os09g38620	



Supplementary data 3.29. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of the long term RGR and root K^+ of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of the medium term RGR and root Na^+ of the 306 accessions as phenotypic data. (**C**) GWAS analysis using ratio of the medium term RGR-reduction and root K^+ of the 306 accessions as phenotypic data. (**D**) GWAS analysis using ratio of the shoot Na^+ and root K^+ of the 306 accessions as phenotypic data. Plants were exposed to medium term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.30. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of the medium term RGR and shoot K^+ of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of the shoot Na^+ and medium term RGR of the 306 accessions as phenotypic data. (**C**) GWAS analysis using ratio of the medium term RGR-reduction and root Na^+ of the 306 accessions as phenotypic data. (**D**) GWAS analysis using ratio of the root K^+ and medium term RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to medium term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.31. Manhattan plots showing the association signals. GWAS analysis using ratio of the shoot K^+ and medium term RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to medium term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.

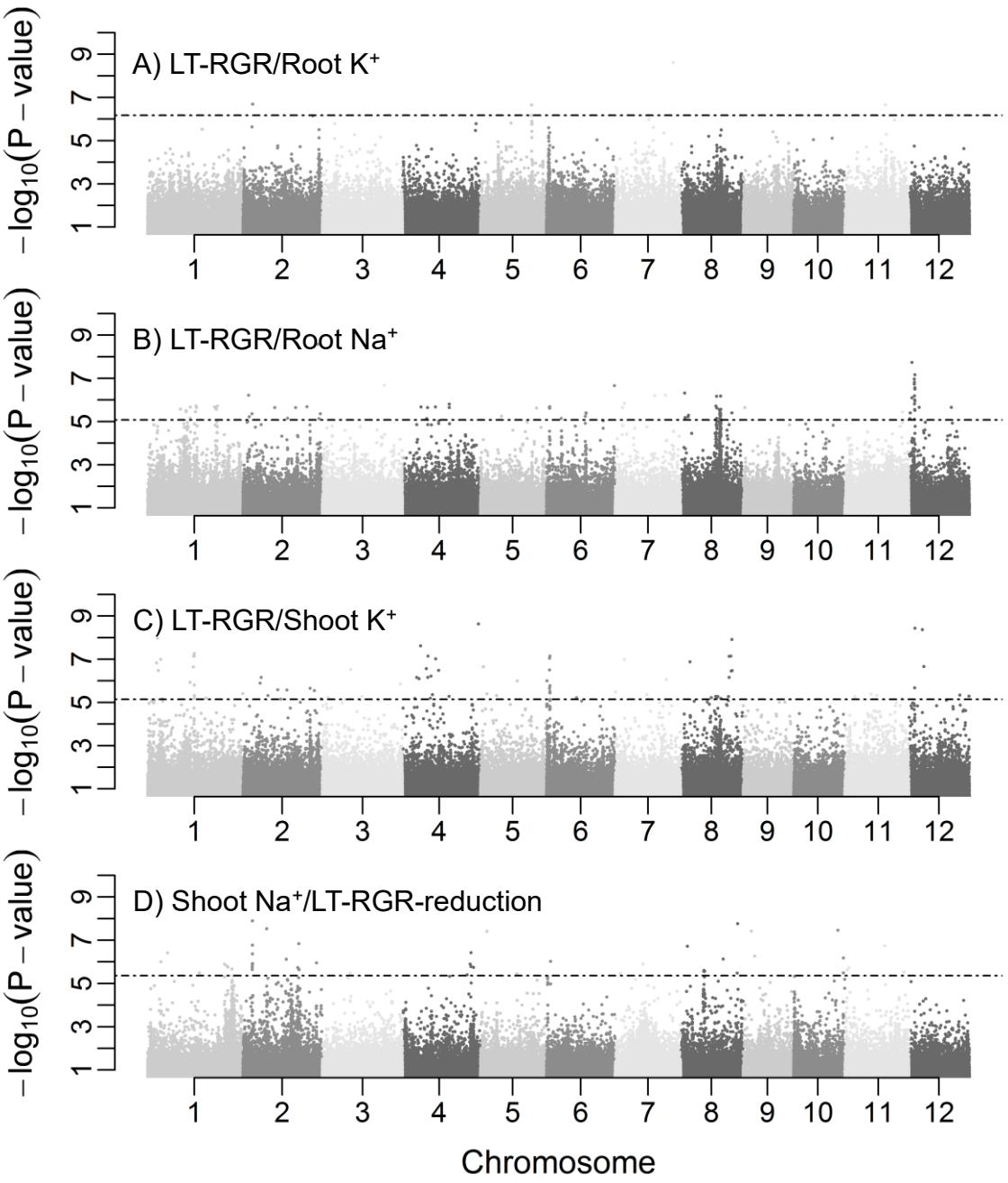
Supplementary data 3.32. Summary of candidate genes identified by GWAS. Significant SNP positions were identified by GWAS using the single and combined traits. Traits were obtained from plants exposed to medium term saline conditions (50 mM NaCl). Table summarises the gene locus of candidate genes.

LOC_Os01g34390	LOC_Os02g48990	LOC_Os06g06300	LOC_Os08g28890
LOC_Os01g34470	LOC_Os02g49030	LOC_Os06g06340	LOC_Os08g28900
LOC_Os01g34480	LOC_Os02g49040	LOC_Os06g06360	LOC_Os08g28940
LOC_Os01g34560	LOC_Os02g49050	LOC_Os06g06390	LOC_Os08g28950
LOC_Os01g34614	LOC_Os02g49060	LOC_Os06g06410	LOC_Os08g28980
LOC_Os01g34620	LOC_Os02g49070	LOC_Os06g06440	LOC_Os08g29020
LOC_Os01g34660	LOC_Os02g49080	LOC_Os06g06460	LOC_Os08g29040
LOC_Os01g34700	LOC_Os02g49090	LOC_Os06g06480	LOC_Os09g21796
LOC_Os01g34770	LOC_Os03g45619	LOC_Os06g06490	LOC_Os09g22000
LOC_Os01g34780	LOC_Os03g45710	LOC_Os06g06510	LOC_Os09g22090
LOC_Os01g62370	LOC_Os03g45720	LOC_Os06g06520	LOC_Os10g40859
LOC_Os01g62380	LOC_Os03g45730	LOC_Os06g06530	LOC_Os10g40880
LOC_Os01g62390	LOC_Os03g45740	LOC_Os06g06540	LOC_Os10g40900
LOC_Os01g62410	LOC_Os03g45750	LOC_Os06g06550	LOC_Os10g40920
LOC_Os01g62420	LOC_Os03g45800	LOC_Os06g06560	LOC_Os10g40934
LOC_Os01g62430	LOC_Os03g45830	LOC_Os06g22010	LOC_Os10g40950
LOC_Os01g62440	LOC_Os03g45850	LOC_Os06g22020	LOC_Os10g40960
LOC_Os01g62460	LOC_Os03g45860	LOC_Os06g22030	LOC_Os10g40990
LOC_Os01g62480	LOC_Os03g45910	LOC_Os06g22050	LOC_Os10g41020
LOC_Os01g62490	LOC_Os03g45920	LOC_Os06g22060	LOC_Os10g41030

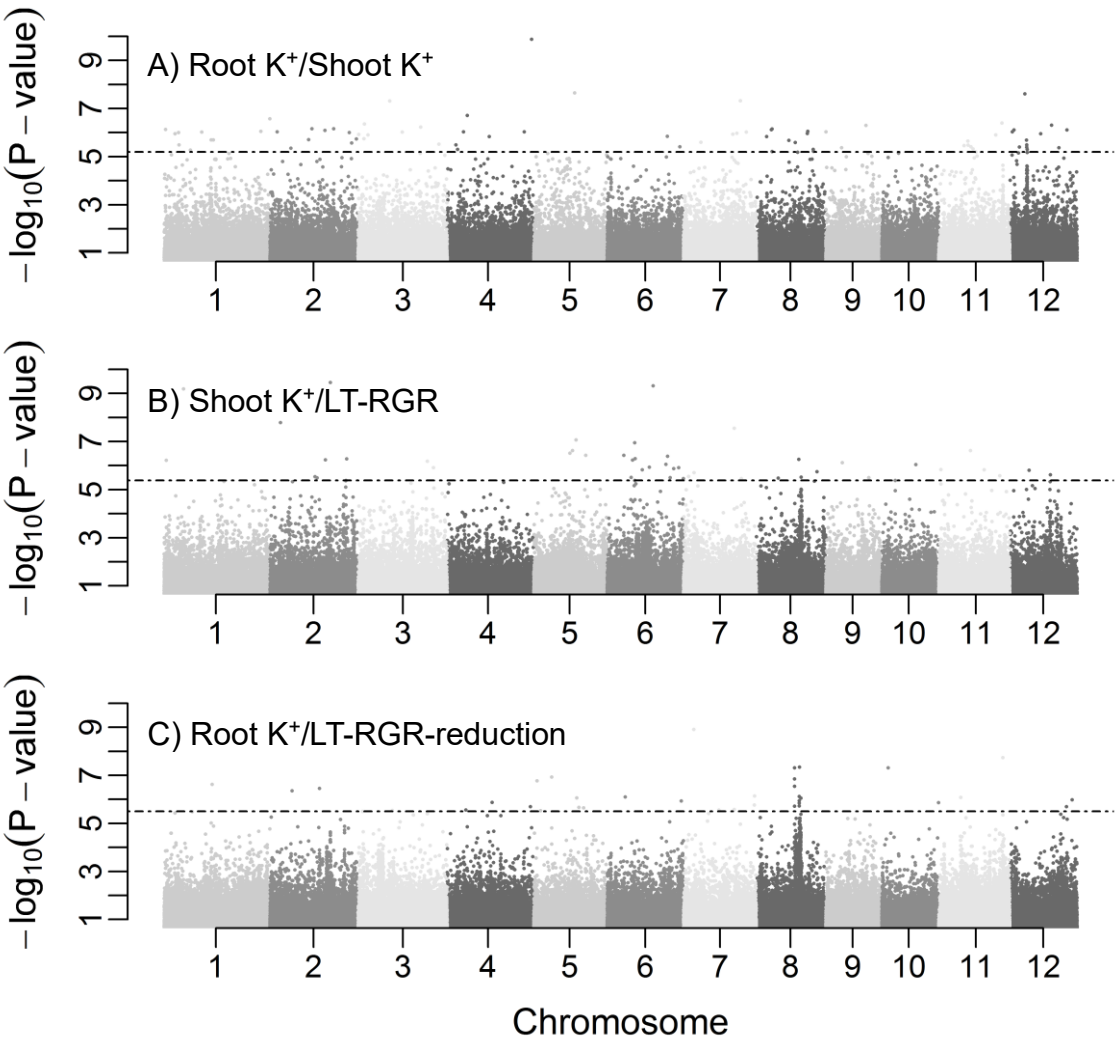
LOC_Os01g62500	LOC_Os03g45960	LOC_Os06g22070	LOC_Os10g41040
LOC_Os01g62510	LOC_Os03g53600	LOC_Os06g22080	LOC_Os10g41050
LOC_Os01g62514	LOC_Os03g53610	LOC_Os06g22100	LOC_Os10g41070
LOC_Os01g62570	LOC_Os03g53620	LOC_Os06g22140	LOC_Os10g41083
LOC_Os01g62584	LOC_Os03g53630	LOC_Os06g22290	LOC_Os10g41100
LOC_Os01g62600	LOC_Os03g53650	LOC_Os06g22340	LOC_Os10g41110
LOC_Os01g62610	LOC_Os03g53660	LOC_Os06g22420	LOC_Os10g41130
LOC_Os01g62620	LOC_Os03g53670	LOC_Os06g22440	LOC_Os10g41150
LOC_Os01g62630	LOC_Os03g53690	LOC_Os06g22460	LOC_Os10g41170
LOC_Os01g62640	LOC_Os03g53700	LOC_Os06g22490	LOC_Os10g41190
LOC_Os01g62650	LOC_Os03g53710	LOC_Os06g22550	LOC_Os10g41200
LOC_Os01g62660	LOC_Os03g53720	LOC_Os06g22560	LOC_Os10g41220
LOC_Os01g62670	LOC_Os03g53730	LOC_Os06g22600	LOC_Os10g41230
LOC_Os01g62720	LOC_Os03g53750	LOC_Os06g22610	LOC_Os10g41240
LOC_Os02g01710	LOC_Os03g53760	LOC_Os06g22650	LOC_Os10g41250
LOC_Os02g01720	LOC_Os03g53770	LOC_Os06g30090	LOC_Os10g41260
LOC_Os02g01730	LOC_Os03g53780	LOC_Os06g30130	LOC_Os10g41270
LOC_Os02g01740	LOC_Os03g53790	LOC_Os06g30310	LOC_Os10g41290
LOC_Os02g01760	LOC_Os03g53800	LOC_Os06g30330	LOC_Os10g41310
LOC_Os02g01800	LOC_Os03g53860	LOC_Os06g30380	LOC_Os10g42670
LOC_Os02g01820	LOC_Os03g53880	LOC_Os06g30179	LOC_Os10g42690
LOC_Os02g01880	LOC_Os03g53890	LOC_Os06g30320	LOC_Os10g42700
LOC_Os02g01890	LOC_Os03g53900	LOC_Os06g30370	LOC_Os10g42710
LOC_Os02g01920	LOC_Os03g53910	LOC_Os06g30390	LOC_Os10g42720
LOC_Os02g01940	LOC_Os03g53920	LOC_Os06g30430	LOC_Os10g42724
LOC_Os02g01960	LOC_Os03g53950	LOC_Os06g30460	LOC_Os10g42750
LOC_Os02g01980	LOC_Os03g53960	LOC_Os06g30420	LOC_Os10g42760
LOC_Os02g01990	LOC_Os03g54000	LOC_Os06g30440	LOC_Os10g42770
LOC_Os02g02000	LOC_Os04g47890	LOC_Os06g30500	LOC_Os10g42780
LOC_Os02g02020	LOC_Os04g47900	LOC_Os06g30640	LOC_Os10g42790
LOC_Os02g02040	LOC_Os04g47906	LOC_Os06g30680	LOC_Os10g42800
LOC_Os02g02050	LOC_Os04g47930	LOC_Os07g06940	LOC_Os10g42820
LOC_Os02g02090	LOC_Os04g47970	LOC_Os07g06950	LOC_Os10g42830
LOC_Os02g02110	LOC_Os04g47990	LOC_Os07g06960	LOC_Os10g42840
LOC_Os02g02120	LOC_Os04g48010	LOC_Os07g06970	LOC_Os10g42850
LOC_Os02g02130	LOC_Os04g48020	LOC_Os07g06980	LOC_Os10g42860
LOC_Os02g02140	LOC_Os04g48030	LOC_Os07g07000	LOC_Os10g42870
LOC_Os02g02470	LOC_Os04g48050	LOC_Os07g07010	LOC_Os10g42900
LOC_Os02g02480	LOC_Os04g48060	LOC_Os07g07020	LOC_Os10g42940
LOC_Os02g02490	LOC_Os04g48070	LOC_Os07g07040	LOC_Os10g42950

LOC_Os02g02500	LOC_Os04g48130	LOC_Os07g07050	LOC_Os10g42960
LOC_Os02g02510	LOC_Os04g48140	LOC_Os07g07060	LOC_Os10g43040
LOC_Os02g02524	LOC_Os04g48160	LOC_Os07g07070	LOC_Os10g43050
LOC_Os02g02530	LOC_Os04g48170	LOC_Os07g07080	LOC_Os11g35210
LOC_Os02g02540	LOC_Os05g35380	LOC_Os07g07170	LOC_Os11g35220
LOC_Os02g02550	LOC_Os05g35400	LOC_Os07g07194	LOC_Os11g35260
LOC_Os02g02560	LOC_Os05g35410	LOC_Os07g07220	LOC_Os11g35274
LOC_Os02g02570	LOC_Os05g35440	LOC_Os07g07230	LOC_Os11g35290
LOC_Os02g02590	LOC_Os05g35460	LOC_Os07g07240	LOC_Os11g35310
LOC_Os02g02600	LOC_Os05g35470	LOC_Os07g07250	LOC_Os11g35330
LOC_Os02g02620	LOC_Os05g35480	LOC_Os07g07260	LOC_Os11g35360
LOC_Os02g02630	LOC_Os05g35500	LOC_Os07g07270	LOC_Os11g35390
LOC_Os02g02640	LOC_Os05g35520	LOC_Os07g47860	LOC_Os11g35320
LOC_Os02g02650	LOC_Os05g35540	LOC_Os07g47960	LOC_Os11g35340
LOC_Os02g02670	LOC_Os05g35570	LOC_Os07g47990	LOC_Os11g35400
LOC_Os02g02700	LOC_Os05g35580	LOC_Os07g48010	LOC_Os11g35450
LOC_Os02g02710	LOC_Os05g35594	LOC_Os07g48020	LOC_Os11g35500
LOC_Os02g02720	LOC_Os05g35650	LOC_Os07g48030	LOC_Os11g35550
LOC_Os02g02740	LOC_Os05g35690	LOC_Os07g48040	LOC_Os11g35430
LOC_Os02g02750	LOC_Os05g35710	LOC_Os07g48050	LOC_Os11g35490
LOC_Os02g02770	LOC_Os05g35720	LOC_Os07g48060	LOC_Os11g35580
LOC_Os02g02780	LOC_Os05g35730	LOC_Os07g48090	LOC_Os11g35660
LOC_Os02g02790	LOC_Os05g35740	LOC_Os07g48100	LOC_Os11g35710
LOC_Os02g02800	LOC_Os05g35760	LOC_Os07g48130	LOC_Os11g38100
LOC_Os02g02820	LOC_Os05g35770	LOC_Os07g48140	LOC_Os11g38120
LOC_Os02g02830	LOC_Os06g03690	LOC_Os07g48150	LOC_Os11g38130
LOC_Os02g02840	LOC_Os06g03700	LOC_Os07g48160	LOC_Os11g38140
LOC_Os02g02850	LOC_Os06g03710	LOC_Os07g48170	LOC_Os11g38160
LOC_Os02g02860	LOC_Os06g03720	LOC_Os07g48180	LOC_Os11g38170
LOC_Os02g02870	LOC_Os06g03740	LOC_Os07g48200	LOC_Os11g38180
LOC_Os02g02890	LOC_Os06g03750	LOC_Os07g48229	LOC_Os11g38200
LOC_Os02g40430	LOC_Os06g03760	LOC_Os07g48244	LOC_Os11g38210
LOC_Os02g40440	LOC_Os06g03770	LOC_Os08g25130	LOC_Os11g38220
LOC_Os02g40450	LOC_Os06g03780	LOC_Os08g25140	LOC_Os11g38230
LOC_Os02g40454	LOC_Os06g03790	LOC_Os08g25200	LOC_Os11g38240
LOC_Os02g40460	LOC_Os06g03800	LOC_Os08g25280	LOC_Os11g38260
LOC_Os02g40500	LOC_Os06g03830	LOC_Os08g25380	LOC_Os11g38330
LOC_Os02g40510	LOC_Os06g03840	LOC_Os08g25430	LOC_Os11g38480
LOC_Os02g40514	LOC_Os06g03850	LOC_Os08g25240	LOC_Os11g38500
LOC_Os02g40530	LOC_Os06g03860	LOC_Os08g25310	LOC_Os11g44430

LOC_Os02g40550	LOC_Os06g03890	LOC_Os08g25390	LOC_Os11g44500
LOC_Os02g40664	LOC_Os06g03900	LOC_Os08g25460	LOC_Os11g44550
LOC_Os02g40680	LOC_Os06g03910	LOC_Os08g25490	LOC_Os11g44560
LOC_Os02g40700	LOC_Os06g03930	LOC_Os08g25570	LOC_Os11g44580
LOC_Os02g40710	LOC_Os06g03940	LOC_Os08g25590	LOC_Os11g44600
LOC_Os02g40730	LOC_Os06g03970	LOC_Os08g25624	LOC_Os11g44630
LOC_Os02g40750	LOC_Os06g03990	LOC_Os08g25710	LOC_Os11g44660
LOC_Os02g40770	LOC_Os06g04000	LOC_Os08g25720	LOC_Os11g44680
LOC_Os02g40784	LOC_Os06g04010	LOC_Os08g25734	LOC_Os11g44690
LOC_Os02g40810	LOC_Os06g04020	LOC_Os08g25799	LOC_Os11g44700
LOC_Os02g40830	LOC_Os06g04030	LOC_Os08g25820	LOC_Os12g05360
LOC_Os02g40840	LOC_Os06g04040	LOC_Os08g25890	LOC_Os12g05370
LOC_Os02g48650	LOC_Os06g04070	LOC_Os08g25900	LOC_Os12g05380
LOC_Os02g48660	LOC_Os06g04080	LOC_Os08g26685	LOC_Os12g05394
LOC_Os02g48670	LOC_Os06g04090	LOC_Os08g26710	LOC_Os12g05410
LOC_Os02g48720	LOC_Os06g06080	LOC_Os08g26820	LOC_Os12g05420
LOC_Os02g48730	LOC_Os06g06090	LOC_Os08g26840	LOC_Os12g05430
LOC_Os02g48740	LOC_Os06g06100	LOC_Os08g26850	LOC_Os12g05440
LOC_Os02g48770	LOC_Os06g06130	LOC_Os08g26870	LOC_Os12g05470
LOC_Os02g48780	LOC_Os06g06150	LOC_Os08g26880	LOC_Os12g05540
LOC_Os02g48790	LOC_Os06g06230	LOC_Os08g28570	LOC_Os12g05550
LOC_Os02g48800	LOC_Os06g06270	LOC_Os08g28600	LOC_Os12g05590
LOC_Os02g48810	LOC_Os06g06290	LOC_Os08g28670	LOC_Os12g05600
LOC_Os02g48820	LOC_Os06g06320	LOC_Os08g28680	LOC_Os12g05609
LOC_Os02g48830	LOC_Os06g06350	LOC_Os08g28700	LOC_Os12g05630
LOC_Os02g48840	LOC_Os06g06380	LOC_Os08g28710	LOC_Os12g05640
LOC_Os02g48850	LOC_Os06g06400	LOC_Os08g28730	LOC_Os12g05650
LOC_Os02g48860	LOC_Os06g06420	LOC_Os08g28780	LOC_Os12g05660
LOC_Os02g48870	LOC_Os06g06450	LOC_Os08g28790	LOC_Os12g05680
LOC_Os02g48880	LOC_Os06g06470	LOC_Os08g28800	LOC_Os12g05709
LOC_Os02g48900	LOC_Os06g06160	LOC_Os08g28820	LOC_Os12g05730
LOC_Os02g48910	LOC_Os06g06180	LOC_Os08g28830	LOC_Os12g05750
LOC_Os02g48920	LOC_Os06g06190	LOC_Os08g28840	LOC_Os12g05760
LOC_Os02g48950	LOC_Os06g06250	LOC_Os08g28860	
LOC_Os02g48964	LOC_Os06g06260	LOC_Os08g28870	
LOC_Os02g48980	LOC_Os06g06280	LOC_Os08g28880	



Supplementary data 3.33. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of long term RGR and root K^+ of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of long term RGR and root Na^+ of the 306 accessions as phenotypic data. (**C**) GWAS analysis using ratio of long term RGR and shoot K^+ of the 306 accessions as phenotypic data. (**D**) GWAS analysis using ratio of the shoot Na^+ and long term RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.



Supplementary data 3.34. Manhattan plots showing the association signals. (**A**) GWAS analysis using ratio of the root K^+ and shoot K^+ of the 306 accessions as phenotypic data. (**B**) GWAS analysis using ratio of the shoot K^+ and long term RGR of the 306 accessions as phenotypic data. (**C**) GWAS analysis using ratio of the root K^+ and long term RGR-reduction of the 306 accessions as phenotypic data. Plants were exposed to long term saline conditions (50 mM NaCl). The X-axis indicates the SNP positions across the 12 rice chromosomes. The y-axis shows the P-value for the association test at each locus on a log scale.

Supplementary data 3.35. Summary of candidate genes identified by GWAS. Significant SNP positions were identified by GWAS using the single and combined traits. Traits were obtained from plants exposed to long term saline conditions (50 mM NaCl). Table summarises the gene locus of candidate genes.

LOC_Os01g11120	LOC_Os02g42170	LOC_Os06g03770	LOC_Os08g29910
LOC_Os01g11130	LOC_Os02g42190	LOC_Os06g03790	LOC_Os08g29970
LOC_Os01g11150	LOC_Os02g42200	LOC_Os06g03830	LOC_Os10g41590
LOC_Os01g11160	LOC_Os02g42210	LOC_Os06g03850	LOC_Os10g41640
LOC_Os01g11200	LOC_Os02g42230	LOC_Os06g03890	LOC_Os10g41650
LOC_Os01g11220	LOC_Os02g42250	LOC_Os06g03910	LOC_Os10g41660
LOC_Os01g11230	LOC_Os02g42270	LOC_Os06g03930	LOC_Os10g41689
LOC_Os01g11240	LOC_Os02g42280	LOC_Os06g03940	LOC_Os10g41696
LOC_Os01g11250	LOC_Os02g42290	LOC_Os06g03970	LOC_Os10g41710
LOC_Os01g11260	LOC_Os02g42310	LOC_Os06g03990	LOC_Os10g41720
LOC_Os01g11270	LOC_Os02g42314	LOC_Os06g04000	LOC_Os10g41740
LOC_Os01g11280	LOC_Os02g42320	LOC_Os06g04010	LOC_Os10g41749
LOC_Os01g11300	LOC_Os02g42330	LOC_Os06g04020	LOC_Os10g41760
LOC_Os01g11340	LOC_Os02g42350	LOC_Os06g04030	LOC_Os10g41780
LOC_Os01g11350	LOC_Os02g52780	LOC_Os06g04040	LOC_Os10g41790
LOC_Os01g11370	LOC_Os02g52790	LOC_Os06g04070	LOC_Os10g41820
LOC_Os01g11414	LOC_Os02g52800	LOC_Os06g04080	LOC_Os10g41829
LOC_Os01g11460	LOC_Os02g52810	LOC_Os06g04090	LOC_Os10g41838
LOC_Os01g11480	LOC_Os02g52820	LOC_Os06g30730	LOC_Os10g41900
LOC_Os01g19290	LOC_Os02g52830	LOC_Os06g30750	LOC_Os10g41930
LOC_Os01g19330	LOC_Os02g52840	LOC_Os06g30770	LOC_Os10g41950
LOC_Os01g19380	LOC_Os02g52850	LOC_Os06g30780	LOC_Os10g41960
LOC_Os01g19390	LOC_Os02g52860	LOC_Os06g30790	LOC_Os10g41970
LOC_Os01g19430	LOC_Os02g52870	LOC_Os06g30810	LOC_Os10g41980
LOC_Os01g19440	LOC_Os02g52880	LOC_Os06g30830	LOC_Os10g41999
LOC_Os01g19450	LOC_Os02g52900	LOC_Os06g30860	LOC_Os10g42020
LOC_Os01g19470	LOC_Os02g52910	LOC_Os06g30901	LOC_Os10g42060
LOC_Os01g19480	LOC_Os02g52920	LOC_Os06g30910	LOC_Os11g02100
LOC_Os01g19490	LOC_Os02g52930	LOC_Os06g30940	LOC_Os11g02130
LOC_Os01g19529	LOC_Os02g52940	LOC_Os06g30950	LOC_Os11g02150
LOC_Os01g19548	LOC_Os02g52960	LOC_Os06g30970	LOC_Os11g02165
LOC_Os01g19610	LOC_Os02g52990	LOC_Os06g31060	LOC_Os11g02180
LOC_Os01g37590	LOC_Os02g53000	LOC_Os07g47710	LOC_Os11g02240
LOC_Os01g37600	LOC_Os02g53030	LOC_Os07g47720	LOC_Os11g02250
LOC_Os01g37630	LOC_Os02g53040	LOC_Os07g47750	LOC_Os11g02260
LOC_Os01g37650	LOC_Os02g53050	LOC_Os07g47760	LOC_Os11g02300
LOC_Os01g37670	LOC_Os02g53060	LOC_Os07g47780	LOC_Os11g02310

LOC_Os01g37690	LOC_Os02g53070	LOC_Os07g47790	LOC_Os11g02320
LOC_Os01g37750	LOC_Os02g53100	LOC_Os07g47820	LOC_Os11g02330
LOC_Os01g37760	LOC_Os02g53120	LOC_Os07g47830	LOC_Os11g02350
LOC_Os01g37770	LOC_Os02g53130	LOC_Os07g47860	LOC_Os11g02369
LOC_Os01g37800	LOC_Os02g53140	LOC_Os07g47960	LOC_Os11g02379
LOC_Os01g37820	LOC_Os03g06240	LOC_Os07g47990	LOC_Os11g02389
LOC_Os01g37825	LOC_Os03g06290	LOC_Os07g48010	LOC_Os11g02400
LOC_Os01g37832	LOC_Os03g06330	LOC_Os07g48020	LOC_Os11g02424
LOC_Os01g37837	LOC_Os03g06340	LOC_Os07g48030	LOC_Os11g02440
LOC_Os01g37870	LOC_Os03g06350	LOC_Os07g48040	LOC_Os11g02450
LOC_Os01g37910	LOC_Os03g06360	LOC_Os07g48050	LOC_Os11g02460
LOC_Os01g48680	LOC_Os03g06370	LOC_Os07g48060	LOC_Os11g02464
LOC_Os01g48690	LOC_Os03g06379	LOC_Os07g48090	LOC_Os11g02470
LOC_Os01g48700	LOC_Os03g06410	LOC_Os07g48100	LOC_Os11g02480
LOC_Os01g48710	LOC_Os03g06440	LOC_Os07g48130	LOC_Os11g02520
LOC_Os01g48720	LOC_Os03g06460	LOC_Os08g08000	LOC_Os11g02530
LOC_Os01g48740	LOC_Os03g06510	LOC_Os08g08030	LOC_Os11g02540
LOC_Os01g48750	LOC_Os03g06520	LOC_Os08g08040	LOC_Os11g02570
LOC_Os01g48760	LOC_Os03g06570	LOC_Os08g08060	LOC_Os11g02580
LOC_Os01g48770	LOC_Os03g06580	LOC_Os08g08070	LOC_Os12g03290
LOC_Os01g48790	LOC_Os03g06620	LOC_Os08g08080	LOC_Os12g03340
LOC_Os01g48800	LOC_Os03g06630	LOC_Os08g08090	LOC_Os12g03360
LOC_Os01g48810	LOC_Os03g06654	LOC_Os08g08110	LOC_Os12g03370
LOC_Os01g48850	LOC_Os03g06670	LOC_Os08g08120	LOC_Os12g03390
LOC_Os01g48874	LOC_Os04g34930	LOC_Os08g08140	LOC_Os12g03420
LOC_Os01g48900	LOC_Os04g34940	LOC_Os08g08200	LOC_Os12g03430
LOC_Os01g48910	LOC_Os04g34970	LOC_Os08g08210	LOC_Os12g03440
LOC_Os01g48920	LOC_Os04g34976	LOC_Os08g08220	LOC_Os12g03470
LOC_Os01g48930	LOC_Os04g34984	LOC_Os08g08230	LOC_Os12g03480
LOC_Os01g48960	LOC_Os04g35010	LOC_Os08g14700	LOC_Os12g03510
LOC_Os01g48980	LOC_Os04g35020	LOC_Os08g14760	LOC_Os12g03530
LOC_Os01g48990	LOC_Os04g35030	LOC_Os08g14770	LOC_Os12g03540
LOC_Os01g49000	LOC_Os04g35060	LOC_Os08g14800	LOC_Os12g03554
LOC_Os01g54670	LOC_Os04g35080	LOC_Os08g14810	LOC_Os12g03594
LOC_Os01g54810	LOC_Os04g35090	LOC_Os08g14830	LOC_Os12g03630
LOC_Os01g54850	LOC_Os04g35100	LOC_Os08g14850	LOC_Os12g03640
LOC_Os01g54860	LOC_Os04g35114	LOC_Os08g14860	LOC_Os12g03650
LOC_Os01g54870	LOC_Os04g35140	LOC_Os08g14940	LOC_Os12g03670
LOC_Os01g54880	LOC_Os04g35160	LOC_Os08g14950	LOC_Os12g03690
LOC_Os01g54890	LOC_Os04g35180	LOC_Os08g14960	LOC_Os12g03710
LOC_Os01g54900	LOC_Os04g35190	LOC_Os08g14970	LOC_Os12g03720

LOC_Os01g54910	LOC_Os04g35210	LOC_Os08g14990	LOC_Os12g03730
LOC_Os01g54920	LOC_Os04g35240	LOC_Os08g15000	LOC_Os12g03740
LOC_Os01g54930	LOC_Os04g36640	LOC_Os08g15020	LOC_Os12g03750
LOC_Os01g54940	LOC_Os04g52260	LOC_Os08g17080	LOC_Os12g05830
LOC_Os01g54969	LOC_Os04g52270	LOC_Os08g17160	LOC_Os12g05840
LOC_Os01g54990	LOC_Os04g52280	LOC_Os08g17220	LOC_Os12g05860
LOC_Os01g55010	LOC_Os04g52290	LOC_Os08g17294	LOC_Os12g05870
LOC_Os01g55030	LOC_Os04g52310	LOC_Os08g17320	LOC_Os12g05880
LOC_Os01g55040	LOC_Os04g52320	LOC_Os08g17370	LOC_Os12g05900
LOC_Os01g55050	LOC_Os04g52361	LOC_Os08g17400	LOC_Os12g05920
LOC_Os01g55070	LOC_Os04g52370	LOC_Os08g17410	LOC_Os12g05980
LOC_Os01g55100	LOC_Os04g52380	LOC_Os08g25570	LOC_Os12g05990
LOC_Os01g65670	LOC_Os04g52390	LOC_Os08g25590	LOC_Os12g06020
LOC_Os01g65680	LOC_Os04g52400	LOC_Os08g25624	LOC_Os12g06030
LOC_Os01g65690	LOC_Os04g52410	LOC_Os08g25710	LOC_Os12g06050
LOC_Os01g65730	LOC_Os04g52440	LOC_Os08g25720	LOC_Os12g06060
LOC_Os01g65740	LOC_Os04g52450	LOC_Os08g25734	LOC_Os12g06100
LOC_Os01g65780	LOC_Os04g52479	LOC_Os08g25799	LOC_Os12g06110
LOC_Os01g65790	LOC_Os04g52500	LOC_Os08g25820	LOC_Os12g06150
LOC_Os01g65800	LOC_Os04g52504	LOC_Os08g25890	LOC_Os12g06160
LOC_Os01g65830	LOC_Os04g52510	LOC_Os08g25900	LOC_Os12g06180
LOC_Os01g65840	LOC_Os04g52520	LOC_Os08g26685	LOC_Os12g12470
LOC_Os01g65850	LOC_Os04g52530	LOC_Os08g26710	LOC_Os12g12514
LOC_Os01g65860	LOC_Os04g52550	LOC_Os08g26820	LOC_Os12g12560
LOC_Os01g65880	LOC_Os04g52580	LOC_Os08g26840	LOC_Os12g12580
LOC_Os01g65890	LOC_Os04g52590	LOC_Os08g26850	LOC_Os12g12590
LOC_Os01g65900	LOC_Os04g52600	LOC_Os08g26870	LOC_Os12g12600
LOC_Os01g65902	LOC_Os04g52606	LOC_Os08g26880	LOC_Os12g12720
LOC_Os01g65920	LOC_Os04g52614	LOC_Os08g28700	LOC_Os12g12730
LOC_Os01g65950	LOC_Os05g39450	LOC_Os08g28710	LOC_Os12g12740
LOC_Os01g65970	LOC_Os05g39500	LOC_Os08g28730	LOC_Os12g12810
LOC_Os01g65986	LOC_Os05g39520	LOC_Os08g28780	LOC_Os12g12830
LOC_Os01g66000	LOC_Os05g39530	LOC_Os08g28790	LOC_Os12g17030
LOC_Os01g66010	LOC_Os05g39540	LOC_Os08g28800	LOC_Os12g17070
LOC_Os01g66020	LOC_Os05g39560	LOC_Os08g28820	LOC_Os12g17080
LOC_Os01g66030	LOC_Os05g39580	LOC_Os08g28830	LOC_Os12g17090
LOC_Os01g66040	LOC_Os05g39590	LOC_Os08g28840	LOC_Os12g17140
LOC_Os01g66050	LOC_Os05g39600	LOC_Os08g28860	LOC_Os12g17160
LOC_Os01g66070	LOC_Os05g39610	LOC_Os08g28870	LOC_Os12g17310
LOC_Os01g66100	LOC_Os05g39650	LOC_Os08g28880	LOC_Os12g17320
LOC_Os02g07830	LOC_Os05g39670	LOC_Os08g28890	LOC_Os12g17340

LOC_Os02g07840	LOC_Os05g39680	LOC_Os08g28900	LOC_Os12g17410
LOC_Os02g07850	LOC_Os05g39690	LOC_Os08g28940	LOC_Os12g17420
LOC_Os02g07870	LOC_Os05g39720	LOC_Os08g28950	LOC_Os12g33954
LOC_Os02g07880	LOC_Os05g39730	LOC_Os08g28980	LOC_Os12g34092
LOC_Os02g07890	LOC_Os05g39760	LOC_Os08g29020	LOC_Os12g34094
LOC_Os02g07900	LOC_Os05g39770	LOC_Os08g29040	LOC_Os12g34108
LOC_Os02g07910	LOC_Os06g03520	LOC_Os08g29100	LOC_Os12g34124
LOC_Os02g07930	LOC_Os06g03530	LOC_Os08g29110	LOC_Os12g34128
LOC_Os02g07960	LOC_Os06g03540	LOC_Os08g29150	LOC_Os12g34154
LOC_Os02g08010	LOC_Os06g03560	LOC_Os08g29160	LOC_Os12g34200
LOC_Os02g08018	LOC_Os06g03570	LOC_Os08g29170	LOC_Os12g34220
LOC_Os02g08030	LOC_Os06g03580	LOC_Os08g29200	LOC_Os12g34240
LOC_Os02g08070	LOC_Os06g03600	LOC_Os08g29340	LOC_Os12g34270
LOC_Os02g08090	LOC_Os06g03610	LOC_Os08g29370	LOC_Os12g34290
LOC_Os02g08100	LOC_Os06g03640	LOC_Os08g29400	LOC_Os12g34300
LOC_Os02g08110	LOC_Os06g03660	LOC_Os08g29500	LOC_Os12g34310
LOC_Os02g08120	LOC_Os06g03670	LOC_Os08g29520	LOC_Os12g34320
LOC_Os02g08130	LOC_Os06g03676	LOC_Os08g29530	LOC_Os12g34330
LOC_Os02g08140	LOC_Os06g03682	LOC_Os08g29570	LOC_Os12g34340
LOC_Os02g08150	LOC_Os06g03690	LOC_Os08g29590	LOC_Os12g34370
LOC_Os02g08160	LOC_Os06g03710	LOC_Os08g29600	LOC_Os12g34380
LOC_Os02g08170	LOC_Os06g03740	LOC_Os08g29650	
LOC_Os02g08180	LOC_Os06g03760	LOC_Os08g29669	
LOC_Os02g08190	LOC_Os06g03780	LOC_Os08g29660	
LOC_Os02g08200	LOC_Os06g03800	LOC_Os08g29710	
LOC_Os02g42040	LOC_Os06g03840	LOC_Os08g29720	
LOC_Os02g42060	LOC_Os06g03860	LOC_Os08g29730	
LOC_Os02g42110	LOC_Os06g03900	LOC_Os08g29760	
LOC_Os02g42134	LOC_Os06g03700	LOC_Os08g29770	
LOC_Os02g42150	LOC_Os06g03720	LOC_Os08g29809	
LOC_Os02g42160	LOC_Os06g03750	LOC_Os08g29854	

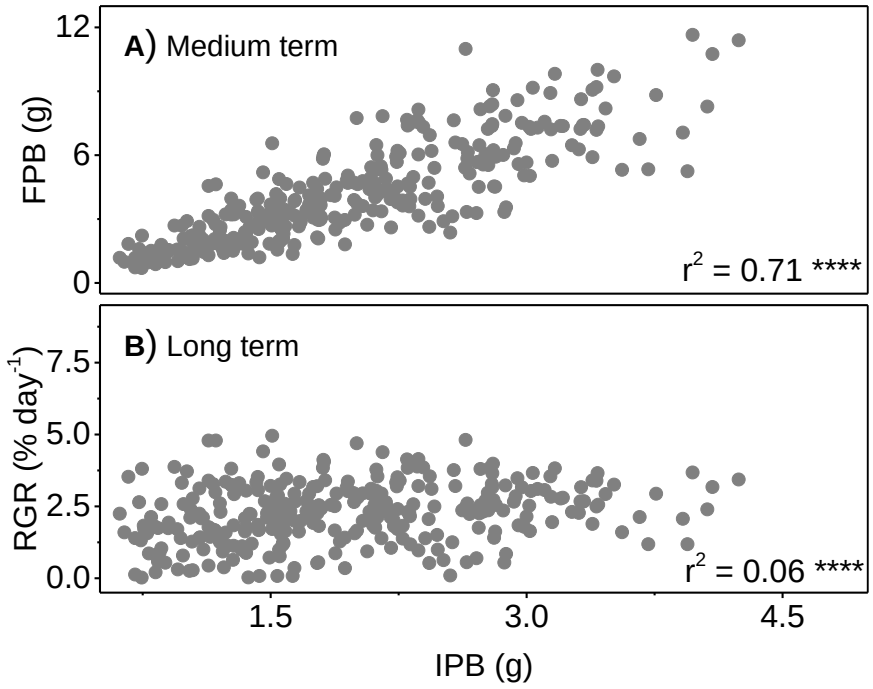
Supplementary data 3.36. Summary of non-synonymous identified by GWAS. The table summarised the changes of amino acids of candidate genes and SNP position identified by GWAS of phenotypic data of plants exposed to short, medium and long term saline conditions.

Chr	SNP position	Locus	SNP effect
1	841234	LOC_Os01g02550	Gln → Leu
1	873200	LOC_Os01g02610	His → Tyr
1	2670143	LOC_Os01g05600	Phe → Leu
1	2813045	LOC_Os01g05870	Arg → His
1	3199294	LOC_Os01g06750	Thr → Ser
1	3203661	LOC_Os01g06760	Asp → Glu
1	3224958	LOC_Os01g06790	Asp → Gly
1	6783030	LOC_Os01g12410	Pro → Leu
1	8065526	LOC_Os01g14420	Ala → Thr
1	16067009	LOC_Os01g28690	Cys → Trp
1	21789050	LOC_Os01g38840	Gly → Ala
1	22376284	LOC_Os01g39670	Asn → Ser
1	22475887	LOC_Os01g39850	Asp → Tyr
1	22899986	LOC_Os01g40540	Ser → Leu
1	23333560	LOC_Os01g41220	Ala → Gly
1	23441347	LOC_Os01g41400	Ala → Thr
1	23684988	LOC_Os01g41820	Val → Ile
1	26455022	LOC_Os01g46510	Gly → Stop
1	30216538	LOC_Os01g52560	Ile → Leu
1	33815682	LOC_Os01g58510	Thr → Lys
1	35912938	LOC_Os01g62060	Glu → Val
2	1155728	LOC_Os02g02950	Pro → Leu
2	3007368	LOC_Os02g06030	Val → Ile
2	8502431	LOC_Os02g15230	Ser → Stop
2	10753297	LOC_Os02g18470	Gly → Asp
2	14355831	LOC_Os02g24740	Cys → Ser
2	24407034	LOC_Os02g40320	Pro → Leu
2	29668062	LOC_Os02g48450	Ala → Glu
2	30560621	LOC_Os02g50010	Thr → Ser
2	33362800	LOC_Os02g54480	Gly → Arg
3	5286111	LOC_Os03g10360	Tyr → Asn
3	7331295	LOC_Os03g13560	Arg → His
3	27839218	LOC_Os03g48880	Asn → Ser
3	27948200	LOC_Os03g49050	Asp → Gly
3	27966965	LOC_Os03g49090	Glu → Lys
3	31006663	LOC_Os03g54084	Met → Ile

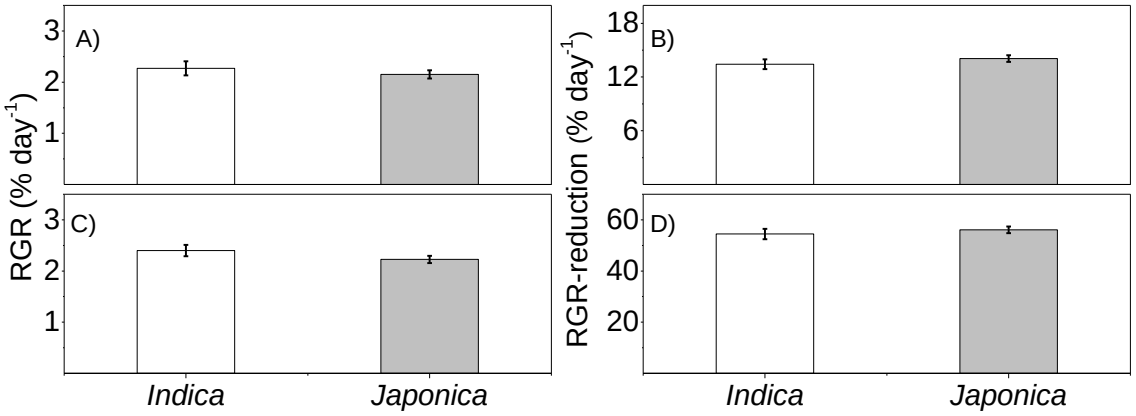
3	35119142	LOC_Os03g61950	Glu → Lys
3	35168215	LOC_Os03g62070	Glu → Lys
4	5432203	LOC_Os04g10060	Leu → Val
4	5664684	LOC_Os04g10420	Asp → Tyr
4	6262987	LOC_Os04g11450	Ser → Leu
4	6442252	LOC_Os04g11760	Ile → Thr
4	6452063	LOC_Os04g11780	Gly → Arg
4	11064006	LOC_Os04g19810	Lys → Asn
4	20095772	LOC_Os04g33210	Arg → His
4	21267182	LOC_Os04g34984	Phe → Ile
4	28239241	LOC_Os04g47590	Ala → Val
4	29783463	LOC_Os04g49930	Ala → Val
4	33194703	LOC_Os04g55760	Arg → Stop
4	35345838	LOC_Os04g59450	Asn → Ser
5	1865342	LOC_Os05g04160	Gln → Leu
5	4330522	LOC_Os05g08010	Asp → Gly
5	5465663	LOC_Os05g09680	Ser → Gly
5	9523737	LOC_Os05g16740	Ala → Ser
5	19410061	LOC_Os05g33100	Pro → Ser
5	24150385	LOC_Os05g41230	Ile → Met
6	3044125	LOC_Os06g06470	Gln → Pro
6	3075080	LOC_Os06g06550	Val → Met
6	6698107	LOC_Os06g12360	Ala → Asp
6	6719213	LOC_Os06g12390	Gln → Lys
6	11270596	LOC_Os06g19730	Tyr → His
6	11728460	LOC_Os06g20410	Ala → Asp
6	12142616	LOC_Os06g21020	Val → Leu
6	12752664	LOC_Os06g22020	Ala → Glu
6	12803227	LOC_Os06g22070	Ala → Val
6	12803590	LOC_Os06g22070	Ile → Met
6	13074710	LOC_Os06g22500	Pro → Ser
6	13108885	LOC_Os06g22550	Ala → Val
6	17197652	LOC_Os06g29844	Met → Ile
6	17200175	LOC_Os06g29844	Cys → Ser
6	17491528	LOC_Os06g30310	Phe → Leu
6	17493338	LOC_Os06g30310	Arg → Gly
6	17499757	LOC_Os06g30310	Asn → Asp
6	17507658	LOC_Os06g30320	Ser → Gly
6	17509098	LOC_Os06g30320	Thr → Arg
6	17511406	LOC_Os06g30320	Glu → Gln
6	17560384	LOC_Os06g30390	Thr → Ala

7	508978	LOC_Os07g01850	Lys → Thr
7	3253918	LOC_Os07g06670	Gly → Ser
7	17642823	LOC_Os07g29960	Pro → Ser
7	22790091	LOC_Os07g37980	Gly → Arg
7	23085133	LOC_Os07g38400	Ser → Cys
7	28865898	LOC_Os07g48310	Thr → Ile
8	5901776	LOC_Os08g10180	Thr → Lys
8	6045827	LOC_Os08g10330	Leu → Ile
8	8033747	LOC_Os08g13500	Glu → Lys
8	9017548	LOC_Os08g14950	Met → Lys
8	10572853	LOC_Os08g17294	Ala → Thr
8	10796978	LOC_Os08g17650	Arg → His
8	17430264	LOC_Os08g28540	Cys → Phe
8	17604953	LOC_Os08g28780	Val → Ile
8	17605046	LOC_Os08g28780	Val → Met
8	17625769	LOC_Os08g28820	Ala → Thr
8	17629747	LOC_Os08g28830	Asp → Asn
8	17678470	LOC_Os08g28900	Ser → Phe
8	17701381	LOC_Os08g28940	Arg → Trp
8	17702198	LOC_Os08g28940	Arg → Gln
8	17702303	LOC_Os08g28940	Leu → Ser
8	17703384	LOC_Os08g28940	Ala → Thr
8	18089227	LOC_Os08g29500	Thr → Lys
8	18157700	LOC_Os08g29590	Thr → Met
8	23724034	LOC_Os08g37456	Val → Ile
8	24205168	LOC_Os08g38200	Lys → Ile
8	24205696	LOC_Os08g38200	Arg → Lys
8	25594957	LOC_Os08g40450	Thr → Met
8	25698328	LOC_Os08g40590	Ile → Val
8	27178176	LOC_Os08g43000	Leu → Arg
8	27182804	LOC_Os08g43010	Ser → Phe
9	850009	LOC_Os09g02160	Val → Leu
9	2128802	LOC_Os09g04110	Glu → Val
9	8540338	LOC_Os09g14450	Met → Leu
9	11882288	LOC_Os09g19850	Arg → Lys
9	13322676	LOC_Os09g22000	Pro → Ser
9	21802754	LOC_Os09g37840	Arg → Met
10	2186495	LOC_Os10g04590	Trp → Stop
10	2549995	LOC_Os10g05200	Ala → Val
10	10729871	LOC_Os10g21110	Ala → Thr
10	10933095	LOC_Os10g21360	Pro → Leu

10	10956585	LOC_Os10g21400	Leu → Ser
10	10958172	LOC_Os10g21400	Gly → Asp
10	10995777	LOC_Os10g21470	Gly → Cys
10	12485105	LOC_Os10g24370	Arg → Pro
10	17331985	LOC_Os10g33080	Gly → Asp
10	19370177	LOC_Os10g36229	Val → Ala
10	21956049	LOC_Os10g40830	Pro → Leu
11	3583009	LOC_Os11g07170	Ala → Gly
11	3583012	LOC_Os11g07170	Met → Thr
11	3591971	LOC_Os11g07180	Pro → Ser
11	5098665	LOC_Os11g09510	Cys → Arg
11	5588597	LOC_Os11g10290	Val → Ala
11	8950420	LOC_Os11g16260	Ala → Val
11	16130006	LOC_Os11g28060	Asp → Asn
11	20505659	LOC_Os11g34990	Val → Leu
11	20525023	LOC_Os11g35030	His → Arg
11	20772865	LOC_Os11g35450	Met → Thr
11	20797374	LOC_Os11g35490	Pro → Leu
11	21472020	LOC_Os11g36410	Asp → Gly
11	22453422	LOC_Os11g37880	Asp → Gly
11	22552734	LOC_Os11g38010	Gly → Glu
11	23629644	LOC_Os11g39670	Val → Ala
11	24914938	LOC_Os11g41540	Phe → Tyr
11	26545426	LOC_Os11g43950	Ala → Val
11	27597068	LOC_Os11g45600	Thr → Ile
12	1398148	LOC_Os12g03530	Glu → Gly
12	2650991	LOC_Os12g05750	Pro → Leu
12	2654491	LOC_Os12g05760	Met → Thr
12	6945466	LOC_Os12g12600	Ala → Gly
12	13102081	LOC_Os12g23170	Lys → Arg
12	18580335	LOC_Os12g30920	Glu → Val
12	20732223	LOC_Os12g34200	Gly → Cys
12	26648506	LOC_Os12g42876	Ser → Thr
12	27399298	LOC_Os12g44170	Asp → Glu



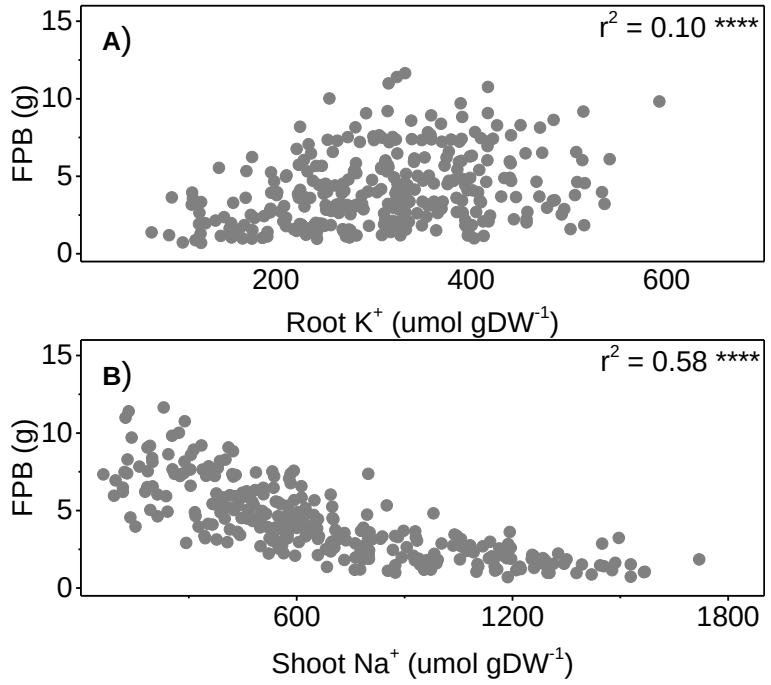
Supplementary data 3.37. Correlations between absolute and relative growth values of accessions. (**A**) Correlation between IPB and FPB. (**B**) Correlation between IPB and RGR. Plants were exposed to medium term saline conditions (50 mM NaCl). ****Correlation is significant at the 0.0001 level (two-tailed).



Supplementary data 3.38. Relative growth values of rice populations. (**A**) Average RGR values of rice indica and japonica populations in medium term saline conditions. (**B**) Average RGR-reduction values of rice indica and japonica populations in medium term saline conditions. (**C**) Average RGR values of rice indica and japonica populations in long term saline conditions. (**D**) Average RGR-reduction values of rice indica and japonica populations in long term saline conditions. Bars show the mean ±SE of 918 plants.

Supplementary data 3.39. Potential tolerant accessions identified from long term saline conditions.

Accession	Name	Subpopulation	Country	RGR-reduction (%)	Shoot Na^+ (umol gDW^{-1})
301254	Baldo	Admixed-jap	Italy	9.94	124
301348	PTB 30	Aus	India	11.49	138
301305	Dawebyan	Ind	Myanmar	15.01	415
301401	IR64	Ind	Philippines	16.72	213
301064	IR 36	Ind	Philippines	18.21	213
301072	Jouiku 393G	Tej	Japan	18.62	92
301163	Zhenshan 2	Ind	China	19.24	62
301152	TeQing	Ind	China	19.41	328
301184	Fossa Av	Trj	Burkina Faso	22.4	255
301342	Bala	Ind	India	22.57	200
301052	Geumobyeo	Tej	South Korea	24	317
301373	CI 11026	Admixed	United States	24.53	522
301347	Surjamkuhi	Aus	India	24.62	293
301271	Patna	Admixed-jap	Morocco	24.92	562
301151	NSF-TV 160	Aromatic	Iran	24.97	381



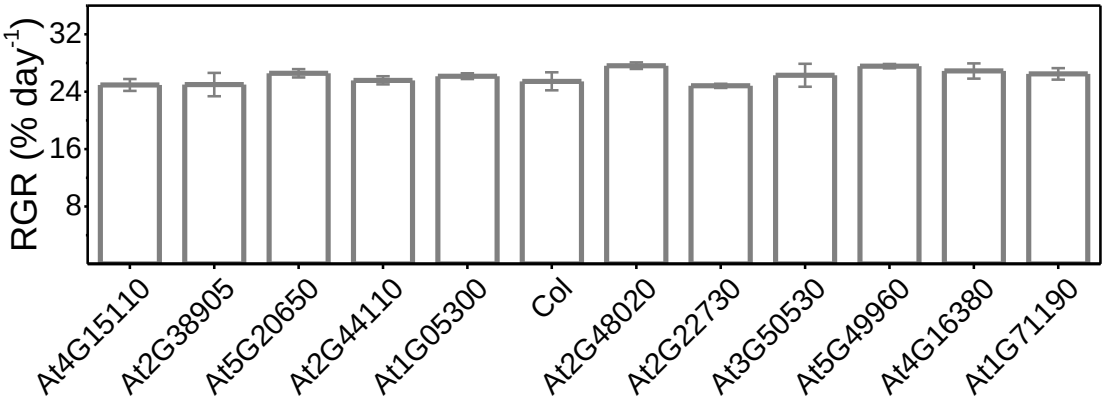
Supplementary data 3.40. Correlations between ion concentrations and FPB of accessions exposed to long term saline conditions. (**A**) Correlation between root K^+ and FPB. (**B**) Correlation between shoot Na^+ and FPB. ****Correlation is significant at the 0.0001 level (two-tailed).

Supplementary data 3.41. Candidate genes identified by GWAS in three terms of salinisation.

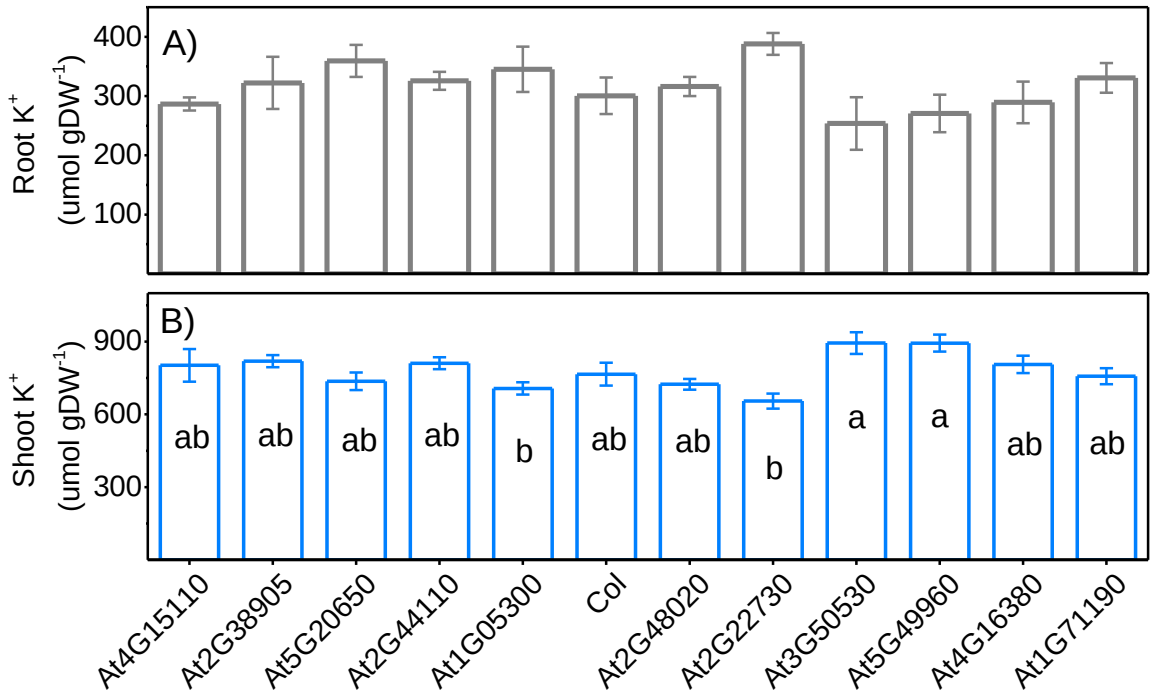
Position	Locus	Gene
15636920	LOC_Os08g25570	Histone deacetylase
15636920	LOC_Os08g25590	Light induced protein like
15636920	LOC_Os08g25624	Phosphoenolpyruvate/phosphate translocator 2
15636920	LOC_Os08g25710	Cellulose synthase-like protein D3
15636920	LOC_Os08g25720	pyrophosphate-dependent phosphofructokinase alpha subunit
15636920	LOC_Os08g25734	Glucose-1-phosphate adenylyltransferase small subunit
15636920	LOC_Os08g25799	Myb transcription factor
15636920	LOC_Os08g25820	Myb-like DNA-binding domain containing protein
15664039	LOC_Os08g25890	Tetratricopeptide repeat, putative, expressed
15664039	LOC_Os08g25900	PsbP family protein, expressed
17659199	LOC_Os08g28700	Putative DnaJ, heat shock protein hsp40
17659199	LOC_Os08g28710	Receptor protein kinase CRINKLY4 precursor
17659199	LOC_Os08g28730	Putative UDP-glucose 4-epimerase
17659199	LOC_Os08g28780	SKP1, putative, expressed
17659199	LOC_Os08g28790	Disease resistance response protein-like
17659199	LOC_Os08g28800	Skp1 family, dimerisation domain containing protein
17659199	LOC_Os08g28820	Putative SKP1-like protein
17659199	LOC_Os08g28830	Selenium-binding protein-like
17659199	LOC_Os08g28840	Putative phosphohydrolase
17659199	LOC_Os08g28860	Hydrolase, NUDIX family, domain containing protein
17659199	LOC_Os08g28870	Putative leucine-rich receptor-like protein kinase
17659199	LOC_Os08g28880	Putative patatin-like protein 1
17659199	LOC_Os08g28890	Serine/threonine protein kinase-like protein
17659199	LOC_Os08g28900	TypeA response regulator 8
17659199	LOC_Os08g28940	OsFBX289 - F-box domain containing protein
17659199	LOC_Os08g28950	Type A response regulator 8
17659199	LOC_Os08g28980	Exonuclease-like protein
17659199	LOC_Os08g29020	Wall-associated kinase 2-like
17659199	LOC_Os08g29040	Protein kinase domain containing protein

Supplementary data 4.1. Selected T-DNA Arabidopsis mutants for third experiment.

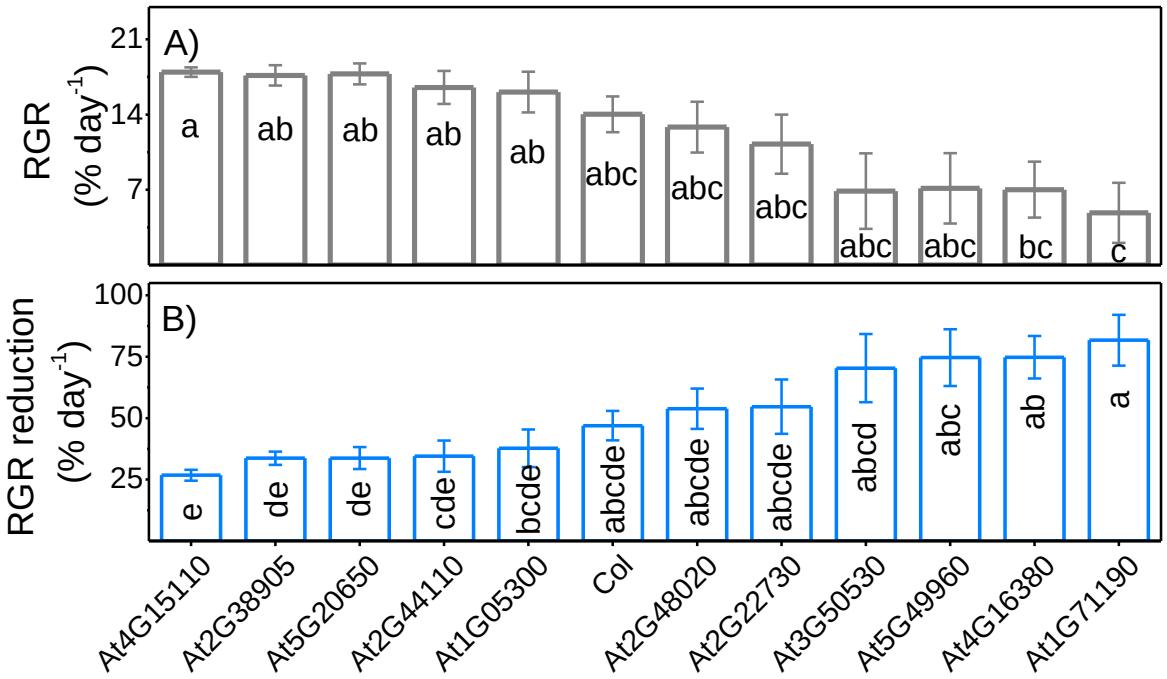
Locus	Gene	Gene function
AT1G05300	AtZIP5	Putative divalent transition metal cation transporter
AT2G22730	AMF1	Major facilitator superfamily protein, ammonium transporter
AT2G38905	AtRCI2H	Low temperature and salt responsive protein family
AT2G44110	AtMLO15	Seven transmembrane MLO family protein
AT3G50530	AtCRK5	CDPK-related kinase
AT4G16380	Cu transporter	Heavy metal transport/detoxification superfamily protein
AT5G20650	AtCOPT5	Copper transporter 5
AT5G49960	K^+ channel	Ion channel Pollux-related
AT1G71190	AtSAG18	Senescence associated gene 18
AT2G48020	AtZIF2	Major facilitator superfamily protein
AT4G15110	AtCYP97B3	Cytochrome P450, family 97, subfamily B, polypeptide 3
Col-0	Wild type	-



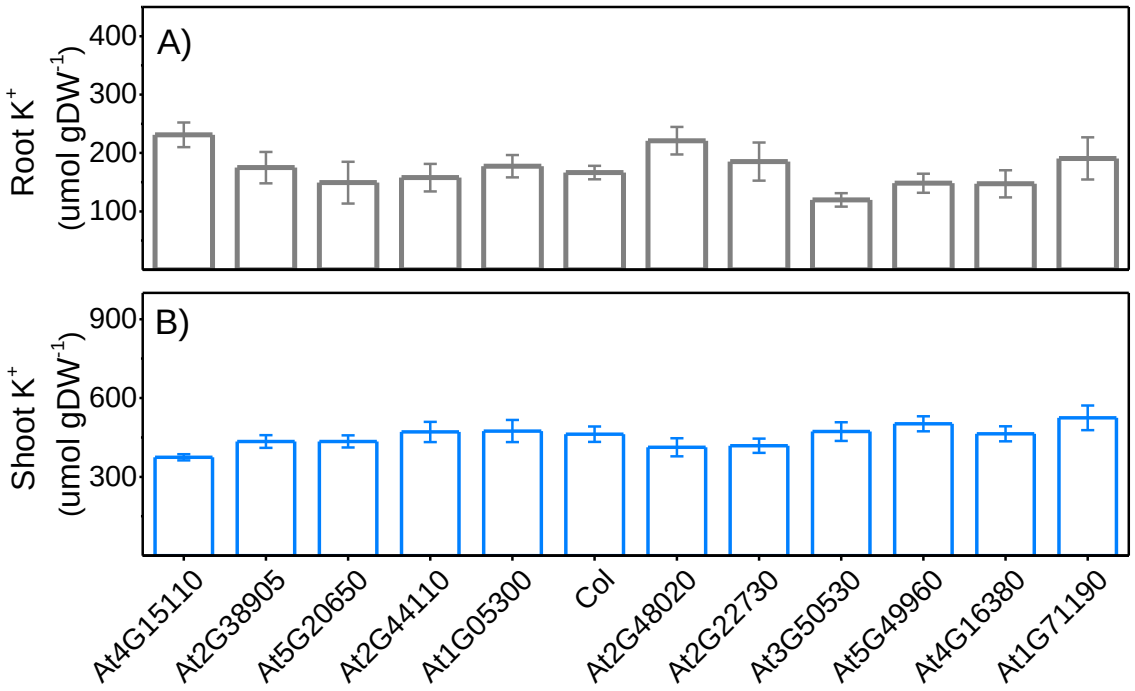
Supplementary data 4.2. RGR of the wild-type Col-0 and 11 T-DNA mutants exposed to control conditions. Bars represent the mean ±SE of six plants.



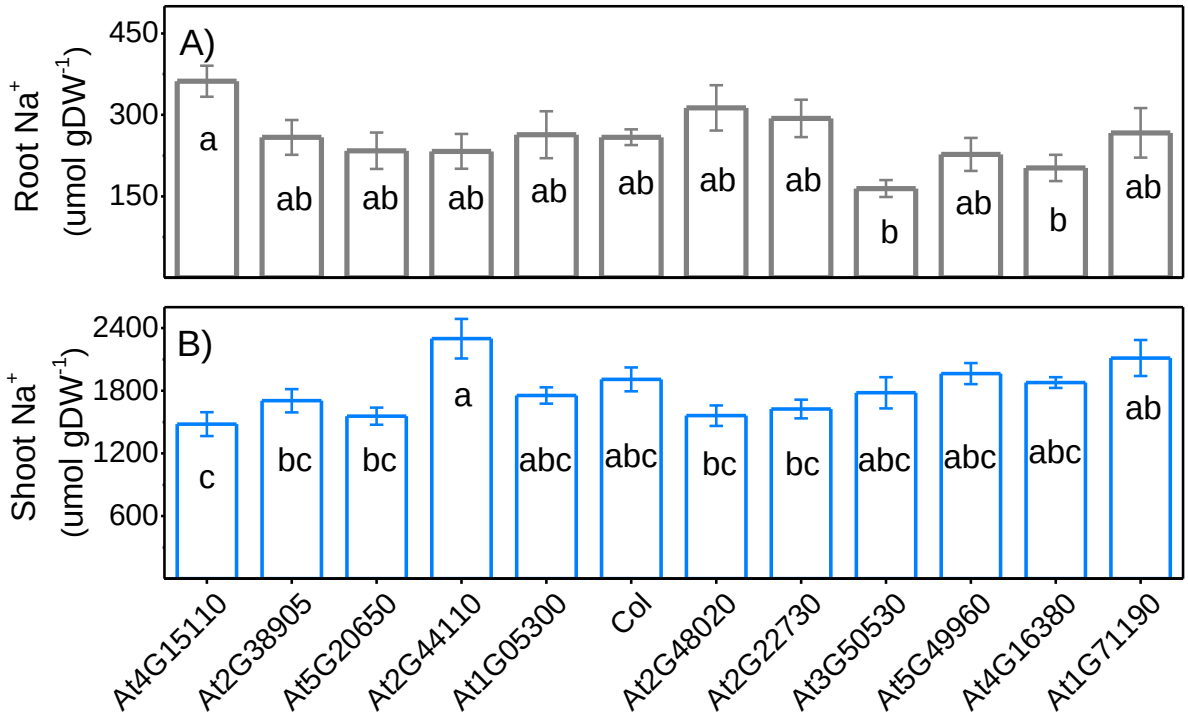
Supplementary data 4.3. K^+ concentrations in root and shoot tissues of Arabidopsis plants exposed to control conditions. (**A**) Root K^+ of the wild-type Col-0 and 11 T-DNA mutants. (**B**) Shoot K^+ of the wild-type Col-0 and 11 T-DNA mutants. Significance was calculated by one-way ANOVA ($P<0.05$). Means followed by different letters are significantly different between genotypes (Tukey's honest significant test HSD, $P<0.05$). Bars represent the mean ±SE of six plants.



Supplementary data 4.4. RGR and RGR-reduction of Arabidopsis plants exposed to saline conditions. (**A**) RGR of the wild-type Col-0 and 11 T-DNA mutants. (**B**) RGR-reduction of the wild-type Col-0 and 11 T-DNA mutants. Significance was calculated by one-way ANOVA ($P<0.05$). Means followed by different letters are significantly different between genotypes (Tukey's honest significant test HSD, $P<0.05$). Bars represent the mean ±SE of six plants.



Supplementary data 4.5. K^+ concentrations in root and shoot tissues of Arabidopsis plants exposed to saline conditions. (**A**) Root K^+ of the wild-type Col-0 and 11 T-DNA mutants. (**B**) Shoot K^+ of the wild-type Col-0 and 11 T-DNA mutants. Significance was calculated by one-way ANOVA ($P<0.05$). Bars represent the mean ±SE of six plants.



Supplementary data 4.6. Na^+ in root and shoot tissues of Arabidopsis plants exposed to saline conditions. (**A**) Root Na^+ of the wild-type Col-0 and 11 T-DNA mutants. (**B**) Shoot Na^+ of the wild-type Col-0 and 11 T-DNA mutants. Significance was calculated by one-way ANOVA ($P<0.05$). Means followed by different letters are significantly different between genotypes (Tukey's honest significant test HSD, $P<0.05$). Bars represent the mean ±SE of six plants.

Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
CAX	Vacuolar Ca^{2+}/H^{+} exchanger
CNGC	Cyclic nucleotide gated channel
CSH	Control standard hydroponic
FASTA	Family-Based Score Test for Association
GAPIT	Genome Association and Prediction Integrated Tool
GLR	Glutamate receptor
GWAS	Genome Wide Association Study
H_2DCFDA	2',7'-dichlorofluorescein diacetate
HAK	High-affinity K^{+} transporters
HKT	High-affinity transporters
KAT	Shaker-type K^{+} channel
MLM	Mixed Linear Model
NaCl	Sodium chloride
NASC	European Arabidopsis Stock Centre
NHX	Sodium hydrogen exchangers
nsSNPs	Nonsynonymous SNPs
PEG	Polyethylene Glycol
QQ	Quantile-quantile
QTL	Quantitative trait loci

RGR	Relative growth rate
ROS	Reactive oxygen species
RPK	Leucine-rich repeat protein kinases
SAG	Senescence associated gene
SAPK	Stress-activated protein kinase
SNPs	Single nucleotide polymorphisms
SOS	Salt overly sensitive
TFs	Transcription factors
WAK	Wall-associated kinase
WL	Water loss by transpiration
WUE	Water use efficiency

References

Abdula, S. E., Lee, H.-J., Ryu, H., Kang, K. K., Nou, I., Sorrells, M. E. & Cho, Y.-G. (2016). Overexpression of BrCIPK1 Gene Enhances Abiotic Stress Tolerance by Increasing Proline Biosynthesis in Rice. *Plant Molecular Biology Reporter* 34(2): 501-511.

Acosta, J. A., Faz, A., Jansen, B., Kalbitz, K. & Martínez-Martínez, S. (2011). Assessment of salinity status in intensively cultivated soils under semiarid climate, Murcia, SE Spain. *Journal of Arid Environments* 75(11): 1056-1066.

Adem, G. D., Roy, S. J., Zhou, M., Bowman, J. P. & Shabala, S. (2014). Evaluating contribution of ionic, osmotic and oxidative stress components towards salinity tolerance in barley. *BMC plant biology* 14(1): 1.

Ahmad, I., Mian, A. & Maathuis, F. J. (2016). Overexpression of the rice AKT1 potassium channel affects potassium nutrition and rice drought tolerance. *Journal of Experimental Botany* 67(9): 2689-2698.

Ahmadi, N., Dzido, J., Vales, M., Rakotoarisoa, J. & Chabanne, A. (2004).Upland rice for highlands: new varieties and sustainable cropping systems for food security promising prospects for the global challenges of rice production. In *Proceedings of the FAO rice conference: rice is life. International Rice Commission Newsletter*, 58-65.

Ahmadizadeh, M., Vispo, N. A., Calapit-Palao, C. D. O., Pangaan, I. D., Viña, C. D. & Singh, R. K. (2016). Reproductive stage salinity tolerance in rice: a complex trait to phenotype. *Indian Journal of Plant Physiology*: 1-9.

Al-Tamimi, N., Brien, C., Oakey, H., Berger, B., Saade, S., Ho, Y. S., Schmöckel, S. M., Tester, M. & Negrão, S. (2016). Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. *Nature Communications* 7: 13342.

Al-Karaki, G. N. (2000). Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress. *Journal of Plant Nutrition* 23(1): 1-8.

Albert, R. (1975). Salt regulation in halophytes. *Oecologia* 21(1): 57-71.

Allu, A. D., Soja, A. M., Wu, A., Szymanski, J. & Balazadeh, S. (2014). Salt stress and senescence: identification of cross-talk regulatory components. *Journal of Experimental Botany*: eru173.

Almeida, D. M., Gregorio, G. B., Oliveira, M. M. & Saibo, N. J. M. (2016). Five novel transcription factors as potential regulators of OsNHX1 gene expression in a salt tolerant rice genotype. *Plant Molecular Biology*: 1-17.

Alscher, R. G., Donahue, J. L. & Cramer, C. L. (1997). Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum* 100(2): 224-233.

Álvarez, S. & Sánchez-Blanco, M. (2014). Long-term effect of salinity on plant quality, water relations, photosynthetic parameters and ion distribution in Callistemon citrinus. *Plant Biology* 16(4): 757-764.

Ammar, M., Pandit, A., Singh, R., Sameena, S., Chauhan, M., Singh, A., Sharma, P., Gaikwad, K., Sharma, T. & Mohapatra, T. (2009). Mapping of QTLs controlling Na^+, K^+ and CI^- ion concentrations in salt tolerant indica rice variety CSR27. *Journal of Plant Biochemistry and Biotechnology* 18(2): 139-150.

An, G., Lee, S., Kim, S.-H. & Kim, S.-R. (2005). Molecular genetics using T-DNA in rice. *Plant and Cell Physiology* 46(1): 14-22.

Anantha, M., Patel, D., Quintana, M., Swain, P., Dwivedi, J. L., Torres, R. O., Verulkar, S. B., Variar, M., Mandal, N. P. & Kumar, A. (2016). Trait Combinations that Improve Rice Yield under Drought: Sahbhagi Dhan and New Drought-Tolerant Varieties in South Asia. *Crop Science* 56(1): 408-421.

Andreola, F., Barbieri, L., Lancellotti, I., Leonelli, C. & Manfredini, T. (2016). Recycling of industrial wastes in ceramic manufacturing: State of art and glass case studies. *Ceramics International* 42(12): 13333-13338.

Anil, V. S., Krishnamurthy, P., Kuruvilla, S., Sucharitha, K., Thomas, G. & Mathew, M. (2005). Regulation of the uptake and distribution of Na^+ in shoots of rice (Oryza sativa) variety Pokkali: role of Ca^{2+} in salt tolerance response. *Physiologia Plantarum* 124(4): 451-464.

Apel, K. & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55: 373-399.

Apse, M. P., Aharon, G. S., Snedden, W. A. & Blumwald, E. (1999). Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in Arabidopsis. *Science* 285(5431): 1256-1258.

Arbelaez, J. D., Moreno, L. T., Singh, N., Tung, C.-W., Maron, L. G., Ospina, Y., Martinez, C. P., Grenier, C., Lorieux, M. & McCouch, S. (2015). Development and GBS-genotyping of introgression lines (ILs) using two wild species of rice, *O. meridionalis* and *O. rufipogon*, in a common recurrent parent, *O. sativa* cv. Curinga. *Molecular Breeding* 35(2): 81.

Arteca, R. N. & Arteca, J. M. (2000). A novel method for growing *Arabidopsis thaliana* plants hydroponically. *Physiologia Plantarum* 108(2): 188-193.

Atwell, S., Huang, Y. S., Vilhjálmsson, B. J., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone, A. M. & Hu, T. T. (2010). Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature* 465(7298): 627-631.

Aulchenko, Y. S., Ripke, S., Isaacs, A. & van Duijn, C. M. (2007). GenABEL: an R library for genome-wide association analysis. *Bioinformatics* 23(10): 1294-1296.

Babu, N. N., Krishnan, S. G., Vinod, K., Krishnamurthy, S., Singh, V. K., Singh, M. P., Singh, R., Ellur, R. K., Rai, V. & Bollinedi, H. (2017). Marker aided incorporation of Saltol, a major QTL associated with seedling stage salt tolerance, into Oryza sativa 'Pusa Basmati 1121'. *Frontiers in Plant Science* 8.

Bado, S., Forster, B. P., Ghanim, A. M. A., Jankowicz-Cieslak, J., Berthold, G. & Luxiang, L. (2016).Protocol for Screening for Salt Tolerance in Barley and Wheat. In *Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley*, 33-37 Cham: Springer International Publishing.

Badran, E. G., Abogadallah, G. M., Nada, R. M. & Alla, M. M. N. (2015). Role of glycine in improving the ionic and ROS homeostasis during NaCl stress in wheat. *Protoplasma* 252(3): 835-844.

Bai, X., Zhao, H., Huang, Y., Xie, W., Han, Z., Zhang, B., Guo, Z., Yang, L., Dong, H. & Xue, W. (2016). Genome-Wide Association Analysis Reveals Different Genetic Control in Panicle Architecture Between and Rice. *The Plant Genome*.

Baisakh, N., RamanaRao, M. V., Rajasekaran, K., Subudhi, P., Janda, J., Galbraith, D., Vanier, C. & Pereira, A. (2012). Enhanced salt stress tolerance of rice plants expressing a vacuolar H^+-ATPase subunit c1 (SaVHAc1) gene from the halophyte grass Spartina alterniflora Löisel. *Plant Biotechnology Journal* 10(4): 453-464.

Banu, M. S. A., Huda, K. M. K., Sahoo, R. K., Garg, B., Tula, S., Islam, S. S., Tuteja, R. & Tuteja, N. (2015). Pea p68 imparts salinity stress tolerance in rice by scavenging of ROS-mediated H_2O_2 and interacts with argonaute. *Plant Molecular Biology Reporter* 33(2): 221-238.

Bañuelos, M. a. A., Garciadeblas, B., Cubero, B. & Rodríguez-Navarro, A. (2002). Inventory and functional characterization of the HAK potassium transporters of rice. *Plant Physiology* 130(2): 784-795.

Bassil, E., Tajima, H., Liang, Y.-C., Ohto, M.-a., Ushijima, K., Nakano, R., Esumi, T., Coku, A., Belmonte, M. & Blumwald, E. (2011). The Arabidopsis Na^+/H^+ antiporters NHX1 and NHX2 control vacuolar pH and K^+ homeostasis to regulate growth, flower development, and reproduction. *The Plant Cell* 23(9): 3482-3497.

Baxter, A., Mittler, R. & Suzuki, N. (2014). ROS as key players in plant stress signalling. *Journal of Experimental Botany* 65(5): 1229-1240.

Begum, H., Spindel, J. E., Lalusin, A., Borromeo, T., Gregorio, G., Hernandez, J., Virk, P., Collard, B. & McCouch, S. R. (2015). Genome-Wide Association Mapping for Yield and Other

Agronomic Traits in an Elite Breeding Population of Tropical Rice (Oryza sativa). *PLoS ONE* 10(3): e0119873.

Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the royal statistical society. Series B (Methodological)*: 289-300.

Bernier, J., Atlin, G. N., Serraj, R., Kumar, A. & Spaner, D. (2008). Breeding upland rice for drought resistance. *Journal of the Science of Food and Agriculture* 88(6): 927-939.

Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. & Atlin, G. (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Science* 47(2): 507-516.

Bertorello, A. M., Aperia, A., Walaas, S. I., Nairn, A. C. & Greengard, P. (1991). Phosphorylation of the catalytic subunit of Na^+, $K^{(+)}$-ATPase inhibits the activity of the enzyme. *Proceedings of the National Academy of Sciences* 88(24): 11359-11362.

Biscarini, F., Cozzi, P., Casella, L., Riccardi, P., Vattari, A., Orasen, G., Perrini, R., Tacconi, G., Tondelli, A. & Biselli, C. (2016). Genome-Wide Association Study for Traits Related to Plant and Grain Morphology, and Root Architecture in Temperate Rice Accessions. *PLoS ONE* 11(5): e0155425.

Bocco, R., Lorieux, M., Seck, P., Futakuchi, K., Manneh, B., Baimey, H. & Ndjiondjop, M. (2012). Agro-morphological characterization of a population of introgression lines derived from crosses between IR64 (*Oryza sativa* indica) and TOG 5681 (*Oryza glaberrima*) for drought tolerance. *Plant Science* 183: 65-76.

Bonilla, P., Mackell, D., Deal, K. & Gregorio, G. (2002). RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (Oryza sativa L.) using recombinant inbred lines. *Philippine Agricultural Scientist (Philippines)*.

Box, S. & Schachtman, D. P. (2000). The effect of low concentrations of sodium on potassium uptake and growth of wheat. *Functional Plant Biology* 27(2): 175-182.

Bozeman, M. (2016). Golden Helix, Inc. *SNP & Variation Suite (Version 7. x)[Software]. Available:* http://www. *goldenhelix. com (Accessed 2013 Jan 13)*.

Bray, E. A., Bailey-Serres, J. & Weretilnyk, E. (2000). Responses to abiotic stresses. *Biochemistry and molecular biology of plants* 11581249.

Bulle, M., Yarra, R. & Abbagani, S. (2016). Enhanced salinity stress tolerance in transgenic chilli pepper (Capsicum annuum L.) plants overexpressing the wheat antiporter (TaNHX2) gene. *Molecular Breeding* 36(4): 36.

Bush, W. S., Oetjens, M. T. & Crawford, D. C. (2016). Unravelling the human genome-phenome relationship using phenome-wide association studies. *Nature Reviews Genetics* 17(3): 129-145.

Cabuslay, G. S., Ito, O. & Alejar, A. A. (2002). Physiological evaluation of responses of rice (Oryza sativa L.) to water deficit. *Plant Science* 163(4): 815-827.

Cai, H. & Morishima, H. (2002). QTL clusters reflect character associations in wild and cultivated rice. *Theoretical and Applied Genetics* 104(8): 1217-1228.

Callaway, E. (2014). Domestication: The birth of rice. *Nature* 514(7524): S58-S59.

Cao, J., Schneeberger, K., Ossowski, S., Günther, T., Bender, S., Fitz, J., Koenig, D., Lanz, C., Stegle, O. & Lippert, C. (2011). Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nature genetics* 43(10): 956-963.

Castillo, E., Tuong, T. P., Ismail, A. & Inubushi, K. (2007). Response to salinity in rice: Comparative effects of osmotic and ionic stresses. *Plant Production Science* 10(2): 159-170.

Caton, B. P., Cope, A. E. & Mortimer, M. (2003). Growth traits of diverse rice cultivars under severe competition: implications for screening for competitiveness. *Field Crops Research* 83(2): 157-172.

Chakrabarty, D., Trivedi, P. K., Misra, P., Tiwari, M., Shri, M., Shukla, D., Kumar, S., Rai, A., Pandey, A., Nigam, D., Tripathi, R. D. & Tuli, R. (2009). Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings. *Chemosphere* 74(5): 688-702.

Chakraborty, N., Singh, N., Kaur, K. & Raghuram, N. (2015). G-protein Signaling Components GCR1 and GPA1 Mediate Responses to Multiple Abiotic Stresses in Arabidopsis. *Frontiers in Plant Science* 6.

Chang, T.-T. (2003). Origin, domestication, and diversification. *Rice: origin, history, technology, and production. New Jersey USA: J. Wiley and Sons, Inc*: 3-25.

Chen, G., Zhang, H., Deng, Z., Wu, R., Li, D., Wang, M. & Tian, J. (2016). Genome-wide association study for kernel weight-related traits using SNPs in a Chinese winter wheat population. *Euphytica* 212(2): 173-185.

Chen, H., Xie, W., He, H., Yu, H., Chen, W., Li, J., Yu, R., Yao, Y., Zhang, W. & He, Y. (2014a). A high-density SNP genotyping array for rice biology and molecular breeding. *Molecular Plant* 7(3): 541-553.

Chen, L.-J., Wuriyanghan, H., Zhang, Y.-Q., Duan, K.-X., Chen, H.-W., Li, Q.-T., Lu, X., He, S.-J., Ma, B. & Zhang, W.-K. (2013). An S-domain receptor-like kinase, *OsSIK2*, confers abiotic stress tolerance and delays dark-induced leaf senescence in rice. *Plant Physiology* 163(4): 1752-1765.

Chen, M., Presting, G., Barbazuk, W. B., Goicoechea, J. L., Blackmon, B., Fang, G., Kim, H., Frisch, D., Yu, Y. & Sun, S. (2002). An integrated physical and genetic map of the rice genome. *The Plant Cell* 14(3): 537-545.

Chen, P. E. & Shapiro, B. J. (2015). The advent of genome-wide association studies for bacteria. *Current opinion in microbiology* 25: 17-24.

Chen, W.-M. & Abecasis, G. R. (2007). Family-based association tests for genomewide association scans. *The American Journal of Human Genetics* 81(5): 913-926.

Chen, W., Gao, Y., Xie, W., Gong, L., Lu, K., Wang, W., Li, Y., Liu, X., Zhang, H. & Dong, H. (2014b). Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. *Nature genetics* 46(7): 714-721.

Chen, X., Wang, Y., Li, J., Jiang, A., Cheng, Y. & Zhang, W. (2009). Mitochondrial proteome during salt stress-induced programmed cell death in rice. *Plant Physiology and Biochemistry* 47(5): 407-415.

Chen, Z., Newman, I., Zhou, M., Mendham, N., Zhang, G. & Shabala, S. (2005). Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. *Plant, Cell & Environment* 28(10): 1230-1246.

Chen, Z., Zhou, M., Newman, I. A., Mendham, N. J., Zhang, G. & Shabala, S. (2007). Potassium and sodium relations in salinised barley tissues as a basis of differential salt tolerance. *Functional Plant Biology* 34(2): 150-162.

Cheng, L., Wang, Y., Meng, L., Hu, X., Cui, Y., Sun, Y., Zhu, L., Ali, J., Xu, J. & Li, Z. (2011). Identification of salt-tolerant QTLs with strong genetic background effect using two sets of reciprocal introgression lines in rice. *Genome* 55(1): 45-55.

Cherry, J. M., Ball, C., Weng, S., Juvik, G., Schmidt, R., Adler, C., Dunn, B., Dwight, S., Riles, L. & Mortimer, R. K. (1997). Genetic and physical maps of Saccharomyces cerevisiae. *Nature* 387(6632 Suppl): 67.

Cooper, H. D. & Clarkson, D. T. (1989). Cycling of Amino-Nitrogen and other Nutrients between Shoots and Roots in Cereals-A Possible Mechanism Integrating Shoot and Root in the Regulation of Nutrient Uptake. *Journal of Experimental Botany* 40(7): 753-762.

Cramer, G. R. (2002).Sodium-calcium interactions under salinity stress. In *Salinity: Environment-plants-molecules*, 205-227: Springer.

Crowell, S., Korniliev, P., Falcão, A., Ismail, A., Gregorio, G., Mezey, J. & McCouch, S. (2016). Genome-wide association and high-resolution phenotyping link Oryza sativa panicle traits to numerous trait-specific QTL clusters. *Nature Communications* 7.

Cui, P., Liu, H., Islam, F., Li, L., Farooq, M. A., Ruan, S. & Zhou, W. (2016). OsPEX11, a Peroxisomal Biogenesis Factor 11, Contributes to Salt Stress Tolerance in Oryza sativa. *Frontiers in Plant Science* 7: 1357.

Cuin, T. A., Betts, S. A., Chalmandrier, R. & Shabala, S. (2008). A root's ability to retain K^+ correlates with salt tolerance in wheat. *Journal of Experimental Botany* 59(10): 2697-2706.

Dagar, J. & Minhas, P. (2016).Global Perspectives on Agroforestry for the Management of Salt-affected Soils. In *Agroforestry for the Management of Waterlogged Saline Soils and Poor-Quality Waters*, 5-32: Springer.

Davenport, R., James, R. A., Zakrisson-Plogander, A., Tester, M. & Munns, R. (2005). Control of sodium transport in durum wheat. *Plant Physiology* 137(3): 807-818.

Davenport, R. J., MUÑOZ-MAYOR, A., Jha, D., Essah, P. A., Rus, A. & Tester, M. (2007). The Na^+ transporter *AtHKT1;1* controls retrieval of Na^+ from the xylem in Arabidopsis. *Plant, Cell & Environment* 30(4): 497-507.

de Oliveira, L. F. V., Christoff, A. P., de Lima, J. C., de Ross, B. C. F., Sachetto-Martins, G., Margis-Pinheiro, M. & Margis, R. (2014). The Wall-associated Kinase gene family in rice genomes. *Plant Science* 229: 181-192.

Degenkolbe, T., Do, P. T., Kopka, J., Zuther, E., Hincha, D. K. & Köhl, K. I. (2013). Identification of Drought Tolerance Markers in a Diverse Population of Rice Cultivars by Expression and Metabolite Profiling. *PLoS ONE* 8(5): e63637.

Demidchik, V. & Tester, M. (2002). Sodium fluxes through nonselective cation channels in the plasma membrane of protoplasts from Arabidopsis roots. *Plant Physiol* 128(2): 379-387.

Desbrosses, G., Josefsson, C., Rigas, S., Hatzopoulos, P. & Dolan, L. (2003). AKT1 and TRH1 are required during root hair elongation in Arabidopsis. *Journal of Experimental Botany* 54(383): 781-788.

Dhar, S., Kibria, M. G., Rahman, M. M. & Hoque, M. A. (2016). Mitigation of the adverse effects of soil salinity in rice using exogenous proline and organic manure. *Asian Journal of Medical and Biological Research* 1(3): 478-486.

Dixit, S., Singh, A., Cruz, M. T. S., Maturan, P. T., Amante, M. & Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC genetics* 15(1): 1.

Dobritsa, A. A., Geanconteri, A., Shrestha, J., Carlson, A., Kooyers, N., Coerper, D., Urbanczyk-Wochniak, E., Bench, B. J., Sumner, L. W. & Swanson, R. (2011). A large-scale genetic screen in Arabidopsis to identify genes involved in pollen exine production. *Plant Physiology* 157(2): 947-970.

Domingo, C., Lalanne, E., Catalá, M. M., Pla, E., Reig-Valiente, J. L. & Talón, M. (2016). Physiological basis and transcriptional profiling of three salt-tolerant mutant lines of rice. *Frontiers in Plant Science* 7.

Doroshenk, K. A., Crofts, A. J., Washida, H., Satoh-Cruz, M., Crofts, N., Sugino, A., Okita, T. W., Morris, R. T., Wyrick, J. J. & Fukuda, M. (2010). Characterization of the rice glup4 mutant suggests a role for the small GTPase Rab5 in the biosynthesis of carbon and nitrogen storage reserves in developing endosperm. *Breeding Science* 60(5): 556-567.

Edwards, J. D., Janda, J., Sweeney, M. T., Gaikwad, A. B., Liu, B., Leung, H. & Galbraith, D. W. (2008). Development and evaluation of a high-throughput, low-cost genotyping platform based on oligonucleotide microarrays in rice. *Plant Methods* 4(1): 1.

El-Osta, H. & Circu, M. L. (2016).Mitochondrial ROS and Apoptosis. In *Mitochondrial Mechanisms of Degeneration and Repair in Parkinson's Disease*, 1-23 (Ed L. M. Buhlman). Cham: Springer International Publishing.

Elzam, O. & Epstein, E. (1969). Salt relations of two grass species differing in salt tolerance. I. Growth and salt content at different salt concentrations. *Agrochimica*.

Erlandsson, G. (1975). Rapid effects on ion and water uptake induced by changes of water potential in young wheat plants. *Physiologia Plantarum* 35(3): 256-262.

Essah, P. A., Davenport, R. & Tester, M. (2003). Sodium Influx and Accumulation in Arabidopsis. *Plant Physiology* 133(1): 307-318.

Evans, G. C. (1972). *The quantitative analysis of plant growth.* Univ of California Press.

Famoso, A. N., Zhao, K., Clark, R. T., Tung, C.-W., Wright, M. H., Bustamante, C., Kochian, L. V. & McCouch, S. R. (2011). Genetic architecture of aluminum tolerance in rice (Oryza sativa) determined through genome-wide association analysis and QTL mapping. *PLoS Genet* 7(8): e1002221.

Fan, Y., Bose, J., Zhou, M. & Shabala, S. (2015). ROS Production, Scavenging, and Signaling under Salinity Stress. *Managing Salt Tolerance in Plants: Molecular and Genomic Perspectives*: 187.

Farquharson, K. L. (2009). Targeted overexpression of a sodium transporter in the root stele increases salinity tolerance. *The Plant Cell* 21(7): 1875-1875.

Feki, K., Quintero, F., Pardo, J. & Masmoudi, K. (2011). Regulation of durum wheat Na^+/H^+ exchanger TdSOS1 by phosphorylation. *Plant Molecular Biology* 76(6): 545-556.

Feng, Y., Lu, Q., Zhai, R., Zhang, M., Xu, Q., Yang, Y., Wang, S., Yuan, X., Yu, H. & Wang, Y. (2016). Genome wide association mapping for grain shape traits in indica rice. *Planta*: 1-12.

Fischer, R. & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research* 29(5): 897-912.

Fitt, B. D., Hughes, D. J. & Stotz, H. U. (2016).Control of Arable Crop Pathogens; Climate Change Mitigation, Impacts and Adaptation. In *Plant Genomics and Climate Change*, 49-66: Springer.

Flam-Shepherd, R. (2016).An Examination of the Role of Silicate in Sodium Transport and Salinity Tolerance in Rice (*O. sativa*).

Flowers, T., Salama, F. & Yeo, A. (1988). Water-use efficiency in rice (*Oryza sativa* L.) in relation to resistance to salinity. *Plant, Cell & Environment* 11(6): 453-459.

Flowers, T. J., Flowers, S. A., Hajibagheri, M. A. & Yeo, A. R. (1990). Salt tolerance in the halophytic wild rice, Porteresia coarctata Tateoka. *New Phytologist* 114(4): 675-684.

Flowers, T. J., Munns, R. & Colmer, T. D. (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Annals of Botany* 115(3): 419-431.

Frei, M. (2015). Breeding of ozone resistant rice: relevance, approaches and challenges. *Environmental Pollution* 197: 144-155.

Fricke, W., Leigh, R. A. & Tomos, A. D. (1996). The intercellular distribution of vacuolar solutes in the epidermis and mesophyll of barley leaves changes in response to NaCl. *Journal of Experimental Botany* 47(9): 1413-1426.

Fu, H.-H. & Luan, S. (1998). AtKUP1: a dual-affinity K^+ transporter from Arabidopsis. *The Plant Cell Online* 10(1): 63-73.

Fuchs, I., Stölzle, S., Ivashikina, N. & Hedrich, R. (2005). Rice K^+ uptake channel *OsAKT1* is sensitive to salt stress. *Planta* 221(2): 212-221.

Gaikwad, K. B., Singh, N., Bhatia, D., Kaur, R., Bains, N. S., Bharaj, T. S. & Singh, K. (2014). Yield-Enhancing Heterotic QTL Transferred from Wild Species to Cultivated Rice Oryza sativa L. *PLoS ONE* 9(6): e96939.

Gain, P., Mannan, M., Pal, P., Hossain, M. M. & Parvin, S. (2004). Effect of salinity on some yield attributes of rice. *Pak. J. Biol. Sci* 7(5).

Galli, V., da Silva Messias, R., Perin, E. C., Borowski, J. M., Bamberg, A. L. & Rombaldi, C. V. (2016). Mild salt stress improves strawberry fruit quality. *LWT - Food Science and Technology* 73: 693-699.

Gao, L., Liu, M., Wang, M., Shen, Q. & Guo, S. (2016). Enhanced Salt Tolerance under Nitrate Nutrition is Associated with Apoplast Na^+ Content in Canola (*Brassica. napus* L.) and Rice (*Oryza sativa* L.) Plants. *Plant and Cell Physiology*: pcw141.

Garciadeblás, B., Senn, M. E., Bañuelos, M. A. & Rodríguez-Navarro, A. (2003). Sodium transport and HKT transporters: the rice model. *The Plant Journal* 34(6): 788-801.

Garg, R., Verma, M., Agrawal, S., Shankar, R., Majee, M. & Jain, M. (2013). Deep Transcriptome Sequencing of Wild Halophyte Rice, Porteresia coarctata, Provides Novel Insights into the Salinity and Submergence Tolerance Factors. *DNA Research*.

Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferrière, N., Thibaud, J.-B. & Sentenac, H. (1998). Identification and Disruption of a Plant Shaker-like Outward Channel Involved in K^+ Release into the Xylem Sap. *Cell* 94(5): 647-655.

George, T., Magbanua, R., Garrity, D. P., Tubana, B. S. & Quiton, J. (2002). Rapid yield loss of rice cropped successively in aerobic soil. *Agronomy Journal* 94(5): 981-989.

Gimhani, D., Gregorio, G. B., Kottearachchi, N. & Samarasinghe, W. (2016). SNP-based discovery of salinity-tolerant QTLs in a bi-parental population of rice (Oryza sativa). *Molecular Genetics and Genomics* 291(6): 2081-2099.

Golldack, D., Su, H., Quigley, F., Kamasani, U. R., Munoz-Garay, C., Balderas, E., Popova, O. V., Bennett, J., Bohnert, H. J. & Pantoja, O. (2002). Characterization of a HKT-type transporter in rice as a general alkali cation transporter. *Plant J* 31(4): 529-542.

Grigore, M.-N., Ivanescu, L. & Toma, C. (2014).General Morphological and Anatomical Adaptations in Halophytes. In *Halophytes: An Integrative Anatomical Study*, 33-37: Springer.

Grotewold, E., Chappell, J. & Kellogg, E. A. (2015). The Shifting Genomic Landscape. *Plant Genes, Genomes and Genetics*: 17-44.

Guan, Q., Wu, J., Yue, X., Zhang, Y. & Zhu, J. (2013). A nuclear calcium-sensing pathway is critical for gene regulation and salt stress tolerance in Arabidopsis. *PLoS Genet* 9(8): e1003755.

Guo, J., Zeng, W., Chen, Q., Lee, C., Chen, L., Yang, Y., Cang, C., Ren, D. & Jiang, Y. (2016). Structure of the voltage-gated two-pore channel TPC1 from *Arabidopsis thaliana*. *Nature* 531(7593): 196-201.

Guo, K.-M., Babourina, O., Christopher, D. A., Borsics, T. & Rengel, Z. (2008). The cyclic nucleotide-gated channel, *AtCNGC10*, influences salt tolerance in Arabidopsis. *Physiologia Plantarum* 134(3): 499-507.

Guo, R., Yang, Z., Li, F., Yan, C., Zhong, X., Liu, Q., Xia, X., Li, H. & Zhao, L. (2015). Comparative metabolic responses and adaptive strategies of wheat (Triticum aestivum) to salt and alkali stress. *BMC plant biology* 15(1): 1.

Gustafsson, B. G. & Westman, P. (2002). On the causes for salinity variations in the Baltic Sea during the last 8500 years. *Paleoceanography* 17(3).

Halfter, U., Ishitani, M. & Zhu, J.-K. (2000). The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. *Proceedings of the National Academy of Sciences* 97(7): 3735-3740.

Hall, D., Evans, A., Newbury, H. & Pritchard, J. (2006). Functional analysis of CHX21: a putative sodium transporter in Arabidopsis. *Journal of Experimental Botany* 57(5): 1201-1210.

Hanson, B., Grattan, S. R. & Fulton, A. (1999). *Agricultural salinity and drainage.* University of California Irrigation Program University of California, Davis.

Hanukoglu, I. (2006). Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. *Drug metabolism reviews* 38(1-2): 171-196.

Harushima, Y., Yano, M., Shomura, A., Sato, M., Shimano, T., Kuboki, Y., Yamamoto, T., Lin, S. Y., Antonio, B. A. & Parco, A. (1998). A high-density rice genetic linkage map with 2275 markers using a single F2 population. *Genetics* 148(1): 479-494.

Hasanuzzaman, M., Nahar, K., Alam, M. M., Bhowmik, P. C., Hossain, M. A., Rahman, M. M., Prasad, M. N. V., Ozturk, M. & Fujita, M. (2014). Potential use of halophytes to remediate saline soils. *BioMed research international* 2014.

Hasegawa, P. M., Bressan, R. A., Zhu, J.-K. & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual review of plant biology* 51(1): 463-499.

Hauser, F. & Horie, T. (2010). A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. *Plant, Cell & Environment* 33(4): 552-565.

Hayes, B. (2013). Overview of statistical methods for genome-wide association studies (GWAS). *Genome-wide association studies and genomic prediction*: 149-169.

Himelblau, E., Gilchrist, E. J., Buono, K., Bizzell, C., Mentzer, L., Vogelzang, R., Osborn, T., Amasino, R. M., Parkin, I. A. & Haughn, G. W. (2009). Forward and reverse genetics of rapid-cycling *Brassica oleracea*. *Theoretical and Applied Genetics* 118(5): 953-961.

Hoque, A., Haque, M. A., Sarker, M. R. A. & Rahman, M. A. (2015). Marker-assisted introgression of saltol locus into genetic background of BRRI Dhan-49. *Int. J. Biosci* 6: 71-80.

Horie, T., Costa, A., Kim, T. H., Han, M. J., Horie, R., Leung, H.-Y., Miyao, A., Hirochika, H., An, G. & Schroeder, J. I. (2007). Rice *OsHKT2;1* transporter mediates large Na^+ influx component into K^+-starved roots for growth. *The EMBO Journal* 26(12): 3003-3014.

Horie, T., Yoshida, K., Nakayama, H., Yamada, K., Oiki, S. & Shinmyo, A. (2001). Two types of HKT transporters with different properties of Na+ and K+ transport in Oryza sativa. *Plant J* 27(2): 129-138.

Hosoo, Y., Kimura, Y., Nanatani, K. & Uozumi, N. (2014). Molecular cloning and expression analysis of a gene encoding KUP/HAK/KT-type potassium uptake transporter from *Cryptomeria japonica*. *Trees* 28(5): 1527-1537.

Hossain, H., Rahman, M., Alam, M. & Singh, R. (2015). Mapping of Quantitative Trait Loci Associated with Reproductive-Stage Salt Tolerance in Rice. *Journal of Agronomy and Crop Science* 201(1): 17-31.

Hossain, M. R., Bassel, G. W., Pritchard, J., Sharma, G. P. & Ford-Lloyd, B. V. (2016). Trait Specific Expression Profiling of Salt Stress Responsive Genes in Diverse Rice Genotypes as Determined by Modified Significance Analysis of Microarrays. *Frontiers in Plant Science* 7: 567.

Hsiao, T. C., Acevedo, E., Fereres, E. & Henderson, D. (1976). Water stress, growth, and osmotic adjustment. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 273(927): 479-500.

Hu, D.-G., Ma, Q.-J., Sun, C.-H., Sun, M.-H., You, C.-X. & Hao, Y.-J. (2016a). Overexpression of MdSOS2L1, a CIPK protein kinase, increases the antioxidant metabolites to enhance salt tolerance in apple and tomato. *Physiologia Plantarum* 156(2): 201-214.

Hu, H., Schrag, T. A., Peis, R., Unterseer, S., Schipprack, W., Chen, S., Lai, J., Yan, J., Prasanna, B. M. & Nair, S. K. (2016b). The genetic basis of haploid induction in maize identified with a novel genome-wide association method. *Genetics* 202(4): 1267-1276.

Hu, J., Ma, Q., Kumar, T., Duan, H.-R., Zhang, J.-L., Yuan, H.-J., Wang, Q., Khan, S. A., Wang, P. & Wang, S.-M. (2016c). ZxSKOR is important for salinity and drought tolerance of *Zygophyllum xanthoxylum* by maintaining K^+ homeostasis. *Plant Growth Regulation* 80(2): 195-205.

Hu, X., Jiang, M., Zhang, A. & Lu, J. (2005). Abscisic acid-induced apoplastic H_2O_2 accumulation up-regulates the activities of chloroplastic and cytosolic antioxidant enzymes in maize leaves. *Planta* 223(1): 57.

Hu, Y. (2015). Statistical Genetics.

Hu, Z.-L., Reecy, J. M. & Wu, X.-L. (2012). Design database for quantitative trait loci (QTL) data warehouse, data mining, and meta-analysis. *Quantitative Trait Loci (QTL) Methods and Protocols*: 121-144.

Huang, N., Parco, A., Mew, T., Magpantay, G., McCouch, S., Guiderdoni, E., Xu, J., Subudhi, P., Angeles, E. R. & Khush, G. S. (1997). RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a doubled haploid rice population. *Molecular Breeding* 3(2): 105-113.

Huang, P., Molina, J., Flowers, J. M., Rubinstein, S., Jackson, S. A., Purugganan, M. D. & Schaal, B. A. (2012a). Phylogeography of Asian wild rice, *Oryza rufipogon*: a genome-wide view. *Molecular ecology* 21(18): 4593-4604.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T. & Zhang, Z. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature genetics* 42(11): 961-967.

Huang, X., Yang, S., Gong, J., Zhao, Q., Feng, Q., Zhan, Q., Zhao, Y., Li, W., Cheng, B., Xia, J., Chen, N., Huang, T., Zhang, L., Fan, D., Chen, J., Zhou, C., Lu, Y., Weng, Q. & Han, B. (2016). Genomic architecture of heterosis for yield traits in rice. *Nature* 537(7622): 629-633.

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, A., Zhao, Q., Li, W., Guo, Y., Deng, L. & Zhu, C. (2012b). Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature genetics* 44(1): 32-39.

Huyen, L. T. N., Cuc, L. M., Ham, L. & Khanh, T. (2013). Introgression the Saltol QTL into Q5DB, the elite variety of Vietnam using marker-assisted-selection (MAS). *Am J BioSci* 1(4): 80À84.

Hwang, H., Yoon, J., Kim, H. Y., Min, M. K., Kim, J.-A., Choi, E.-H., Lan, W., Bae, Y.-M., Luan, S. & Cho, H. (2013). Unique features of two potassium channels, OsKAT2 and OsKAT3, expressed in rice guard cells. *PLoS ONE* 8(8): e72541.

Hwang, J. E., Jang, D.-S., Lee, K. J., Ahn, J.-W., Kim, S. H., Kang, S.-Y., Kim, D. S. & Kim, J.-B. (2016). Identification of gamma ray irradiation-induced mutations in membrane transport genes in a rice population by TILLING. *Genes & Genetic Systems*: 15-00052.

Inupakutika, M. A., Sengupta, S., Devireddy, A. R., Azad, R. K. & Mittler, R. (2016). The evolution of reactive oxygen species metabolism. *Journal of Experimental Botany*: erw382.

IRRI (2016). The International Genebank.

Islam, M. R., Sarker, M. R. A., Sharma, N., Rahman, M. A., Collard, B. C. Y., Gregorio, G. B. & Ismail, A. M. (2016). Assessment of adaptability of recently released salt tolerant rice varieties in coastal regions of South Bangladesh. *Field Crops Research* 190: 34-43.

Jabeen, N. & Ahmad, R. (2016). Growth response and nitrogen metabolism of sunflower (Helianthus annuus L.) to vermicompost and biogas slurry under salinity stress. *Journal of Plant Nutrition*: 1-11.

Jabnoune, M., Espeout, S., Mieulet, D., Fizames, C., Verdeil, J.-L., Conéjéro, G., Rodríguez-Navarro, A., Sentenac, H., Guiderdoni, E. & Abdelly, C. (2009). Diversity in expression patterns and functional properties in the rice HKT transporter family. *Plant Physiology* 150(4): 1955-1971.

Jackson, S. A. (2016). Rice: The First Crop Genome. *Rice* 9(1): 1.

Jajic, I., Sarna, T. & Strzalka, K. (2015). Senescence, Stress, and Reactive Oxygen Species. *Plants* 4(3): 393.

James, R. A., Davenport, R. J. & Munns, R. (2006). Physiological Characterization of Two Genes for Na^+ Exclusion in Durum Wheat, Nax1 and Nax2. *Plant Physiology* 142(4): 1537-1547.

Jan, A., Maruyama, K., Todaka, D., Kidokoro, S., Abo, M., Yoshimura, E., Shinozaki, K., Nakashima, K. & Yamaguchi-Shinozaki, K. (2013). OsTZF1, a CCCH-tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. *Plant Physiology* 161(3): 1202-1216.

Jangam, A. P., Pathak, R. R. & Raghuram, N. (2016). Microarray Analysis of Rice d1 (RGA1) Mutant Reveals the Potential Role of G-Protein Alpha Subunit in Regulating Multiple Abiotic Stresses Such as Drought, Salinity, Heat, and Cold. *Frontiers in Plant Science* 7.

Jia, W., Wang, Y., Zhang, S. & Zhang, J. (2002). Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. *Journal of Experimental Botany* 53(378): 2201-2206.

Jibrin, J., Oikeh, S., Hussaini, M., Miko, S., Shehu, B. & Sie, M. (2010). Responses of Lowland NERICA and Improved Oryza sativa Rice to Phosphorus and Nitrogen in Sudan Savanna Agroecosystem. *Nong Ye Ke Xue Yu Ji Shu* 4(4): 47.

Jin, J., Huang, W., Gao, J.-P., Yang, J., Shi, M., Zhu, M.-Z., Luo, D. & Lin, H.-X. (2008). Genetic control of rice plant architecture under domestication. *Nature genetics* 40(11): 1365-1369.

JMP®, V. (2012). SAS Institute Inc., Cary, NC, 1989-2007.

Jones, M. P., Dingkuhn, M., Aluko, G. K. & Semon, M. (1997). Interspecific *Oryza Sativa* L. X *O. Glaberrima* Steud. progenies in upland rice improvement. *Euphytica* 94(2): 237-246.

Joshi, R., Karan, R., Singla-Pareek, S. L. & Pareek, A. (2016). Ectopic expression of Pokkali phosphoglycerate kinase-2 (OsPGK2-P) improves yield in tobacco plants under salinity stress. *Plant Cell Reports* 35(1): 27-41.

Kader, M. A. & Lindberg, S. (2005). Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, Oryza sativa L. determined by the fluorescent dye SBFI. *Journal of Experimental Botany* 56(422): 3149-3158.

Kang, H., Wang, Y., Peng, S., Zhang, Y., Xiao, Y., Wang, D., Qu, S., Li, Z., Yan, S. & Wang, Z. (2015). Dissection of the genetic architecture of rice resistance to the blast fungus Magnaporthe oryzae. *Molecular plant pathology*.

Kao, C. H. & Yang, S. F. (1983). Role of Ethylene in the Senescence of Detached Rice Leaves. *Plant Physiology* 73(4): 881-885.

Kasprowicz, A. (2011).Osmosensing. In *Mechanical Integration of Plant Cells and Plants*, 225-240: Springer.

Katschnig, D., Bliek, T., Rozema, J. & Schat, H. (2015). Constitutive high-level SOS1 expression and absence of HKT1;1 expression in the salt-accumulating halophyte Salicornia dolichostachya. *Plant Science* 234: 144-154.

Katsura, K., Maeda, S., Lubis, I., Horie, T., Cao, W. & Shiraiwa, T. (2008). The high yield of irrigated rice in Yunnan, China: 'A cross-location analysis'. *Field Crops Research* 107(1): 1-11.

Kaur, R. & Singh, R. (2011). Assessment for different groundwater quality parameters for irrigation purposes in Bikaner City, Rajasthan. *Journal of Applied Sciences in Environmental Sanitation* 6(3): 385-392.

Kavitha, P., Miller, A., Mathew, M. & Maathuis, F. J. (2012). Rice cultivars with differing salt tolerance contain similar cation channels in their root cells. *Journal of Experimental Botany* 63(8): 3289-3296.

Keller, B. U., Hedrich, R. & Raschke, K. (1989). Voltage-dependent anion channels in the plasma membrane of guard cells. *Nature* 341(6241): 450-453.

Khush, G. S. (1997).Origin, dispersal, cultivation and variation of rice. In *Oryza: From molecule to plant*, 25-34: Springer.

Khush, G. S. (2005). What it will take to Feed 5.0 Billion Rice consumers in 2030. *Plant Molecular Biology* 59(1): 1-6.

Kikuta, M., Makihara, D., Arita, N., Miyazaki, A. & Yamamoto, Y. (2016). Growth and yield responses of upland NERICAs to variable water management under field conditions. *Plant Production Science*: 1-11.

Kim, B. G., Waadt, R., Cheong, Y. H., Pandey, G. K., Dominguez-Solis, J. R., Schültke, S., Lee, S. C., Kudla, J. & Luan, S. (2007). The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis. *The Plant Journal* 52(3): 473-484.

Kim, H., Jung, J., Singh, N., Greenberg, A., Doyle, J. J., Tyagi, W., Chung, J.-W., Kimball, J., Hamilton, R. S. & McCouch, S. R. (2016). Population Dynamics Among six Major Groups of the Oryza rufipogon Species Complex, Wild Relative of Cultivated Asian Rice. *Rice* 9(1): 56.

Kintzer, A. F. & Stroud, R. M. (2016). Structure, inhibition and regulation of two-pore channel TPC1 from Arabidopsis thaliana. *Nature* 531(7593): 258-264.

Kobayashi, S., Abe, N., Yoshida, K. T., Liu, S. & Takano, T. (2012). Molecular cloning and characterization of plasma membrane- and vacuolar-type Na^+/H^+ antiporters of an alkaline-salt-tolerant monocot, *Puccinellia tenuiflora*. *Journal of Plant Research* 125(4): 587-594.

Koh, S., Lee, S.-C., Kim, M.-K., Koh, J. H., Lee, S., An, G., Choe, S. & Kim, S.-R. (2007). T-DNA tagged knockout mutation of rice OsGSK1, an orthologue of Arabidopsis BIN2, with enhanced tolerance to various abiotic stresses. *Plant Molecular Biology* 65(4): 453-466.

Kohli, A., Sreenivasulu, N., Lakshmanan, P. & Kumar, P. P. (2013). The phytohormone crosstalk paradigm takes center stage in understanding how plants respond to abiotic stresses. *Plant Cell Reports* 32(7): 945-957.

Kohorn, B. & Kohorn, S. (2012a). The cell wall-associated kinases, WAKs, as pectin receptors. *Frontiers in Plant Science* 3(88).

Kohorn, B. D. & Kohorn, S. L. (2012b).The Cell Wall-Associated Kinases, WAKs, Regulate Cell Expansion and the Stress Response. In *Receptor-like Kinases in Plants: From Development to Defense*, 109-124 (Eds F. Tax and B. Kemmerling). Berlin, Heidelberg: Springer Berlin Heidelberg.

Kooke, R., Kruijer, W., Bours, R., Becker, F. F., Kuhn, A., Buntjer, J., Doeswijk, T., Guerra, J., Bouwmeester, H. J. & Vreugdenhil, D. (2016). Genome-wide association mapping and genomic prediction elucidate the genetic architecture of morphological traits in *Arabidopsis thaliana*. *Plant Physiology*: pp. 00997.02015.

Korte, A. & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant methods* 9(1): 1.

Koutroubas, S. D. & Ntanos, D. A. (2003). Genotypic differences for grain yield and nitrogen utilization in *Indica* and *Japonica* rice under Mediterranean conditions. *Field Crops Research* 83(3): 251-260.

Koyama, M. L., Levesley, A., Koebner, R. M., Flowers, T. J. & Yeo, A. R. (2001). Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiology* 125(1): 406-422.

Krishnamurthy, S., Sharma, P., Sharma, S., Batra, V., Kumar, V. & Rao, L. (2016). Effect of salinity and use of stress indices of morphological and physiological traits at the seedling stage in rice. *Indian Journal of Experimental Biology* 54: 843-850.

Krysan, P. J., Young, J. C., Tax, F. & Sussman, M. R. (1996). Identification of transferred DNA insertions within Arabidopsis genes involved in signal transduction and ion transport. *Proceedings of the National Academy of Sciences* 93(15): 8145-8150.

Kumar, V., Singh, A., Mithra, S. A., Krishnamurthy, S., Parida, S. K., Jain, S., Tiwari, K. K., Kumar, P., Rao, A. R. & Sharma, S. (2015). Genome-wide association mapping of salinity tolerance in rice (Oryza sativa). *DNA Research*: dsu046.

Kurata, N. a., Nagamura, Y., Yamamoto, K., Harushima, Y., Sue, N., Wu, J., Antonio, B., Shomura, A., Shimizu, T. & Lin, S. Y. (1994). A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nature genetics* 8(4): 365-372.

Kurowska, M., Daszkowska-Golec, A., Gruszka, D., Marzec, M., Szurman, M., Szarejko, I. & Maluszynski, M. (2011). TILLING-a shortcut in functional genomics. *Journal of applied genetics* 52(4): 371-390.

Lafitte, H., Li, Z., Vijayakumar, C., Gao, Y., Shi, Y., Xu, J., Fu, B., Yu, S., Ali, A. & Domingo, J. (2006). Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. *Field Crops Research* 97(1): 77-86.

Lafitte, H., Price, A. & Courtois, B. (2004). Yield response to water deficit in an upland rice mapping population: associations among traits and genetic markers. *Theoretical and Applied Genetics* 109(6): 1237-1246.

Lan, T., Duan, Y., Wang, B., Zhou, Y. & Wu, W. (2011). Molecular cloning and functional characterization of a Na^+/H^+ antiporter gene from halophyte *Spartina anglica*. *Turkish Journal of Agriculture and Forestry* 35(5): 535-543.

Land, F. (2008).Plant Nutrition Management Service.

Le Gall, H., Philippe, F., Domon, J.-M., Gillet, F., Pelloux, J. & Rayon, C. (2015). Cell wall metabolism in response to abiotic stress. *Plants* 4(1): 112-166.

Le Rudulier, D., Strom, A., Dandekar, A., Smith, L. & Valentine, R. (1984). Molecular biology of osmoregulation. *Science* 224(4653): 1064-1068.

Lee, C.-Y., Kim, T.-S., Lee, S. & Park, Y.-J. (2015).Concept of Genome-Wide Association Studies. In *Current Technologies in Plant Molecular Breeding*, 175-204: Springer.

Lee, K.-S., Choi, W.-Y., Ko, J.-C., Kim, T.-S. & Gregorio, G. B. (2003). Salinity tolerance of *japonica* and *indica* rice (Oryza sativa L.) at the seedling stage. *Planta* 216(6): 1043-1046.

Lee, R. H., Wang, C. H., Huang, L. T. & Chen, S. C. G. (2001). Leaf senescence in rice plants: cloning and characterization of senescence up-regulated genes. *Journal of Experimental Botany* 52(358): 1117-1121.

Li, C., Zhou, A. & Sang, T. (2006). Rice domestication by reducing shattering. *Science* 311(5769): 1936-1939.

Li, D., Wang, L., Wang, M., Xu, Y. Y., Luo, W., Liu, Y. J., Xu, Z. H., Li, J. & Chong, K. (2009). Engineering OsBAK1 gene as a molecular tool to improve rice architecture for high yield. *Plant Biotechnology Journal* 7(8): 791-806.

Li, F., Chen, B., Xu, K., Wu, J., Song, W., Bancroft, I., Harper, A. L., Trick, M., Liu, S. & Gao, G. (2014). Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (Brassica napus L.). *DNA Research* 21(4): 355-367.

Li, J., Lee, Y.-R. J. & Assmann, S. M. (1998). Guard cells possess a calcium-dependent protein kinase that phosphorylates the KAT1 potassium channel. *Plant Physiology* 116(2): 785-795.

Liang, C., Wang, Y., Zhu, Y., Tang, J., Hu, B., Liu, L., Ou, S., Wu, H., Sun, X., Chu, J. & Chu, C. (2014). OsNAP connects abscisic acid and leaf senescence by fine-tuning abscisic acid biosynthesis and directly targeting senescence-associated genes in rice. *Proceedings of the National Academy of Sciences* 111(27): 10013-10018.

Liang, S., Wu, L., Ren, G., Zhao, X., Zhou, M., McNeil, D. & Ye, G. (2016). Genome-wide association study of grain yield and related traits using a collection of advanced indica rice breeding lines for irrigated ecosystems. *Field Crops Research* 193: 70-86.

Liao, Y.-D., Lin, K.-H., Chen, C.-C. & Chiang, C.-M. (2016). Oryza sativa protein phosphatase 1a (OsPP1a) involved in salt stress tolerance in transgenic rice. *Molecular Breeding* 36(3): 22.

Lin, H., Yanagihara, S., Zhuang, J., Senboku, T., Zheng, K. & Yashima, S. (1997). Identification of QTL for salt tolerance in rice via molecular markers. *Zhongguo shuidao kexue* 12(2): 72-78.

Lin, H., Zhu, M., Yano, M., Gao, J., Liang, Z., Su, W., Hu, X., Ren, Z. & Chao, D. (2004). QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. *Theoretical and Applied Genetics* 108(2): 253-260.

Linh, L. H., Linh, T. H., Xuan, T. D., Ham, L. H., Ismail, A. M. & Khanh, T. D. (2012). Molecular breeding to improve salt tolerance of rice (Oryza sativa L.) in the Red River Delta of Vietnam. *International journal of plant genomics* 2012.

Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., Gore, M. A., Buckler, E. S. & Zhang, Z. (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics* 28(18): 2397-2399.

Liu, F. & Stützel, H. (2002). Leaf expansion, stomatal conductance, and transpiration of vegetable amaranth (Amaranthus sp.) in response to soil drying. *Journal of the american Society for Horticultural Science* 127(5): 878-883.

Liu, J., Shen, J., Xu, Y., Li, X., Xiao, J. & Xiong, L. (2016a). Ghd2, a CONSTANS-like gene, confers drought sensitivity through regulation of senescence in rice. *Journal of Experimental Botany* 67(19): 5785-5798.

Liu, L., Mei, Q., Yu, Z., Sun, T., Zhang, Z. & Chen, M. (2013). An integrative bioinformatics framework for genome-scale multiple level network reconstruction of rice. *J. Integr. Bioinform* 10(223): 10.2390.

Liu, W.-Z., Deng, M., Li, L., Yang, B., Li, H., Deng, H. & Jiang, Y.-Q. (2015a). Rapeseed calcineurin B-like protein CBL4, interacting with CBL-interacting protein kinase CIPK24, modulates salt tolerance in plants. *Biochemical and Biophysical Research Communications* 467(3): 467-471.

Liu, Y., Qi, X., Gealy, D., Olsen, K. M., Caicedo, A. L. & Jia, Y. (2015b). QTLs analysis for resistance to blast disease in US weedy rice. *Molecular Plant-Microbe Interactions* (ja).

Liu, Y., Sun, J. & Wu, Y. (2016b). Arabidopsis ATAF1 enhances the tolerance to salt stress and ABA in transgenic rice. *Journal of Plant Research*: 1-8.

Londo, J. P., Chiang, Y.-C., Hung, K.-H., Chiang, T.-Y. & Schaal, B. A. (2006). Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. *Proceedings of the National Academy of Sciences* 103(25): 9578-9583.

Lu, C., ZHANG, J., PAN, X.-b., ZHANG, F., ZHENG, T.-q., ZHAO, X.-q., WANG, W.-s., Jauhar, A., XU, J.-l. & LI, Z.-k. (2014). Advanced backcross QTL analysis for the whole plant growth duration salt tolerance in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture* 13(8): 1609-1620.

Lu, Z. & Neumann, P. M. (1998). Water-stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. *Journal of Experimental Botany* 49(329): 1945-1952.

Luu TN, H., Luu M, C., Abdelbagi M, I. & Le H, H. (2012). Introgression the salinity tolerance QTLs Saltol into AS996, the elite rice variety of Vietnam. *American Journal of Plant Sciences* 2012.

Lv, S., Jiang, P., Chen, X., Fan, P., Wang, X. & Li, Y. (2012). Multiple compartmentalization of sodium conferred salt tolerance in Salicornia europaea. *Plant Physiology and Biochemistry* 51(0): 47-52.

Maathuis, F. J. (2013). Sodium in plants: perception, signalling, and regulation of sodium fluxes. *Journal of Experimental Botany*: ert326.

Maathuis, F. J. & Amtmann, A. (1999). K^+ Nutrition and Na^+Toxicity: The Basis of Cellular K^+/Na^+ Ratios. *Annals of Botany* 84(2): 123-133.

Maathuis, F. J. & Sanders, D. (1993). Energization of potassium uptake in Arabidopsis thaliana. *Planta* 191(3): 302-307.

Maathuis, F. J. M. & Diatloff, E. (2013).Roles and Functions of Plant Mineral Nutrients. In *Plant Mineral Nutrients: Methods and Protocols*, 1-21 (Ed F. J. M. Maathuis). Totowa, NJ: Humana Press.

Mahmuduzzaman, M., Ahmed, Z. U., Nuruzzaman, A. & Ahmed, F. R. S. (2014). Causes of salinity intrusion in coastal belt of Bangladesh. *International Journal of Plant Research* 4(4A): 8-13.

Manning, K. & Timpson, A. (2014). The demographic response to Holocene climate change in the Sahara. *Quaternary Science Reviews* 101: 28-35.

Mao, G., Seebeck, T., Schrenker, D. & Yu, O. (2013). CYP709B3, a cytochrome P450 monooxygenase gene involved in salt tolerance in Arabidopsis thaliana. *BMC Plant Biology* 13(1): 1-13.

Marathi, B., Guleria, S., Mohapatra, T., Parsad, R., Mariappan, N., Kurungara, V. K., Atwal, S. S., Prabhu, K. V., Singh, N. K. & Singh, A. K. (2012). QTL analysis of novel genomic regions associated with yield and yield related traits in new plant type based recombinant inbred lines of rice (Oryza sativa L.). *BMC plant biology* 12(1): 1.

Mäser, P., Hosoo, Y., Goshima, S., Horie, T., Eckelman, B., Yamada, K., Yoshida, K., Bakker, E. P., Shinmyo, A. & Oiki, S. (2002). Glycine residues in potassium channel-like selectivity filters determine potassium selectivity in four-loop-per-subunit HKT transporters from plants. *Proceedings of the National Academy of Sciences* 99(9): 6428-6433.

Masood, M. S., Seiji, Y., Shinwari, Z. K. & Anwar, R. (2004). Mapping quantitative trait loci (QTLs) for salt tolerance in rice (Oryza sativa) using RFLPs. *Pak. J. Bot* 36(4): 825-834.

Matoh, T., Kairusmee, P. & Takahashi, E. (1986). Salt-Induced Damage to Rice Plants and Alleviation Effect of Silicate. *Soil Science and Plant Nutrition* 32(2): 295-304.

Matsubara, K., Yamamoto, E., Kobayashi, N., Ishii, T., Tanaka, J., Tsunematsu, H., Yoshinaga, S., Matsumura, O., Yonemaru, J.-i. & Mizobuchi, R. (2016). Improvement of Rice Biomass Yield through QTL-Based Selection. *PLoS ONE* 11(3): e0151830.

Matsunami, M., Toyofuku, K., Ishikawa–Sakurai, J., Ogawa, A., Matsunami, T. & Kokubun, M. (2016). Root development and the expression of aquaporin genes in rice seedlings under osmotic stress. *Plant Production Science* 19(2): 315-322.

McCouch, S. R., Wright, M. H., Tung, C.-W., Maron, L. G., McNally, K. L., Fitzgerald, M., Singh, N., DeClerck, G., Agosto-Perez, F. & Korniliev, P. (2016). Open access resources for genome-wide association mapping in rice. *Nature Communications* 7.

Mei, H., Li, Z., Shu, Q., Guo, L., Wang, Y., Yu, X., Ying, C. & Luo, L. (2005). Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross populations. *Theoretical and Applied Genetics* 110(4): 649-659.

Meyer, R. S., Choi, J. Y., Sanches, M., Plessis, A., Flowers, J. M., Amas, J., Dorph, K., Barretto, A., Gross, B. & Fuller, D. Q. (2016). Domestication history and geographical adaptation inferred from a SNP map of African rice. *Nature Genetics*.

Mickelbart, M. V., Hasegawa, P. M. & Bailey-Serres, J. (2015). Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nature Reviews Genetics* 16(4): 237-251.

Miller, G., Suzuki, N., CIFTCI-YILMAZ, S. & Mittler, R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment* 33(4): 453-467.

Miller, G., Suzuki, N., Rizhsky, L., Hegie, A., Koussevitzky, S. & Mittler, R. (2007). Double mutants deficient in cytosolic and thylakoid ascorbate peroxidase reveal a complex mode of interaction between reactive oxygen species, plant development, and response to abiotic stresses. *Plant Physiology* 144(4): 1777-1785.

Miller, J. D., Arteca, R. N. & Pell, E. J. (1999). Senescence-associated gene expression during ozone-induced leaf senescence in Arabidopsis. *Plant Physiology* 120(4): 1015-1024.

Mitchell, J., Siamhan, D., Wamala, M., Risimeri, J., Chinyamakobvu, E., Henderson, S. & Fukai, S. (1998). The use of seedling leaf death score for evaluation of drought resistance of rice. *Field Crops Research* 55(1): 129-139.

Miyamoto, T., Ochiai, K., Nonoue, Y., Matsubara, K., Yano, M. & Matoh, T. (2015). Expression level of the sodium transporter gene *OsHKT2;1* determines sodium accumulation of rice cultivars under potassium-deficient conditions. *Soil Science and Plant Nutrition* (ahead-of-print): 1-12.

Mizrahi, Y., Blumenfeld, A. & Richmond, A. E. (1970). Abscisic acid and transpiration in leaves in relation to osmotic root stress. *Plant Physiology* 46(1): 169.

Møller, I. S., Gilliham, M., Jha, D., Mayo, G. M., Roy, S. J., Coates, J. C., Haseloff, J. & Tester, M. (2009). Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type–specific alteration of Na^+ transport in *Arabidopsis*. *The Plant Cell Online* 21(7): 2163-2178.

Moradi, F. & Ismail, A. M. (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Annals of Botany* 99(6): 1161-1173.

Moukoumbi, Y., Sie, M., Vodouhe, R., N'dri, B., Toulou, B., Ogunbayo, S. & Ahanchede, A. (2011). Assessing phenotypic diversity of interspecific rice varieties using agro-morphological characterization. J. *Plant Breed. Crop Sci* 3: 74-86.

Moumeni, A., Satoh, K., Kondoh, H., Asano, T., Hosaka, A., Venuprasad, R., Serraj, R., Kumar, A., Leung, H. & Kikuchi, S. (2011). Comparative analysis of root transcriptome profiles of two pairs of drought-tolerant and susceptible rice near-isogenic lines under different drought stress. *BMC plant biology* 11(1): 174.

Mühling, K. H. & Läuchli, A. (2002). Determination of apoplastic Na^+ in intact leaves of cotton by in vivo fluorescence ratio-imaging. *Functional Plant Biology* 29(12): 1491-1499.

Munasinghe, M. & Price, A. H. (2016). Genetic and root phenotype diversity in Sri Lankan rice landraces may be related to drought resistance. *Rice* 9(1): 24.

Munns, R., James, R. A. & Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany* 57(5): 1025-1043.

Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59: 651-681.

Nagarajan, Y., Rongala, J., Luang, S., Singh, A., Shadiac, N., Hayes, J., Sutton, T., Gilliham, M., Tyerman, S. D. & McPhee, G. (2016). A barley efflux transporter operates in a Na+-dependent manner, as revealed by a multidisciplinary platform. *The Plant Cell* 28(1): 202-218.

Nakamura, R. L., McKendree Jr, W. L., Hirsch, R. E., Sedbrook, J. C., Gaber, R. F. & Sussman, M. R. (1995). Expression of an Arabidopsis potassium channel gene in guard cells. *Plant Physiology* 109(2): 371-374.

Nath, M., Yadav, S., Sahoo, R. K., Passricha, N., Tuteja, R. & Tuteja, N. (2016). PDH45 transgenic rice maintain cell viability through lower accumulation of Na^+, ROS and calcium homeostasis in roots under salinity stress. *Journal of Plant Physiology* 191: 1-11.

Nawaz, Z., Kakar, K. U., Saand, M. A. & Shu, Q.-Y. (2014). Cyclic nucleotide-gated ion channel gene family in rice, identification, characterization and experimental analysis of expression response to plant hormones, biotic and abiotic stresses. *BMC Genomics* 15(1): 1.

Neale, B. M., Medland, S. E., Ripke, S., Asherson, P., Franke, B., Lesch, K.-P., Faraone, S. V., Nguyen, T. T., Schäfer, H. & Holmans, P. (2010). Meta-analysis of genome-wide association studies of attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child & Adolescent Psychiatry* 49(9): 884-897.

Negrão, S., Cecília Almadanim, M., Pires, I. S., Abreu, I. A., Maroco, J., Courtois, B., Gregorio, G. B., McNally, K. L. & Margarida Oliveira, M. (2013). New allelic variants found in key rice salt-tolerance genes: an association study. *Plant Biotechnology Journal* 11(1): 87-100.

Negrão, S., Schmöckel, S. M. & Tester, M. (2016). Evaluating physiological responses of plants to salinity stress. *Annals of Botany*.

Nguyen, T. X. & Sticklen, M. (2013). Barley HVA1 Gene Confers Drought and Salt Tolerance in Transgenic Maize Zea Mays L.). *Advances in Crop Science and Technology* 2013.

Ni, J., Colowit, P. M. & Mackill, D. J. (2002). Evaluation of Genetic Diversity in Rice Subspecies Using Microsatellite Markers. *Crop Science* 42(2): 601-607.

Niones, J. M. (2004). Five mapping of the salinity tolerance gene on chromosome 1 of rice (Oryza sativa L.) using near-isogenic lines.

Ntanos, D. A. & Koutroubas, S. D. (2002). Dry matter and N accumulation and translocation for Indica and Japonica rice under Mediterranean conditions. *Field Crops Research* 74(1): 93-101.

Nutan, K. K., Kushwaha, H. R., Singla-Pareek, S. L. & Pareek, A. (2017). Transcription dynamics of Saltol QTL localized genes encoding transcription factors, reveals their differential regulation in contrasting genotypes of rice. *Functional & Integrative Genomics* 17(1): 69-83.

Obata, T., Kitamoto, H. K., Nakamura, A., Fukuda, A. & Tanaka, Y. (2007). Rice shaker potassium channel OsKAT1 confers tolerance to salinity stress on yeast and rice cells. *Plant Physiology* 144(4): 1978-1985.

Ohta, M., Hayashi, Y., Nakashima, A., Hamada, A., Tanaka, A., Nakamura, T. & Hayakawa, T. (2002). Introduction of a Na^+/H^+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. *FEBS Letters* 532(3): 279-282.

Oikeh, S., Touré, A., Sidibé, B., Niang, A., Semon, M., Sokei, Y. & Mariko, M. (2009). Responses of upland NERICA® rice varieties to nitrogen and plant density. *Archives of Agronomy and Soil Science* 55(3): 301-314.

Olías, R., Eljakaoui, Z., Pardo, J. M. & Belver, A. (2009). The Na^+/H^+ exchanger SOS1 controls extrusion and distribution of Na^+ in tomato plants under salinity conditions. *Plant Signaling & Behavior* 4(10): 973-976.

Oliveira, P. C. P. d., Gloaguen, T. V., Gonçalves, R. A. B., Santos, D. L. & Couto, C. F. (2016). Soil Chemistry after Irrigation with Treated Wastewater in Semiarid Climate. *Revista Brasileira de Ciência do Solo* 40.

Oliver, M. J., Cushman, J. C. & Koster, K. L. (2010).Dehydration tolerance in plants. In *Plant stress tolerance*, 3-24: Springer.

Olyaei, F., Baninasab, B., Ghobadi, C. & Gholami, M. (2016). Ion content and its correlation with some physiological parameters in olive cultivars in response to salinity. *Iran Agricultural Research* 34(2): 61-70.

Oppenheimer, M. & Anttila-Hughes, J. K. (2016). The Science of Climate Change. *The Future of Children*: 11-30.

Page, D. R. & Grossniklaus, U. (2002). The art and design of genetic screens: Arabidopsis thaliana. *Nature Reviews Genetics* 3(2): 124-136.

Pandey, G. K., Cheong, Y. H., Kim, K. N., Grant, J. J., Li, L., Hung, W., D'Angelo, C., Weinl, S., Kudla, J. & Luan, S. (2004). The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in Arabidopsis. *Plant Cell* 16(7): 1912-1924.

Pandit, A., Rai, V., Bal, S., Sinha, S., Kumar, V., Chauhan, M., Gautam, R. K., Singh, R., Sharma, P. C. & Singh, A. K. (2010). Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (Oryza sativa L.). *Molecular Genetics and Genomics* 284(2): 121-136.

Pantalião, G. F., Narciso, M., Guimarães, C., Castro, A., Colombari, J. M., Breseghello, F., Rodrigues, L., Vianello, R. P., Borba, T. O. & Brondani, C. (2016). Genome wide association study (GWAS) for grain yield in rice cultivated under water deficit. *Genetica*: 1-14.

Pantoja, O., Dainty, J. & Blumwald, E. (1989). Ion channels in vacuoles from halophytes and glycophytes. *FEBS Letters* 255(1): 92-96.

Pasam, R. K., Sharma, R., Malosetti, M., van Eeuwijk, F. A., Haseneyer, G., Kilian, B. & Graner, A. (2012). Genome-wide association studies for agronomical traits in a world wide spring barley collection. *BMC plant biology* 12(1): 16.

Passricha, N., Saifi, S., Ansari, M. W. & Tuteja, N. (2016). Prediction and validation of cis-regulatory elements in 5′ upstream regulatory regions of lectin receptor-like kinase gene family in rice. *Protoplasma*: 1-16.

Patade, V. Y., Bhargava, S. & Suprasanna, P. (2009). Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. *Agriculture, Ecosystems & Environment* 134(1–2): 24-28.

Peiter, E., Maathuis, F. J., Mills, L. N., Knight, H., Pelloux, J., Hetherington, A. M. & Sanders, D. (2005). The vacuolar Ca^{2+}-activated channel TPC1 regulates germination and stomatal movement. *Nature* 434(7031): 404-408.

Peleg, Z., Reguera, M., Tumimbang, E., Walia, H. & Blumwald, E. (2011). Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnology Journal* 9(7): 747-758.

Pértille, F., Guerrero-Bosagna, C., da Silva, V. H., Boschiero, C., da Silva Nunes, J. d. R., Ledur, M. C., Jensen, P. & Coutinho, L. L. (2016). High-throughput and Cost-effective Chicken Genotyping Using Next-Generation Sequencing. *Scientific Reports* 6: 26929.

Platten, J. D., Egdane, J. A. & Ismail, A. M. (2013). Salinity tolerance, Na^+ exclusion and allele mining of HKT1;5 in *Oryza sativa* and *O. glaberrima*: many sources, many genes, one mechanism? *BMC plant biology* 13(1): 32.

Podar, D. (2013). Plant Growth and Cultivation. *Plant Mineral Nutrients: Methods and Protocols*: 23-45.

Qi, Z., Hampton, C. R., Shin, R., Barkla, B. J., White, P. J. & Schachtman, D. P. (2008). The high affinity K^+ transporter *AtHAK5* plays a physiological role in planta at very low K^+ concentrations and provides a caesium uptake pathway in Arabidopsis. *Journal of Experimental Botany* 59(3): 595-607.

Qiao, H., Chang, K. N., Yazaki, J. & Ecker, J. R. (2009). Interplay between ethylene, ETP1/ETP2 F-box proteins, and degradation of EIN2 triggers ethylene responses in Arabidopsis. *Genes & development* 23(4): 512-521.

Qu, Y., Mu, P., Zhang, H., Chen, C. Y., Gao, Y., Tian, Y., Wen, F. & Li, Z. (2008). Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica* 133(2): 187-200.

R Development Core Team (2016). A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.*

Raghavendra, A. S., Gonugunta, V. K., Christmann, A. & Grill, E. (2010). ABA perception and signalling. *Trends in Plant Science* 15(7): 395-401.

Rahman, M. A., Thomson, M. J., Shah-E-Alam, M., de Ocampo, M., Egdane, J. & Ismail, A. M. (2016). Exploring novel genetic sources of salinity tolerance in rice through molecular and physiological characterization. *Annals of Botany*.

Ramensky, V., Bork, P. & Sunyaev, S. (2002). Human non-synonymous SNPs: server and survey. *Nucleic acids research* 30(17): 3894-3900.

Rasband, W. S. (2008). ImageJ. http://rsbweb. *nih. gov/ij/*.

Rasool, S., Hameed, A., Azooz, M., Siddiqi, T. & Ahmad, P. (2013). Salt stress: causes, types and responses of plants. In *Ecophysiology and Responses of Plants under Salt Stress*, 1-24: Springer.

Ren, Z.-H., Gao, J.-P., Li, L.-G., Cai, X.-L., Huang, W., Chao, D.-Y., Zhu, M.-Z., Wang, Z.-Y., Luan, S. & Lin, H.-X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature genetics* 37(10): 1141-1146.

Rodenburg, J., Diagne, A., Oikeh, S., Futakuchi, K., Kormawa, P., Semon, M., Akintayo, I., Cissé, B., Sié, M. & Narteh, L. (2006). Achievements and impact of NERICA on sustainable rice production in sub-Saharan Africa. *International Rice Commission Newsletter* 55(1): 45-58.

Rodríguez-Navarro, A. & Rubio, F. (2006). High-affinity potassium and sodium transport systems in plants. *Journal of Experimental Botany* 57(5): 1149-1160.

Romero-Aranda, R., Soria, T. & Cuartero, J. (2001). Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science* 160(2): 265-272.

Rosas-Santiago, P., Lagunas-Gómez, D., Barkla, B. J., Vera-Estrella, R., Lalonde, S., Jones, A., Frommer, W. B., Zimmermannova, O., Sychrová, H. & Pantoja, O. (2015). Identification of rice cornichon as a possible cargo receptor for the Golgi-localized sodium transporter *OsHKT1;3*. *Journal of Experimental Botany* 66(9): 2733-2748.

Rosenvasser, S., Mayak, S. & Friedman, H. (2006). Increase in reactive oxygen species (ROS) and in senescence-associated gene transcript (SAG) levels during dark-induced senescence of Pelargonium cuttings, and the effect of gibberellic acid. *Plant Science* 170(4): 873-879.

Rubio, F., Gassmann, W. & Schroeder, J. I. (1995). Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science* 270(5242): 1660-1663.

Ruijter, A. J. M. d., GENNIP, A. H. v., CARON, H. N., KEMP, S. & KUILENBURG, A. B. P. v. (2003). Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochemical Journal* 370(3): 737-749.

Ruiz-Sola, M. Á. & Rodríguez-Concepción, M. (2012). Carotenoid biosynthesis in Arabidopsis: a colorful pathway. *The Arabidopsis Book*: e0158.

Russell, R. S. & Shorrocks, V. (1959). The relationship between transpiration and the absorption of inorganic ions by intact plants. *Journal of Experimental Botany* 10(2): 301-316.

Sakai, T., Duque, M. C., Vallejo Cabrera, F. A., Martínez, C. P. & Ishitani, M. (2010). Establishment of drought screening protocols for rice under field conditions. *Acta Agronómica* 59(3): 338-346.

Sakina, A., Ahmed, I., Shahzad, A., Iqbal, M. & Asif, M. (2016). Genetic Variation for Salinity Tolerance in Pakistani Rice (Oryza sativa L.) Germplasm. *Journal of Agronomy and Crop Science* 202(1): 25-36.

Sakuraba, Y., Han, S.-H., Yang, H.-J., Piao, W. & Paek, N.-C. (2016). Mutation of Rice Early Flowering3.1 (OsELF3.1) delays leaf senescence in rice. *Plant Molecular Biology* 92(1): 223-234.

Sakuraba, Y., Jeong, J., Kang, M.-Y., Kim, J., Paek, N.-C. & Choi, G. (2014). Phytochrome-interacting transcription factors PIF4 and PIF5 induce leaf senescence in Arabidopsis. *Nature Communications* 5: 4636.

SAS, I. (1999). *SAS/STAT user's guide, version 8.* SAS Institute.

SAS Institute, I. (2011).SAS 9.3. Software. SAS Institute, Inc. Cary, NC.

Schachtman, D. P. & Schroeder, J. I. (1994). Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. *Nature* 370(6491): 655-658.

Schuler, G., Boguski, M., Stewart, E. & Stein, L. (1996). A gene map of the human genome. *Science* 274(5287): 540.

Semon, M., Nielsen, R., Jones, M. P. & McCouch, S. R. (2005). The Population Structure of African Cultivated Rice Oryza glaberrima (Steud.). *Genetics* 169(3): 1639-1647.

Sessions, A., Burke, E., Presting, G., Aux, G., McElver, J., Patton, D., Dietrich, B., Ho, P., Bacwaden, J. & Ko, C. (2002). A high-throughput Arabidopsis reverse genetics system. *The Plant Cell* 14(12): 2985-2994.

Sewelam, N., Kazan, K., Thomas-Hall, S. R., Kidd, B. N., Manners, J. M. & Schenk, P. M. (2013). Ethylene response factor 6 is a regulator of reactive oxygen species signaling in Arabidopsis. *PLoS ONE* 8(8): e70289.

Shabala, S. (2000). Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant, Cell & Environment* 23(8): 825-837.

Shabala, S., Shabala, S., Cuin, T. A., Pang, J., Percey, W., Chen, Z., Conn, S., Eing, C. & Wegner, L. H. (2010). Xylem ionic relations and salinity tolerance in barley. *The Plant Journal* 61(5): 839-853.

Shannon, M. C. & Grieve, C. M. (1998). Tolerance of vegetable crops to salinity. *Scientia Horticulturae* 78(1–4): 5-38.

Sharoni, A. M., Nuruzzaman, M., Satoh, K., Moumeni, A., Attia, K., Venuprasad, R., Serraj, R., Kumar, A., Leung, H. & Islam, A. R. (2012). Comparative transcriptome analysis of AP2/EREBP gene family under normal and hormone treatments, and under two drought stresses in NILs setup by Aday Selection and IR64. *Molecular Genetics and Genomics* 287(1): 1-19.

Shen, Y., Shen, L., Shen, Z., Jing, W., Ge, H., Zhao, J. & Zhang, W. (2015). The potassium transporter OsHAK21 functions in the maintenance of ion homeostasis and tolerance to salt stress in rice. *Plant, Cell & Environment*.

Shimizu, A., Yanagihara, S., Kawasaki, S. & Ikehashi, H. (2004). Phosphorus deficiency-induced root elongation and its QTL in rice (Oryza sativa L.). *Theoretical and Applied Genetics* 109(7): 1361-1368.

Shinada, H., Yamamoto, T., Sato, H., Yamamoto, E., Hori, K., Yonemaru, J., Sato, T. & Fujino, K. (2015). Quantitative trait loci for rice blast resistance detected in a local rice breeding population by genome-wide association mapping. *Breeding Science* 65(5): 388.

Si, L., Chen, J., Huang, X., Gong, H., Luo, J., Hou, Q., Zhou, T., Lu, T., Zhu, J. & Shangguan, Y. (2016). OsSPL13 controls grain size in cultivated rice. *Nature genetics* 48(4): 447-456.

Sikirou, M., Shittu, A., Konaté, K. A., Maji, A. T., Ngaujah, A. S., Sanni, K. A., Ogunbayo, S. A., Akintayo, I., Saito, K., Dramé, K. N., Ahanchédé, A. & Venuprasad, R. (2016). Screening African rice (*Oryza glaberrima*) for tolerance to abiotic stresses: I. Fe toxicity. *Field Crops Research*.

Singh, A., Gopalakrishnan, S., Singh, V., Prabhu, K., Mohapatra, T., Singh, N., Sharma, T., Nagarajan, M., Vinod, K. & Singh, D. (2011). Marker assisted selection: a paradigm shift in Basmati breeding. *Indian Journal of Genetics and Plant Breeding* 71(2): 120.

Singh, A., Kanwar, P., Yadav, A. K., Mishra, M., Jha, S. K., Baranwal, V., Pandey, A., Kapoor, S., Tyagi, A. K. & Pandey, G. K. (2014). Genome-wide expressional and functional analysis of calcium transport elements during abiotic stress and development in rice. *Febs Journal* 281(3): 894-915.

Singh, A. K., Kumar, R., Tripathi, A. K., Gupta, B. K., Pareek, A. & Singla-Pareek, S. L. (2015a). Genome-wide investigation and expression analysis of Sodium/Calcium exchanger gene family in rice and Arabidopsis. *Rice* 8(1): 1-16.

Singh, B. P., Jayaswal, P. K., Singh, B., Singh, P. K., Kumar, V., Mishra, S., Singh, N., Panda, K. & Singh, N. K. (2015b). Natural allelic diversity in OsDREB1F gene in the Indian wild rice germplasm led to ascertain its association with drought tolerance. *Plant Cell Reports* 34(6): 993-1004.

Singh, D., Singh, B., Mishra, S., Singh, A. K., Sharma, T. R. & Singh, N. K. (2016a). Allelic diversity for salt stress responsive candidate genes among Indian rice landraces.

Singh, K., Neelam, K., Kaur, A. & Kaur, K. (2016b).Rice. In *Broadening the Genetic Base of Grain Cereals*, 27-65 (Eds M. Singh and S. Kumar). New Delhi: Springer India.

Sitton, D., Itai, C. & Kende, H. (1967). Decreased cytokinin production in the roots as a factor in shoot senescence. *Planta* 73(3): 296-300.

Smedema, L. K. & Shiati, K. (2002). Irrigation and salinity: a perspective review of the salinity hazards of irrigation development in the arid zone. *Irrigation and drainage systems* 16(2): 161-174.

Smyth, D. J., Cooper, J. D., Bailey, R., Field, S., Burren, O., Smink, L. J., Guja, C., Ionescu-Tirgoviste, C., Widmer, B. & Dunger, D. B. (2006). A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nature genetics* 38(6): 617-619.

Sobahan, M. A., Akter, N., Ohno, M., Okuma, E., Hirai, Y., Mori, I. C., Nakamura, Y. & Murata, Y. (2012). Effects of exogenous proline and glycinebetaine on the salt tolerance of rice cultivars. *Bioscience, biotechnology, and biochemistry* 76(8): 1568-1570.

Soda, N., Ephrath, J. E., Dag, A., Beiersdorf, I., Presnov, E., Yermiyahu, U. & Ben-Gal, A. (2016). Root growth dynamics of olive (Olea europaea L.) affected by irrigation induced salinity. *Plant and Soil*: 1-14.

Stamatiou, G., Vidaurre, D. P., Shim, I., Tang, X., Moeder, W., Bonetta, D. & McCourt, P. (2013). Forward genetic screening for the improved production of fermentable sugars from plant biomass. *PLoS ONE* 8(1): e55616.

Strange, R. N. & Scott, P. R. (2005). Plant disease: a threat to global food security. *Phytopathology* 43.

Suzuki, K., Yamaji, N., Costa, A., Okuma, E., Kobayashi, N. I., Kashiwagi, T., Katsuhara, M., Wang, C., Tanoi, K. & Murata, Y. (2016). OsHKT1;4-mediated Na^+ transport in stems contributes to Na^+ exclusion from leaf blades of rice at the reproductive growth stage upon salt stress. *BMC plant biology* 16(1): 1.

Svishcheva, G. R., Axenovich, T. I., Belonogova, N. M., Van Duijn, C. M. & Aulchenko, Y. S. (2012). Rapid variance components-based method for whole-genome association analysis. *Nature genetics* 44(10): 1166-1170.

Sweeney, M. & McCouch, S. (2007). The complex history of the domestication of rice. *Annals of Botany* 100(5): 951-957.

Talla, S. K., Panigrahy, M., Kappara, S., Nirosha, P., Neelamraju, S. & Ramanan, R. (2016). Cytokinin delays dark-induced senescence in rice by maintaining the chlorophyll cycle and photosynthetic complexes. *Journal of Experimental Botany* 67(6): 1839-1851.

Tan, L., Li, X., Liu, F., Sun, X., Li, C., Zhu, Z., Fu, Y., Cai, H., Wang, X. & Xie, D. (2008). Control of a key transition from prostrate to erect growth in rice domestication. *Nature genetics* 40(11): 1360-1364.

Tanner, W. & Beevers, H. (1990). Does transpiration have an essential function in long-distance ion transport in plants? *Plant, Cell & Environment* 13(8): 745-750.

Tapken, D. & Hollmann, M. (2008). Arabidopsis thaliana Glutamate Receptor Ion Channel Function Demonstrated by Ion Pore Transplantation. *Journal of Molecular Biology* 383(1): 36-48.

Tello-Ruiz, M. K., Stein, J., Wei, S., Preece, J., Olson, A., Naithani, S., Amarasinghe, V., Dharmawardhana, P., Jiao, Y. & Mulvaney, J. (2015). Gramene 2016: comparative plant genomics and pathway resources. *Nucleic acids research*: gkv1179.

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

Thomson, M. J., de Ocampo, M., Egdane, J., Rahman, M. A., Sajise, A. G., Adorada, D. L., Tumimbang-Raiz, E., Blumwald, E., Seraj, Z. I. & Singh, R. K. (2010). Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. *Rice* 3(2-3): 148-160.

Till, B. J., Reynolds, S. H., Weil, C., Springer, N., Burtner, C., Young, K., Bowers, E., Codomo, C. A., Enns, L. C. & Odden, A. R. (2004). Discovery of induced point mutations in maize genes by TILLING. *BMC plant biology* 4(1): 12.

Tiwari, S., Krishnamurthy, S., Kumar, V., Singh, B., Rao, A., Rai, V., Singh, A. K. & Singh, N. K. (2016). Mapping QTLs for Salt Tolerance in Rice (Oryza sativa L.) by Bulked Segregant Analysis of Recombinant Inbred Lines Using 50K SNP Chip. *PLoS ONE* 11(4): e0153610.

Tol, N., Pinas, J., Schat, H., Hooykaas, P. J. & Zaal, B. J. (2016). Genome interrogation for novel salinity tolerant Arabidopsis mutants. *Plant, Cell & Environment*.

Tyerman, S. D., Skerrett, M., Garrill, A., Findlay, G. P. & Leigh, R. A. (1997). Pathways for the permeation of Na^+ and Cl^- into protoplasts derived from the cortex of wheat roots. *Journal of Experimental Botany* 48(Special Issue): 459-480.

Ueda, Y., Frimpong, F., Qi, Y., Matthus, E., Wu, L., Höller, S., Kraska, T. & Frei, M. (2015). Genetic dissection of ozone tolerance in rice (Oryza sativa L.) by a genome-wide association study. *Journal of Experimental Botany* 66(1): 293-306.

Umezawa, T., Nakashima, K., Miyakawa, T., Kuromori, T., Tanokura, M., Shinozaki, K. & Yamaguchi-Shinozaki, K. (2010). Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. *Plant and Cell Physiology* 51(11): 1821-1839.

Usatov, A., Alabushev, A., Kostylev, P., Azarin, K., Makarenko, M. & Usatova, O. (2015). Introgression the SalTol QTL into the Elite Rice Variety of Russia by Marker-Assisted Selection. *American Journal of Agricultural and Biological Sciences* 10(4): 165-169.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. & Thomas, G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.)—differential response in salt-tolerant and sensitive varieties. *Plant Science* 165(6): 1411-1418.

Vaughan, D. A., Morishima, H. & Kadowaki, K. (2003). Diversity in the Oryza genus. *Current Opinion in Plant Biology* 6(2): 139-146.

Vaughan, J. (2015).Investigating the genetic basis of natural leaf shape variation in Arabidopsis thaliana. University of York.

Venuprasad, R., Dalid, C., Del Valle, M., Zhao, D., Espiritu, M., Cruz, M. S., Amante, M., Kumar, A. & Atlin, G. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics* 120(1): 177-190.

Venuprasad, R., Impa, S., Gowda, R., Atlin, G. & Serraj, R. (2011). Rice near-isogenic-lines (NILs) contrasting for grain yield under lowland drought stress. *Field Crops Research* 123(1): 38-46.

Venuprasad, R., Lafitte, H. & Atlin, G. (2007). Response to direct selection for grain yield under drought stress in rice. *Crop Science* 47(1): 285-293.

Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J. & Zhu, J. K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal* 45(4): 523-539.

Verulkar, S., Mandal, N., Dwivedi, J., Singh, B., Sinha, P., Mahato, R., Dongre, P., Singh, O., Bose, L. & Swain, P. (2010). Breeding resilient and productive genotypes adapted to drought-prone rainfed ecosystem of India. *Field Crops Research* 117(2): 197-208.

Vibhuti, C. S., Bargali, K. & Bargali, S. (2015). Seed germination and seedling growth parameters of rice (Oryza sativa L.) varieties as affected by salt and water stress. *Indian Journal of Agricultural Sciences* 85(1): 102-108.

Vidaurre, D. & Bonetta, D. (2012). Accelerating forward genetics for cell wall deconstruction. *Frontiers in Plant Science* 3: 119.

Vu, H. T. T., Le, D. D., Ismail, A. M. & Le, H. H. (2012). Marker-assisted backcrossing (MABC) for improved salinity tolerance in rice (Oryza sativa L.) to cope with climate change in Vietnam. *Australian Journal of Crop Science* 6(12): 1649.

Wada, T., Miyahara, K., Sonoda, J.-y., Tsukaguchi, T., Miyazaki, M., Tsubone, M., Ando, T., Ebana, K., Yamamoto, T. & Iwasawa, N. (2015). Detection of QTLs for white-back and basal-white grains caused by high temperature during ripening period in japonica rice. *Breeding Science* 65(3): 216.

Wang, C., Yang, Y., Wang, H., Ran, X., Li, B., Zhang, J. & Zhang, H. (2016a). Ectopic expression of a cytochrome P450 monooxygenase gene PtCYP714A3 from Populus trichocarpa reduces shoot growth and improves tolerance to salt stress in transgenic rice. *Plant Biotechnology Journal*.

Wang, D., Zhu, J., Li, Z. & Paterson, A. (1999). Mapping QTLs with epistatic effects and QTL× environment interactions by mixed linear model approaches. *Theoretical and Applied Genetics* 99(7-8): 1255-1264.

Wang, H., Xu, X., Vieira, F. G., Xiao, Y., Li, Z., Wang, J., Nielsen, R. & Chu, C. (2016b). The power of inbreeding: NGS based GWAS of rice reveals convergent evolution during rice domestication. *Molecular Plant*.

Wang, M., Yu, Y., Haberer, G., Marri, P. R., Fan, C., Goicoechea, J. L., Zuccolo, A., Song, X., Kudrna, D. & Ammiraju, J. S. (2014). The genome sequence of African rice (Oryza glaberrima) and evidence for independent domestication. *Nature genetics* 46(9): 982-988.

Wang, R., Jing, W., Xiao, L., Jin, Y., Shen, L. & Zhang, W. (2015). The Rice High-Affinity Potassium Transporter1;1 Is Involved in Salt Tolerance and Regulated by an MYB-Type Transcription Factor. *Plant Physiology* 168(3): 1076-1090.

Wang, Y., Peng, X., Xu, W., Luo, Y., Zhao, W., Hao, J., Liang, Z., Zhang, Y. & Huang, K. (2011). Transcript and protein profiling analysis of OTA-induced cell death reveals the regulation of the toxicity response process in Arabidopsis thaliana. *Journal of Experimental Botany*: err447.

Wang, Y., Zang, J., Sun, Y., Ali, J., Xu, J. & Li, Z. (2013). Background-independent quantitative trait loci for drought tolerance identified using advanced backcross introgression lines in rice. *Crop Science* 53(2): 430-441.

Wang, Z., Chen, Z., Cheng, J., Lai, Y., Wang, J., Bao, Y., Huang, J. & Zhang, H. (2012). QTL analysis of Na^+ and K^+ concentrations in roots and shoots under different levels of NaCl stress in rice (*Oryza sativa* L.). *PLoS ONE* 7(12): e51202.

Wang, Z. & Sherwood, D. R. (2011). Dissection of genetic pathways in C. elegans. *Methods in cell biology* 106: 113.

Ward Jr, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American statistical association* 58(301): 236-244.

Waziri, A., Kumar, P. & Purty, R. (2016). Saltol QTL and their role in salinity tolerance in rice. *Austin J. Biotechnol. Bioeng* 3: 1067-1072.

Weerakoon, W. M. W., Mutunayake, M. M. P., Bandara, C., Rao, A. N., Bhandari, D. C. & Ladha, J. K. (2011). Direct-seeded rice culture in Sri Lanka: Lessons from farmers. *Field Crops Research* 121(1): 53-63.

Wegner, L. H. & De Boer, A. H. (1997). Properties of two outward-rectifying channels in root xylem parenchyma cells suggest a role in K^+ homeostasis and long-distance signaling. *Plant Physiology* 115(4): 1707-1719.

Wei, J., Li, C., Li, Y., Jiang, G., Cheng, G. & Zheng, Y. (2013). Effects of External Potassium (K) Supply on Drought Tolerances of Two Contrasting Winter Wheat Cultivars. *PLoS ONE* 8(7): e69737.

Weigel, D. (2012). Natural variation in Arabidopsis: from molecular genetics to ecological genomics. *Plant Physiology* 158(1): 2-22.

Windt, C. W., Vergeldt, F. J., De Jager, P. A. & Van As, H. (2006). MRI of long-distance water transport: a comparison of the phloem and xylem flow characteristics and dynamics in poplar, castor bean, tomato and tobacco. *Plant, Cell & Environment* 29(9): 1715-1729.

Wolf, O. & Dieter Jeschke, W. (1987). Modeling of Sodium and Potassium Flows via Phloem and Xylem in the Shoot of Salt-stressed Barley. *Journal of Plant Physiology* 128(4–5): 371-386.

Wolf, O., JESCHKE, W. D. & HARTUNG, W. (1990). Long Distance Transport of Abscisic Acid in NaCI-Treated Intact Plants of Lupinus albus. *Journal of Experimental Botany* 41(5): 593-600.

Wu, J., Zhang, Z., Zhang, Q., Liu, Y., Zhu, B., Cao, J., Li, Z., Han, L., Jia, J. & Zhao, G. (2015). Generation of Wheat Transcription Factor FOX Rice Lines and Systematic Screening for Salt and Osmotic Stress Tolerance. *PLoS ONE* 10(7): e0132314.

Wu, S.-J., Ding, L. & Zhu, J.-K. (1996). SOS1, a genetic locus essential for salt tolerance and potassium acquisition. *The Plant Cell Online* 8(4): 617-627.

Wu, T., Yang, C., Ding, B., Feng, Z., Wang, Q., He, J., Tong, J., Xiao, L., Jiang, L. & Wan, J. (2016). Microarray-based gene expression analysis of strong seed dormancy in rice cv. N22 and less dormant mutant derivatives. *Plant Physiology and Biochemistry* 99: 27-38.

Wu, Y., Jiang, L., Zheng, Y., Wang, C. & Zhao, Z. (2014). Morphological trend analysis of rice phytolith during the early Neolithic in the Lower Yangtze. *Journal of Archaeological Science* 49: 326-331.

Xiong, L., Wang, R.-G., Mao, G. & Koczan, J. M. (2006). Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiology* 142(3): 1065-1074.

Xu, J., Li, H.-D., Chen, L.-Q., Wang, Y., Liu, L.-L., He, L. & Wu, W.-H. (2006a). A protein kinase, interacting with two calcineurin B-like proteins, regulates K^+ transporter AKT1 in Arabidopsis. *Cell* 125(7): 1347-1360.

Xu, K., Xu, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R., Heuer, S., Ismail, A. M., Bailey-Serres, J., Ronald, P. C. & Mackill, D. J. (2006b). Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442(7103): 705-708.

Xu, M.-R., Huang, L.-Y., Zhang, F., Zhu, L.-H., Zhou, Y.-L. & Li, Z.-K. (2013). Genome-wide phylogenetic analysis of stress-activated protein kinase genes in rice (OsSAPKs) and expression profiling in response to Xanthomonas oryzae pv. oryzicola infection. *Plant Molecular Biology Reporter* 31(4): 877-885.

Xuan, T. D., Minh, T. N., Anh, L. H. & Khanh, T. D. (2016). Allelopathic momilactones A and B are implied in rice drought and salinity tolerance, not weed resistance. *Agronomy for Sustainable Development* 36(3): 52.

Xue, Y., Warburton, M. L., Sawkins, M., Zhang, X., Setter, T., Xu, Y., Grudloyma, P., Gethi, J., Ribaut, J.-M. & Li, W. (2013). Genome-wide association analysis for nine agronomic traits in maize under well-watered and water-stressed conditions. *Theoretical and Applied Genetics* 126(10): 2587-2596.

Xue, Z.-Y., Zhi, D.-Y., Xue, G.-P., Zhang, H., Zhao, Y.-X. & Xia, G.-M. (2004). Enhanced salt tolerance of transgenic wheat (*Tritivum aestivum* L.) expressing a vacuolar Na^+/H^+ antiporter gene with improved grain yields in saline soils in the field and a reduced level of leaf Na^+. *Plant Science* 167(4): 849-859.

Yamada, H., Takimoto, S., Toshinari, D., Kataoka, K. & Habu, T. (2015). Effect of Seawater Irrigation on Water Relations and Fruit Quality in Potted Citrus Trees. *The Horticulture Journal* (0).

Yang, L., Zu, Y.-G. & Tang, Z.-H. (2013). Ethylene improves *Arabidopsis* salt tolerance mainly via retaining K^+ in shoots and roots rather than decreasing tissue Na^+ content. *Environmental and Experimental Botany* 86: 60-69.

Yang, Q., Chen, Z.-Z., Zhou, X.-F., Yin, H.-B., Li, X., Xin, X.-F., Hong, X.-H., Zhu, J.-K. & Gong, Z. (2009). Overexpression of SOS (Salt Overly Sensitive) genes increases salt tolerance in transgenic Arabidopsis. *Molecular Plant* 2(1): 22-31.

Yeo, A., Caporn, S. & Flowers, T. (1985). The effect of salinity upon photosynthesis in rice (Oryza sativa L.): gas exchange by individual leaves in relation to their salt content. *Journal of Experimental Botany* 36(8): 1240-1248.

Yeo, A., Izard, P., Boursier, P. & Flowers, T. (1991). Short-and long-term effects of salinity on leaf growth in rice (Oryza sativa L.). *Journal of Experimental Botany* 42(7): 881-889.

Yoshida, R., Umezawa, T., Mizoguchi, T., Takahashi, S., Takahashi, F. & Shinozaki, K. (2006). The regulatory domain of SRK2E/OST1/SnRK2. 6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis. *Journal of Biological Chemistry* 281(8): 5310-5318.

Yoshida, S., Forno, D., Cock, J. & Gomez, K. (1976). Laboratory manual for physiological studies of rice.: Manila (Philippines): International Rice Research Institute.

Yoshida, S. & Hara, T. (1977). Effects of air temperature and light on grain filling of an indica and a japonica rice (Oryza sativa L.) under controlled environmental conditions. *Soil Science and Plant Nutrition* 23(1): 93-107.

Yu, J.-K., La Rota, M., Kantety, R. & Sorrells, M. (2004). EST derived SSR markers for comparative mapping in wheat and rice. *Molecular Genetics and Genomics* 271(6): 742-751.

Yuan, X., Sun, H., Tang, Z., Tang, H., Zhang, H. & Huang, J. (2016). A Novel Little Membrane Protein Confers Salt Tolerance in Rice (Oryza sativa L.). *Plant Molecular Biology Reporter* 34(2): 524-532.

Yue, Y., Zhang, M., Zhang, J., Duan, L. & Li, Z. (2012). SOS1 gene overexpression increased salt tolerance in transgenic tobacco by maintaining a higher K^+/Na^+ ratio. *Journal of Plant Physiology* 169(3): 255-261.

Zahir, Z. A., Asghar, H. N. & Arshad, M. (2001). Cytokinin and its precursors for improving growth and yield of rice. *Soil Biology and Biochemistry* 33(3): 405-408.

Zaidi, I., Ebel, C., Belgaroui, N., Ghorbel, M., Amara, I. & Hanin, M. (2016). The wheat MAP kinase phosphatase 1 alleviates salt stress and increases antioxidant activities in Arabidopsis. *Journal of Plant Physiology* 193: 12-21.

Zeng, L. & Shannon, M. C. (2000). Salinity effects on seedling growth and yield components of rice.

Zhang, C., Bruce, H., Yang, T., Charagu, P., Kemp, R. A., Boddicker, N., Miar, Y., Wang, Z. & Plastow, G. (2016). Genome Wide Association Studies (GWAS) Identify QTL on SSC2 and SSC17 Affecting Loin Peak Shear Force in Crossbred Commercial Pigs. *PLoS ONE* 11(2): e0145082.

Zhang, J., Jia, W., Yang, J. & Ismail, A. M. (2006). Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Research* 97(1): 111-119.

Zhang, K., Xia, X., Zhang, Y. & Gan, S.-S. (2012). An ABA-regulated and Golgi-localized protein phosphatase controls water loss during leaf senescence in Arabidopsis. *The Plant Journal* 69(4): 667-678.

Zhang, Q., Maroof, M. A. S., Lu, T. Y. & Shen, B. Z. (1992). Genetic diversity and differentiation of indica and japonica rice detected by RFLP analysis. *Theoretical and Applied Genetics* 83(4): 495-499.

Zhao, F.-Y., Zhang, X.-J., Li, P.-H., Zhao, Y.-X. & Zhang, H. (2006a). Co-expression of the Suaeda salsa SsNHX1 and Arabidopsis AVP1 confer greater salt tolerance to transgenic rice than the single SsNHX1. *Molecular Breeding* 17(4): 341-353.

Zhao, F., Wang, Z., Zhang, Q., Zhao, Y. & Zhang, H. (2006b). RETRACTED ARTICLE: Analysis of the physiological mechanism of salt-tolerant transgenic rice carrying a vacuolar Na^+/H^+ antiporter gene from Suaeda salsa. *Journal of Plant Research* 119(2): 95-104.

Zhao, J., Li, M., Gu, D., Liu, X., Zhang, J., Wu, K., Zhang, X., Teixeira da Silva, J. A. & Duan, J. (2016a). Involvement of rice histone deacetylase HDA705 in seed germination and in

response to ABA and abiotic stresses. *Biochemical and Biophysical Research Communications* 470(2): 439-444.

Zhao, K., Tung, C.-W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., Norton, G. J., Islam, M. R., Reynolds, A. & Mezey, J. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. *Nature Communications* 2: 467.

Zhao, Y., Chan, Z., Gao, J., Xing, L., Cao, M., Yu, C., Hu, Y., You, J., Shi, H. & Zhu, Y. (2016b). ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proceedings of the National Academy of Sciences* 113(7): 1949-1954.

Zheng, H., Zhao, H., Liu, H., Wang, J. & Zou, D. (2015). QTL analysis of Na^+ and K^+ concentrations in shoots and roots under NaCl stress based on linkage and association analysis in japonica rice. *Euphytica* 201(1): 109-121.

Zhou, X., Jiang, Y. & Yu, D. (2011). WRKY22 transcription factor mediates dark-induced leaf senescence in Arabidopsis. *Molecules and Cells* 31(4): 303-313.

Zhu, D., Kang, H., Li, Z., Liu, M., Zhu, X., Wang, Y., Wang, D., Wang, Z., Liu, W. & Wang, G.-L. (2016a). A Genome-Wide Association Study of Field Resistance to Magnaporthe Oryzae in Rice. *Rice* 9(1): 44.

Zhu, J.-K. (2000). Genetic analysis of plant salt tolerance using Arabidopsis. *Plant Physiology* 124(3): 941-948.

Zhu, J., Fu, X., Koo, Y. D., Zhu, J.-K., Jenney, F. E., Adams, M. W., Zhu, Y., Shi, H., Yun, D.-J. & Hasegawa, P. M. (2007). An enhancer mutant of Arabidopsis salt overly sensitive 3 mediates both ion homeostasis and the oxidative stress response. *Molecular and cellular biology* 27(14): 5214-5224.

Zhu, M., Zhou, M., Shabala, L. & Shabala, S. (2016b). Physiological and molecular mechanisms mediating xylem Na^+ loading in barley in the context of salinity stress tolerance. *Plant, Cell & Environment*.

Zong, J.-M., Li, X.-W., Zhou, Y.-H., Wang, F.-W., Wang, N., Dong, Y.-Y., Yuan, Y.-X., Chen, H., Liu, X.-M., Yao, N. & Li, H.-Y. (2016). The AaDREB1 Transcription Factor from the Cold-Tolerant Plant Adonis amurensis Enhances Abiotic Stress Tolerance in Transgenic Plant. *International Journal of Molecular Sciences* 17(4): 611.