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Molecular Genetics of Salinity Tolerance in Rice (Oryza sativa L.)

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MOLECULAR GENETICS OF SALINITY TOLERANCE IN RICE (*ORYZA SATIVA L.*)

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LIST OF ABBREVIATIONS

µl	Microliter
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
Ave	Average
B	Bengal
Can1	Canonical discriminant function 1
Can2	Canonical discriminant function 2
CHL	Chlorophyll content
Chl_R	% Chlorophyll reduction
cM	centi-Morgan
conc	Concentration
CORR	Correlation
CTAB	Cetyl trimethylammonium bromide
Ctr	Control
df	Degree of freedom
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
DPS	Days post salinization
DWT	Dry weight
F_6	6^{th} filial generation
FLDA	Fisher linear discriminant analysis
GBS	Genotyping-by-sequencing
GRIN	Germplasm Resources Information Network
H	Broad sense heritability computed on family mean basis
Ha	Hectare
HKT	High-affinity K^+ transporter
HS	Highly sensitive
HT	Highly tolerant
ICIM-ADD	Inclusive composite interval mapping for additive QTLs
ILs	Introgression lines
IM-ADD	Interval mapping for additive QTLs
Ion_leak	Index of injury by ion leakage
IRRI	International Rice Research Institute
K^+	Potassium concentration
Kb	Kilobase
LOD	Logarithm of the odds
LRT	Stepwise regression-based likelihood ratio test
LS	Least square
M	Million
Max	Maximum
Mb	Megabase
Min	Minimum
ml	Milliliter

mM	Millimolar
MS	Mean square
MT	Moderately tolerant
Na^+	Sodium concentration
NaK	Ratio of the shoot sodium and shoot potassium content
NHX	Na^+/H^+ transporters
P (rand perm.999)	Significance of genetic distance at 999 random permutations
P	Pokkali
PCR	Polymerase chain reaction
PhiPT	Estimate of genetic distance among populations
PVE	Percent variance explained
qCHL	QTL for chlorophyll content
QDA	Quadratic discriminant analysis
qDWT	QTL for dry weight
qK	QTL for shoot potassium concentration
qNa	QTL for shoot sodium concentration
qNaK	QTL for ratio of the shoot sodium and shoot potassium content
qRTL	QTL for root length
qSHL	QTL for shoot length
qSIS	QTL for salt injury score
qSKC1	QTL for shoot K^+ concentration on chromosome 1
qSRR	QTL for shoot length to root length ratio
QTLs	Quantitative trait loci
R	RIL
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
Ro	Ion leakage in control treatment
Rt	Ion leakage in saline treatment
Rt_Na	Sodium concentration in root
Rt_Na/K	Na/K ratio in root
RTL	Root length
RtL_R	% root length reduction
S	Sensitive
Sal	Saline treatment
Saltol	QTL for salt tolerance
SDLs	Segregation distortion loci
SFR	Super fine resolution
Sh_K	Shoot potassium concentration (mmol/kg)
Sh_Na	Shoot sodium concentration (mmol/kg)
Sh_Na/K	Na^+/K^+ ratio in shoot
SHL	Shoot length
ShL_R	% Shoot length reduction
SIS	Salt injury score
SMA	Single marker analysis
SNP	Single nucleotide polymorphism
SRR	Shoot length to root length ratio

SS	Sum of squares
SSR	Simple sequence repeats
T	Tolerant
Taq	*Thermus aquaticus*
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

ABSTRACT

Due to the threat of salinity stress to Louisiana rice production, an effort was made to understand the molecular genetics of salinity tolerance with the overall goal of developing salt tolerant varieties. The objectives of this study were to 1) determine if salinity tolerance exist in the US rice varieties, 2) map the additive and epistatic QTLs for traits related to seedling salinity tolerance in recombinant inbred lines (RILs) using genotyping-by-sequencing (GBS)-derived SNP markers, and 3) identify and validate stable QTLs and their effects in introgression lines (ILs) of Pokkali in Bengal background. All experiments for phenotypic characterization were conducted in hydroponics at salinity level of 12dSm^{-1} in the greenhouse. Among the thirty Southern US rice varieties characterized under salt stress, LAH10, R609, and Cheniere were tolerant. Additionally, CL162, Jupiter, Jazzman, Templeton, Cypress, Neptune, and Caffey were identified as moderately tolerant based on clustering and discriminant analyses using the linear combination of six traits. On the other hand, clustering based on DNA profiles did not correspond to the varietal grouping based on salinity responses. Nona Bokra, Pokkali, and Pokkali-derived lines remained the donors of choice for highest salt tolerance. Alternatively, TCCP266, Geumgangbyeo, and R609 with few undesirable agronomic traits were recommended as donors for rice improvement. For QTL mapping, 189 lines of F_6 RIL population were phenotyped and characterized by GBS. A total of 9303 SNP markers were used for construction of genetic map. Eighty-five QTLs with small and large effects were identified for nine traits. Of which, 11 QTLs co-localized with 14 reported QTLs. Epistatic QTLs were also mapped and indicated the complexity of salinity tolerance. Based on the annotation of candidate genes within QTL intervals, ion transporters, osmotic regulators, transcription factors, and protein kinases may play important roles in salinity tolerance. On the other hand, at least 14 QTLs in RILs were

validated in the IL population. Our study emphasized the importance of salt injury score (SIS) and seedling vigor-QTLs for salinity tolerance. Based on tolerant ILs, the probable mechanisms of tolerance are Na^+ dilution in leaves, Na^+ ion compartmentation, and by synthesis of compatible solutes. The tolerant ILs will serve as improved variety of Bengal or donor breeding lines for transferring salinity tolerance to other US elite varieties.

CHAPTER 1. INTRODUCTION

1.1 Rice: importance and production

Rice (*Oryza sativa*) is one of the most important crops in the world. It is a staple food for more than half of the world's population. It is planted and produced in 77 countries worldwide. This year, the world's rice production is about 713.8 million (M) tons harvested from 161 M hectares (ha). China, India, and Indonesia are the three largest producers and consumers of rice. These three countries alone account for the 60% of the world rice production and consumption. China, Nigeria, and the Philippines are the top three importers of rice while Thailand, India, and Vietnam are the largest exporters. The USA ranks 11th in production and consumption of rice, with a total of 7.3 M tons of milled rice produced on 1.2 M ha (USDA, 2016). Additionally, the USA is the fifth largest exporter, with about 50% of its rice being exported to Latin America, Asia, Europe, Middle East, and Africa (USARice, 2016). Among the rice-producing states, Louisiana is the third largest producer of rice, with 1.46 M tons of rice harvested from 170, 000 ha of land (USDA, 2016).

There are about 27 species of *Oryza* that are annual or perennial, diploid or tetraploid, and with genome composition grouped in AA, BB, CC, DD, EE, FF, GG, HH, JJ, KK, LL and combinations of these (GRiSP, 2013). However, there are only two widely cultivated rice species, the *Oryza sativa* that originated in Asia and *Oryza glaberrima* from Africa. Under *O. sativa*, there are two subspecies, the *japonica* and *indica* type. *O. sativa* is diplod with two sets of 12 chromosomes (2n=24) (Izawa and Shimamoto, 1996). In 2002, rice genomes were sequenced, with a genome size of 430 Mb and 460 Mb for *japonica* and *indica*, repectively (Goff et al., 2002; Yu et al., 2002). Since then, numerous genomic studies followed. More molecular markers were developed and more QTL mapping studies were conducted. Sequence variations between

cultivated and wild species of rice were compared, and more gene cloning and transformation studies were conducted for the development of more resilient rice (Jackson, 2016).

1.2 Salinity stress and effects on rice plants

There are several constraints to rice production. Among the abiotic stresses, water and soil salinity is a worldwide problem in both irrigated and non-irrigated crop production. Salinity is a condition in which the soil or water has a concentration greater than 4dSm^{-1} of soluble salts, predominantly by sodium. According to the Food and Agriculture Organization of the United Nations (FAO), there are about 397 M ha of land affected by salinity worldwide. Among the 230 M ha of irrigated land used in crop production, 20% (45M ha) were affected by salinity, leading to an estimated cost of about USD 11billion yr^{-1} (Thomas and Morini, 2005).

Natural climatic factors are usually the cause of salinity problem. In the coastal areas, sea water intrusion may contaminate rivers and aquifers. In the arid and semi-arid regions, weathering of basalt rocks releases various types of soluble salts and accumulates over time causing elevated salinity. Secondary salinization, on the other hand, occurs due to irrigation using salt-rich water and poor drainage system (Yadav et al., 2011).

During salinity stress, the high concentration of Na^+ ions in soil reduces the ability of plants to take up water and nutrients (osmotic stress). Later on, plants affected by excessive salt concentration often show reduced growth, leaf damage, necrosis, and eventual death of the crop (ionic stress), resulting to significant yield loss (Flowers, 2004; Munns and Tester, 2008). Among the economically important cereals, rice is most sensitive to salinity stress (Munns and Tester, 2008). Seedlings die at salt level of 10dSm^{-1} (Munns et al., 2006), and yield loss can be as high as ninety percent at 3.5 dSm^{-1} salt stress during the reproductive stage (Asch et al., 2000).

1.3 Mechanisms of salinity tolerance

Plants experience osmotic and ionic stress during salinity stress. Under osmotic stress, the availability of water and nutrients to the plants is limited due to low water potential caused by higher concentration of salts in water or soil surrounding the roots. The mechanism of tolerance under this condition is called osmotic tolerance which is speculated to be regulated by long-distance signaling and perception of salts (Munns and Tester, 2008; Roy et al., 2014). Plants respond to osmotic stress by stomal closure to conserve water and reduced transpiration stream that drives Na^+ ion influx from roots to the shoots of plants (Flowers and Flowers, 2005). At the cellular level, water and low molecular weight compounds enter the plant cells through membrane proteins called aquaporins. Down regulation and over expression of genes encoding aquaporins suggests important role of membrane proteins in water homeostasis during osmotic stress. Additionally, plants accumulate compatible solutes like sucrose, glycine betaine, mannitol, and proline for cellular osmotic adjustment, and for restoration of water uptake to prevent dehydration (Horie et al., 2012).

Ionic stress or Na^+ accumulation in leaves is toxic to plants. The increased accumulation of Na^+ ions was correlated to reduced survival of plants under salinity stress (Yeo et al., 1990). There is no Na^+-selective membrane channel identified in plants. Na^+ ions are believed to be taken up into cells by the same K^+ transporters or nonselective cation channels (Flowers and Flowers, 2005; Demidchik and Maathius, 2007). For this reason, membrane channels and transporters that reduced the accumulation of Na^+ ions in leaves were involved in salinity tolerance by ion exclusion (Negrão et al., 2011). High-affinity K^+ transporters or HKT family is one of the most studied transporters associated to salinity tolerance. In *Arabidopsis*, at high concentration of Na^+, *AtHKT1;1* was shown to be selectively permeable to Na^+ ions and helped

in the removal and recirculation of Na^+ from leaves to roots (Sunarpi et al., 2005). Similarly, Ren et al. (2005) reported *OsHKT1;5* gene in rice with the same function for Na^+/K^+ homeostasis. Both genes were reported to mediate Na^+ exclusion in shoots by unloading Na^+ ions from xylem sap and then reloading them into phloem for transport to the roots. However, recirculation model of Na^+ ions from shoots to roots were not validated using radioactive tracer $^{22}Na^+$, and thus, raised controversy on the role of *HKT* in unloading Na^+ ions from the shoot (Davenport et al., 2007; Munns and Tester, 2008).

Another well known mechanism of salinity tolerance is by compartmentation of Na^+ ions in vacuoles which is considered as tissue tolerance. Na^+/H^+ transporters (NHX) in tonoplast were responsible for selective sequestration Na^+ ions in vacuoles (Negrão et al., 2011). Together with this process is the coordinated cytosolic increased accumulation of K^+ ions and other compatible solutes to balance the osmotic pressure of ions in the vacuoles (Munns and Tester, 2008). Additionally, secretion of Na^+ ions in leaves through specialized modified cells were observed in halophytes (Flowers and Flowers, 2005). In wild relatives of rice, the *Oryza coarctata*, a tetraploid with KKLL genome, was found highly tolerant to salinity stress due to its characteric trichomes (salt hairs) that burst for salt excretion (Bal and Dutt, 1986).

1.4 Genetics and QTL mapping of salinity tolerance

With the variety of plant's responses to salinity stress, it is not surprising to expect the complexity of salinity tolerance. Phenotypic charaterization of mapping populations in rice for morphological and physiological traits under salt stress showed continuous distribution, presence of transgressive segregants, and significant interaction of genotype with the environment. All of these are indications of a quantitative trait and polygenic nature of salinity tolerance (Gregorio and Senadhira, 1993; Koyama et al., 2001; Flowers, 2004). Additionally, QTL mapping for

salinity tolerance-related traits indicated the presence of many small-effect QTLs and very few large-effect QTLs. The list of QTLs for salinity-related traits in rice are available in www.gramene.org. To date, there are around 80 reported QTLs for Na^+, K^+ concentrations, Na^+/K^+ ratio, salt injury score, survival rate, root and shoot lengths, and chlorophyll content under salinity stress. A QTL on chromosome 1 was consistently reported for shoot K^+ concentration. The QTL was named *qSKC1* and was located between 9.82 and13.30 Mb region (Koyama et al., 2001; Lin et al., 2002; Thomson et al., 2010; Wang et al., 2012) from which, an HKT1;5 gene was cloned from Nona Bokra for salinity tolerance (Ren et al., 2005). In a separate study using Pokkali as a salinity tolerance donor, the same locus was associated with low Na^+/K^+ ratio and was named *Saltol* (Gregorio, 1997; Bonilla et al., 2002). For other traits, QTLs were detected on chromosomes 2, 3, 4, 6, 7, 9, 11, 12 and no QTLs were reported yet on chromosomes 5, 8, and 10.

1.5 Rationale for research

While good farming and integrated management practices can be applied, the use of salt tolerant rice varieties is another option to address the problem of salinity. Several traditional rice genotypes were salt tolerant and may provide opportunities to improve salt tolerance of rice through breeding (Gregorio et al., 2002). However, with many factors contributing to salt tolerance, understanding the molecular genetics, physiology, and mechanisms of salinity tolerance are thus essential for the development of salt tolerant rice varieties. Since the magnitude of salinity stress and adaptation of rice vary with different environments, breeding for salt tolerance should be targeted to the growing environments. In this study, the specific objectives were:

1. To characterize the thirty Southern USA rice varieties for salinity tolerance along with 19 donor genotypes of varying levels of salt tolerance;

2. To map the additive and epistatic QTLs for traits related to seedling salinity tolerance in recombinant inbred lines (RILs) using genotyping-by-sequencing (GBS)-derived SNP markers; and

3. To identify and validate stable QTLs and their effects in introgression lines (ILs) of Pokkali in Bengal background.

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CHAPTER 2. GENETIC VARIATION IN SOUTHERN USA RICE GENOTYPES FOR SEEDLING SALINITY TOLERANCE*

2.1 Introduction

The USA is a major exporter of rice to Latin America and Asia. Among the US states, Louisiana is the third largest producer of rice (USDA National Statistics Service, 2013). However, its proximity to the Gulf of Mexico makes it vulnerable to salinity stress. During the hurricane season, salt water intrusion normally occurs in coastal areas. Moreover, if reduced rainfall follows the year of salt water flooding, fresh water gets contaminated with brackish water and recovery of affected areas is hindered (Leonards, 2012). After hurricanes Katrina, Rita, Gustav, and Ike, soil salinity increased in coastal areas of Louisiana. Soil salinity sampled from 2005 to 2008 ranged from 286-4329 parts per million (ppm) (Breitenbeck et al., 2007; Saichuk and Gauthier, 2011; Viator et al., 2011), while water salinity rose to as high as 7,000 ppm between 2001 and 2003 (Branch, 2004).

Louisiana has considerable success for breeding high-yielding rice varieties. However, continuous progress is necessary to meet the demand of the world's increasing population in conjunction with changing climate, environment, and pests. Successful targeted trait improvement depends on the availability of donor genotypes, efficient screening methods, and a thorough understanding of the genetics and physiology of salinity tolerance (Negrão et al., 2011). Despite the establishment of a screening procedure for salinity tolerance by IRRI (Gregorio et al., 1997), consistency and reproducibility of results between laboratories worldwide remains a challenge due to the lack of uniform growth environments. Several studies have been published on the screening method (Yeo et al., 1990; Aslam et al., 1993; Asch et al., 2000), but only a few were in large scale (Yeo at al., 1990; Kanawapee et al., 2012). Although salinity tolerance is

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polygenic, most studies still treat salinity tolerance as a single trait and commonly use visual scoring (Gregorio et al., 1997) or the Na^+/K^+ ratio for classification. Yeo et al. (1990) suggested pyramiding of favorable morphological and physiological traits to increase salinity tolerance. Therefore, a statistical model combining morphological and physiological traits would be more appropriate. Previously, cluster analysis using agronomic and physiological parameters has been employed in genotypic classification for salinity tolerance. (Zeng et al., 2002). However, cross-validation of the clustering method was not employed to evaluate the accuracy of the classification. In addition, attempts to define the differences among levels of tolerance are not well established due to the complexity of tolerance and limited genotypic screening techniques (Platten et al., 2013). To address these concerns, we classified 49 rice genotypes for salinity tolerance based on the linear combination of morphological and physiological traits using the combined power of clustering and discriminant analyses. We employed MANOVA and canonical discriminant functions to define the differences in salinity tolerance. Lastly, we genotyped the 49 rice varieties to identify ideal tolerant genotypes suited for breeding programs in the Southern USA. To our knowledge, this is the first time these Southern USA rice varieties were evaluated for salinity tolerance and genetic relatedness.

2.2 Materials and methods

2.2.1 Plant materials

Forty-nine rice genotypes were screened for salinity tolerance at the seedling stage (Table 2.1). Thirty varieties were grown in the Southern USA and fourteen genotypes were acquired from IRRI, including the sensitive check IR29 and the highly tolerant check Pokkali. The other five genotypes were acquired from the Germplasm Resources Information Network (GRIN).

Table 2.1. List of genotypes used in the experiment, their source, and some key agronomic attributes.

Genotype	Source[#]	Subspecies	Photosensitivity	Presence of awn	Grain type	Pericarp color
Hasawi (IRGC 16817)	IRRI Genebank	Indica	no	awned	medium grain	red
Cheriviruppu	IRRI Genebank	Indica	yes	awned	medium grain	red
Pokkali (IRGC 108921)	IRRI Genebank	Indica	yes	awned	medium grain	red
Nona Bokra (IRGC 01231)	IRRI Genebank	Indica	yes	no	medium grain	red
FL478	IRRI Genebank	Indica	no	awned	long grain	red
FL378	IRRI Genebank	Indica	yes	awned	long grain	red
TCCP-266-1-38-13-1-3	IRRI Genebank	Indica	no	awned	long grain	white
IRRI 147	IRRI Genebank	Indica	no	no	medium grain	white
Ketumbar (IRGC 13516)	IRRI Genebank	Indica	yes	no	short grain	white
Damodar (IRGC 17038)	IRRI Genebank	Indica	yes	no	medium grain	white
Getu (IRGC 17041)	IRRI Genebank	Indica	yes	no	medium grain	white
CSR II (IRGC 83240)	IRRI Genebank	Indica	no	no	medium grain	white
PSBRC50 (IRGC 99706)	IRRI Genebank	Indica	no	no	long grain	white
IR 1702-74-3-2 (PI 399813)	GRIN	Indica	yes	awned	long grain	white
IR 944-102-2-3-2 (PI 408628)	GRIN	Indica	no	awned	long grain	white
IR 2706-11-2 (PI 408508)	GRIN	Indica	no	no	long grain	white
Nipponbare (GSOR# 70)	USDA (Arkansas)	Japonica	no	no	medium grain	white
Geumgangbyeo	GRIN	Indica	no	no	medium grain	white
IR29 (IRGC 30412)	IRRI Genebank	Indica	no	no	long grain	white
Cocodrie	LRRS	Japonica	no	no	long grain	white
R609 (MG)	LRRS	Indica	no	no	medium grain	white
LAH 10	LRRS	Indica	no	no	long grain	white
LA 0802140	LRRS	Japonica	no	no	long grain	white
Cheniere	LRRS	Japonica	no	no	long grain	white

(Table 2.1 continued)

Genotype	Source[#]	Subspecies	Photosensitivity	Presence of awn	Grain type	Pericarp color
Bengal	LRRS	Japonica	no	no	medium grain	white
CL152	LRRS	Japonica	no	no	long grain	white
Roy J	LRRS	Japonica	no	awned[$]	long grain	white
Rex	LRRS	Japonica	no	awned[$]	long grain	white
CL142	LRRS	Japonica	no	awned[$]	long grain	white
Mermentau	LRRS	Japonica	no	no	long grain	white
Jupiter	LRRS	Japonica	no	no	medium grain	white
Wells	LRRS	Japonica	no	awned[$]	long grain	white
Catahoula	LRRS	Japonica	no	no	long grain	white
CL151	LRRS	Japonica	no	no	long grain	white
Jazzman	LRRS	Japonica	no	awned[$]	long grain	white
Neptune	LRRS	Japonica	no	no	medium grain	white
Caffey	LRRS	Japonica	no	no	medium grain	white
Templeton	LRRS	Japonica	no	awned[$]	long grain	white
Taggert	LRRS	Japonica	no	awned[$]	long grain	white
Jazzman-2	LRRS	Japonica	no	awned[$]	long grain	white
Jes	LRRS	Indica	no	awned[$]	long grain	white
CL162	LRRS	Japonica	no	awned[$]	long grain	white
CL181	LRRS	Japonica	no	no	long grain	white
CL111	LRRS	Japonica	no	no	long grain	white
CL131	LRRS	Japonica	no	no	long grain	white
Cypress	LRRS	Japonica	no	no	long grain	white
CL161	LRRS	Japonica	no	no	long grain	white
LA 0702085	LRRS	Japonica	no	no	long grain	white
CL261	LRRS	Japonica	no	no	medium grain	white

[#] GRIN, Germplasm Resources Information Network; LRRS, LSU Agricultural Center Rice Research Station

[$] These cultivars developed short awns in greenhouse conditions in this study.

2.2.2 Screening for salinity tolerance at seedling stage

Unimbibed seeds of the 49 rice genotypes were incubated at 50°C for five days to break any residual seed dormancy. The IRRI standard evaluation technique (Gregorio et al., 1997) for salinity tolerance was followed with some modifications. Ten seeds from each genotype were pre-germinated in a paper towel for 2 days at 35°C and then transferred into a styrofoam trays suspended on a basin containing tap water. After 3 days, the seedlings were allowed to grow for 2 weeks in a hydroponic nutrient solution containing 1 g/l of Jack's Professional fertilizer 20-20-20 (J.R. Peters, Inc.) supplemented with 300mg/l ferrous sulfate. NaCl was added to the nutrient solution at 12dSm^{-1} with the pH maintained between 5.0-5.1. Control plants were grown at the same time in nutrient solutions without NaCl. All experiments were conducted in a greenhouse with temperatures set between 25-29°C.

The entire experiment was conducted in a randomized block design and was replicated three times. Ten seedlings were grown, but only five plants of uniform growth per genotype for every replication were considered for data collection. The mean value of the trait for 5 seedlings per genotype was considered one replicate of a treatment.

- Ion leakage

Early responses of rice genotypes to salinity stress were investigated by measuring the concentration of the ions that leaked from the leaf tissue using a conductivity meter (VWR Traceable). After 2 days in saline solution, 100mg of leaf tissue were collected from the second youngest leaf of each genotype. The tissue was cut into 10mm long strips, placed in 10 ml distilled deionized water, and incubated at room temperature for 2 h before autoclaving. The electrical conductance of the solution was measured before and after autoclaving for $EC_{initial}$ and EC_{final} values, respectively. Since ion leakage could vary between genotypes, the index of salt

injury was estimated with respect to the ion leakage of the corresponding genotype grown in control conditions, following the formula of Flint et al., (1967): Ion_leak= 100 (Rt-Ro) / (1-Ro); where Ion_leak is the index of injury by ion leakage; Ro = $EC_{initial}/EC_{final}$ of the control plant, and Rt = $EC_{initial}/EC_{final}$ of the stressed plant.

- Chlorophyll concentration

Leaf yellowing was observed in plants 4 days after salinization. To compare the differences among genotypes, the relative chlorophyll concentration was measured nondestructively from the mid-part of the second youngest leaf in control and stressed rice genotypes using the SPAD 502 chlorophyll meter (Spectrum Technologies, Inc.) after 4 days. The relative percent reduction in chlorophyll concentration was computed by the formula: Chl_R = 100 ($Chl_{control}$ – $Chl_{stress}/Chl_{control}$).

- Growth parameters

Changes in shoot and root length in response to salinity stress were measured for each genotype 7 days post salinization (DPS). Shoot length was measured from the base of the plant to the tip of the longest leaf, while the root length was measured from the base of the plant to the tip of the root mass. To account for genotypic differences, all comparisons were done with respect to the control. Hence, the percent reduction in root and shoots were computed by a formula similar to the chlorophyll percent reduction.

- Visual salt injury score (SIS)

Plant responses to salinity stress were evident 7 DPS. For visual scoring, the IRRI standard evaluation scoring was followed (Gregorio et al., 1997). The plant scored 3 if it showed little to no leaf damage, but was stunted compared to the corresponding genotype grown in the control solution. A score of 5 was given if the plant was stunted with green rolled leaves having a few

whitish tips. A plant showing only green culm with dried leaves was scored 7, and a score of 9 was given if the plant was completely dead. All visual scoring was done when the sensitive check IR29 had a score of 7 or 9. The mean SIS score of each genotype was computed from 10 individual plants per experiment.

- Na-K analysis

The concentration of sodium and potassium in the root and shoot were determined for each genotype grown in saline conditions after 7 days. Five plants per genotype were rinsed with distilled water and then dried for 2 days at 65°C. Each dried tissue was ground by mortar and pestle and 100 mg were digested with 5ml of nitric acid and 3ml hydrogen peroxide at 152-155°C for 3 hours in a hood. The digested tissue was diluted to a final volume of 12.5 ml, and the concentration of sodium and potassium were quantified using a flame photometer (model PFP7, Bibby Scientific Ltd, Staffordshire, UK). The estimated concentration was calculated from a standard curve. The absolute concentration was computed based on the dilution of the sample.

2.2.3 Statistical analyses

To evaluate the genotypic differences for each trait, ANOVA and comparison of means by Dunnett's test were conducted using the GLIMMIX procedure against IR29 or Pokkali. The genotype was entered as the fixed effect and the replication as a random effect. To improve the normality of the data for analysis of genotypic differences, values were anchored to 1, then log transformed prior to data analysis. Correlation among traits was computed using the CORR procedure of SAS Version 9.3 for Windows (SAS Institute, 2011), based on the pooled least square (LS) mean of three replications per trait.

To characterize the level of salinity tolerance of the 49 varieties, the LS mean values of genotypes for six traits (SIS, ion_leak, chlorophyll and shoot length reduction, shoot K concentration, and shoot Na^+/K^+ ratio) were used in multivariate cluster analysis of NTSYSpc 2.10t (Rohlf, 2000). Because of different scaling and to give equal importance among the trait variables, the data were standardized to have a mean of 0 and a variance of 1. Euclidean distances between all pairs of genotypes were computed from standardized six seedling traits, and the phenogram of rice genotypes was constructed based on the UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Based on the ranking of the group mean SIS, the clusters were classified as highly tolerant (HT), tolerant (T), moderately tolerant (MT), sensitive (S) and highly sensitive (HS). To confirm the classification of genotypes, the same data for clustering were used in discriminant analyses with the group assignment for each genotype. The six seedling traits were considered as dependent variables, and the salinity clusters (HT, T, MT, S, and HS) were considered as independent variables. All genotypes were then given an equal prior probability to be grouped into the five levels of salinity tolerance. The PROC DISCRIM, PROC CANDISC, and the GLM procedures for MANOVA were run in SAS v9.3 (SAS Institute, 2011) to determine the differences among the levels of salinity groupings.

2.2.4 Genetic diversity analysis

Plants were grown in non-saline growth medium, and leaf tissues were harvested from a single plant of each genotype. The genomic DNA from each genotype was isolated following the CTAB method (Chen and Ronald, 1999). The DNA concentration was quantified by a spectrophotometer (NanoDrop ND-1000) and was adjusted to a final concentration of 25ng/µl for PCR amplification.

One hundred forty-six SSR markers, evenly spaced across the 12 chromosomes of rice, were used in PCR amplification for genetic diversity (B.2). For each 25µl reaction, the PCR mixture contained 12.8µl water, 2.5µl 10X PCR buffer, 2.5µl 25mM $MgCl_2$, 2.5µl 2mM dNTPs, 1.25µl 50ng/µl reverse and forward primers, and 1U Taq polymerase (Promega Corporation, Madison, USA). The reactions were run for 35 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min with a final extension at 72°C for 5 minutes. The PCR products were analyzed by 4.5% SFR agarose gel electrophoresis. Four hundred twenty-seven alleles were then scored as 1 or 0 for the presence or absence of a PCR band. The pairwise distance matrix was computed among genotypes using the dice coefficient, and then used in tree construction by unweighted neighbor-joining as implemented in DARwin 6.0 (Perrier et al., 2003). AMOVA, genetic distance, and Mantel's test were performed using GenAlEx (Peakall and Smouse, 2012) to evaluate genetic diversity**.**

2.3 Results

2.3.1 Phenotypic response to salinity stress

During the experiment, greenhouse temperature ranged between 24-29°C during the day. Plants assigned to control and the corresponding genotypes to salinity treatment grew uniformly after two weeks in non-saline hydroponic solution. Upon addition of sodium chloride at $12dSm^{-1}$, most of the rice genotypes showed leaf rolling after 2 to 3 hours. Growth of the plants stopped by the 2^{nd} or 3^{rd} day, followed by chlorosis and leaf bleaching from the tip of the leaf blade to the leaf base on the 4^{th} or 5^{th} day. By the 7^{th} to 9^{th} day post salinization, susceptible seedlings of IR29 were dead. Tolerant varieties also showed the same early response to salinity stress, but at 4^{th} or 5^{th} day, they showed some signs of recovery, such as leaf greening and growing of the youngest leaf.

Significant differences among genotypes were observed for some of the traits investigated (B.1). However, the differences across genotypes were not significant in the root length reduction (RtL_R), root sodium concentration in (Rt_Na), root potassium concentration (Rt_K), root sodium: potassium ratio (Rt_Na/K), and shoot sodium concentration (Sh_Na) at $P<0.05$ level of significance. For ion leakage, genotypic differences were highly significant ($P<0.0001$). It ranged from 27% to 72%, indicating a wide variation in the membrane permeability across 49 genotypes under salt stress. The exotic donor cultivars from IRRI showed low ion leakage not greater than 42%, while IR29 had 52%. Among the USA varieties, salt tolerant lines were CL162, Cypress, and CL261, with ion leakage values of 33%, 40%, and 40%, respectively.

Percent reduction in chlorophyll concentration (Chl_R) among genotypes was highly significant ($P<0.0001$). Pokkali had 35% reduction while IR29 had 52%. Among the donor genotypes from IRRI, CSR II had the lowest chlorophyll reduction of 4%. FL478, IR944-102-2-3-2, TCCP-266, and Geumgangbyeo had 18%, 19%, 24%, and 26 % chlorophyll reduction, respectively. Among the USA genotypes, Cheniere, R609, LAH10, Cypress, Neptune, Caffey, and Templeton showed less than 40% chlorophyll reduction.

At the 7^{th} day post salinization, salt injury scores (SIS) were significantly different between genotypes ($P<0.0001$). Pokkali had a mean SIS of 2.9 and IR29 had a score of 7.7. The donor genotypes showed varying levels of tolerance with SIS range between 2.9 and 6.1. The USA genotypes were sensitive, except for R609, LAH10, and Cypress with SIS of 4.4, 4.4 and 5.1, respectively. In addition, Cheniere, Roy J, Jupiter, Neptune, Caffey, Templeton, Taggert, and CL162 showed an intermediate response with SIS of 5.9 to 6.2. The rest of the USA genotypes were highly sensitive to salt stress with SIS more than 7.0.

Other morphological responses to salinity such as root and shoot length, showed variation among genotypes. Root growth was inhibited in all genotypes, and the reduction was as high as 56%. However, analysis of variance for the percent root length reduction (RtL_R) did not show significant genotypic differences (P=0.9927). In contrast, percent shoot length reduction (ShL_R) was significantly different (P<0.0001) among genotypes. Pokkali and Hasawi had the lowest growth reduction (34%) while IR29 was reduced by 40%. All USA genotypes displayed shoot growth reduction that ranged from 44-58%, indicating the sensitivity of USA genotypes to salt stress.

The Na^+ and K^+ concentration were determined in roots and shoots of the 49 genotypes. All genotypes grown in salinized medium showed an increased Na^+ concentration in roots and shoots, while the K^+ concentration was reduced when compared to non-salinized condition (data not shown). Varying concentrations of Na^+ were observed among the genotypes. In general, shoot Na^+ concentration was about two times the concentration of Na^+ in roots. Analysis of variance showed that neither root Na^+ nor shoot Na^+ concentration was significantly different among genotypes, despite the higher concentration of Na^+ in susceptible IR29 than Pokkali. The genotypic differences in root K^+ concentration were also not statistically significant (P=0.3763) at 5% level of significance although the 49 genotypes showed differences in concentrations. In contrast, significant genotypic differences for shoot K^+ concentration was observed among genotypes (P=0.0492). Donor genotypes from IRRI had shoot K^+ concentrations that ranged from 900 to 1300 $mmolkg^{-1}$. FL378 and Damodar had the highest shoot K^+ concentration (1336 and 1333 $mmolkg^{-1}$), while Pokkali and IR29 had shoot K^+ concentrations of 995 and 821 $mmolkg^{-1}$, respectively. On the other hand, all USA genotypes except Jazzman had low shoot K^+ concentration ranging from 600 to 900 $mmolkg^{-1}$. Variation in root Na^+/K^+ ratio (Rt_Na/K) was

not significant (P=0.2619), but in the shoot Na^+/K^+ ratio (Sh_Na/K), the difference was significant (P=0.0160) among genotypes. Donor cultivars and Geumgangbyeo had lower shoot Na^+/K^+ ratios compared to USA genotypes. IR29 had a Na^+/K^+ ratio of 4 while Pokkali had a ratio of 2.7. Interestingly, LAH10, which showed a SIS of 4.4, had a ratio of 2.9, while Cocodrie, CL162, Rex, Cheniere, LA0702085, and Jazzman-2 had shoot Na^+/K^+ ratios between 3.0 and 3.5.

2.3.2 Correlation of traits related to salinity tolerance

To better understand the physiological traits that best describe salinity tolerance, relationships among all traits were analyzed (Table 2.2). Individual correlation of traits showed that SIS was positive and highly correlated to ion_leak, chlorophyll % reduction, shoot length % reduction, and shoot Na^+/K^+ ratio, but negatively correlated to shoot K^+ concentration. The patterns of correlations were the same for shoot Na^+/K^+ ratio and other traits. Shoot Na^+/K^+ ratio was positive and highly correlated to ion_leak, chlorophyll % reduction, and shoot length % reduction; it was highly but negatively correlated to shoot K^+ concentration. Shoot length reduction was also positive and highly correlated to ion_leak and chlorophyll reduction. Shoot K^+ was negatively correlated to ion_leak and shoot length reduction, but significantly and positively correlated to shoot Na^+ and root Na^+. Root Na^+/K^+ ratio was positively correlated to root Na^+ and negatively correlated to root K^+. Taken together, ANOVA and correlation results indicated that SIS, ion leakage, chlorophyll reduction, shoot length reduction, shoot K^+ concentration, and shoot Na^+/K^+ ratio are important parameters in defining the levels of salinity tolerance.

2.3.3 Classification of 49 rice genotypes for salinity tolerance

Because of the significant genotypic differences and high correlations in SIS, ion leakage, chlorophyll reduction, shoot length reduction, shoot K^+ concentration, and shoot Na^+/K^+

Table 2.2. Pearson correlation matrix of seedling traits in response to salt stress at 12 dSm^{-1} in rice genotypes.

	SIS	RtL_R	Rt_Na	Rt_K	Rt_Na/K	Ion_leak	Chl_R	ShL_R	Sh_Na	Sh_K	Sh_Na/K
SIS	1										
RtL_R	-0.006	1									
Rt_Na	0.0542	-0.136	1								
Rt_K	-0.123	-0.173	0.258	1							
Rt_Na/K	0.115	0.125	0.446***	-0.350**	1						
Ion_leak	0.474***	0.0689	-0.075	-0.184	0.105	1					
Chl_R	0.771***	0.0547	0.111	-0.128	0.208	0.289*	1				
ShL_R	0.538***	0.124	-0.233	-0.106	0.011	0.470***	0.442***	1			
Sh_Na	0.106	-0.338*	0.281	0.068	0.109	-0.138	0.257	-0.003	1		
Sh_K	-0.540***	-0.039	0.346**	0.222	0.083	-0.563***	-0.254	-0.435***	0.318*	1	
Sh_Na/K	0.644***	-0.208	-0.102	-0.265	0.038	0.473***	0.431***	0.373**	0.221	-0.746***	1

SIS=salt injury score; Chl_R=% chlorophyll reduction; ShL_R= % shoot length reduction; RtL_R=% root length reduction; Ion_leak=index of injury by ion leakage; Rt_Na=root sodium concentration; Rt_K=root potassium concentration; Rt_Na/K=N/K ratio in root; Sh_Na=shoot sodium concentration; Sht_K=shoot potassium concentration; Sh_Na/K=Na/K ratio in shoot.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

ratio, we decided to use these parameters in the cluster analysis for the phenotypic classification of rice genotypes in response to salinity stress. The phenogram generated by UPGMA computed from the six traits (SIS, Ion_leak, Chl_R, ShL_R, Sh_K, and Sh_Na/K) produced 5 major clusters (Figure 2.1). From the ranking of their group SIS means, cluster I was assigned as highly tolerant (HT), with the lowest group mean of 4.3. As expected, cluster I grouped the known highly tolerant genotypes such as Pokkali, Nona Bokra, FL478, TCCP266, FL378, Hasawi, and Cheriviruppu. Cluster II had a group SIS mean of 5.8 and was classified as moderately tolerant (MT). The USA genotypes such as CL162, Jupiter, Jazzman, Templeton, Cypress, Neptune, and Caffey grouped together in cluster II. The highest group SIS mean (7.4) was observed for cluster III and hence classified as highly sensitive (HS). It included the sensitive check IR29 and ten other USA genotypes. Cluster IV had a group SIS mean of 4.7 and was considered as tolerant (T) group, which contained CSRII, Nipponbare, Geumgangbyeo, R609, and LAH10. Cluster V was classified as sensitive (S) with a group SIS mean of 7.4, where popular genotypes such as Roy J, Cocodrie, Bengal, Mermentau, and Jazzman2 were placed.

The Fisher linear discriminant analysis (FLDA) is an approach similar to logistic regression, but the computation is more like the MANOVA or canonical correlation. The procedure initially computes the Mahalanobis distance of each genotype to a group and then uses it to classify the genotype into a group to which it has the smallest generalized squared distance (Truxillo, 2008). Results of FLDA indicated an error rate of 6.9%, owing to the three genotypes that were misclassified (B.3). IR1702, which was classified as moderately tolerant, should be placed in the tolerant group; Nipponbare should be classified as moderately tolerant instead of tolerant, and Jazzman2 should be grouped into the highly sensitive group instead of sensitive group. In FLDA,

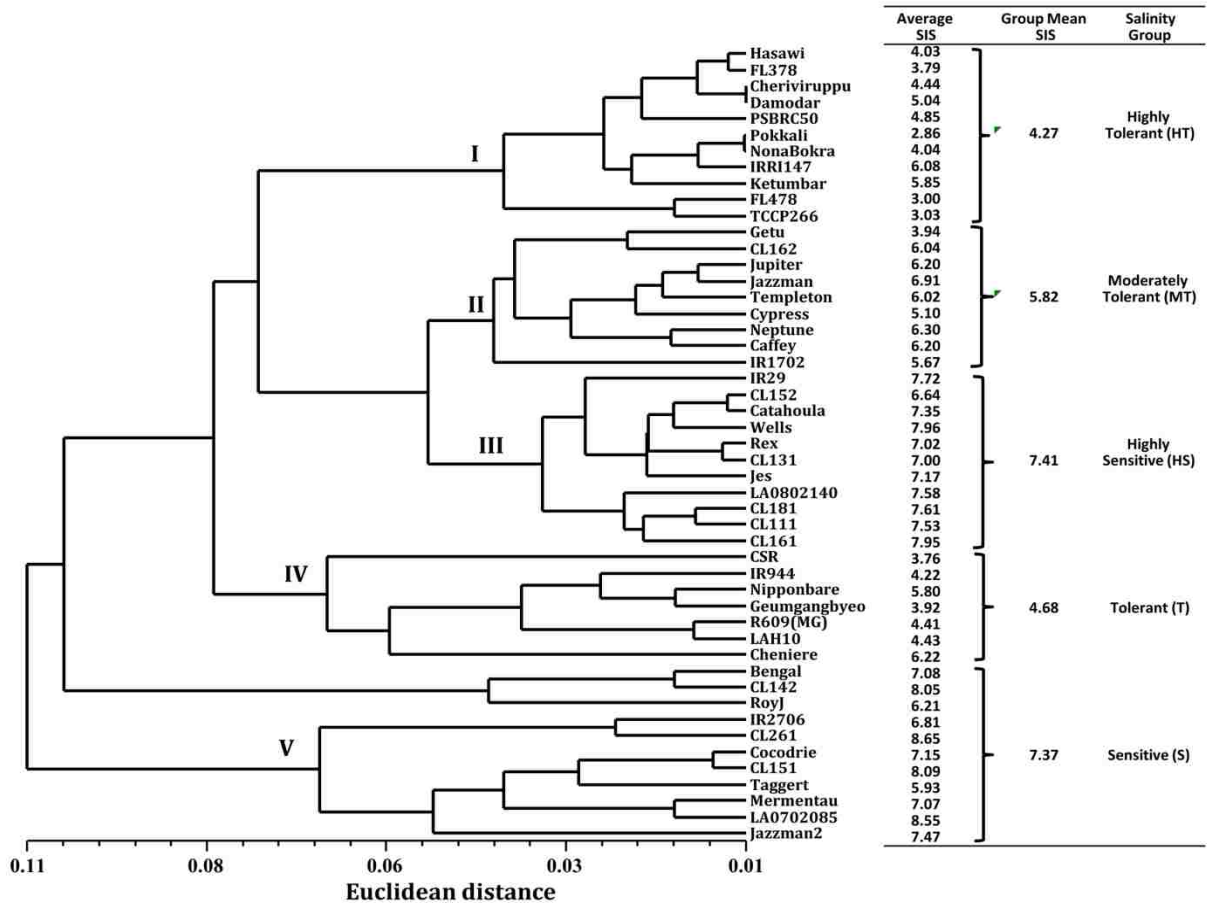

Figure 2.1. Clustering of forty-nine genotypes by UPGMA based on Euclidean distance of six morphological and physiological trait responses to salinity stress.

however, the test of homogeneity of covariance matrices was significant ($P<0.0001$). Hence, we were prompted to use quadratic discriminant analysis (QDA) instead of FLDA. In QDA, the result indicated a 0 % error rate, confirming that our genotype classification based on the clustering method was robust.

2.3.4 Differentiation of salinity groups by canonical discriminant function and MANOVA

To further understand the grouping and to assess the extent of differences between salinity groups, canonical discriminant analysis was employed. Multivariate test statistics of nonlinear prediction of group membership based on the six physiological traits was highly significant in all

statistics, thus confirming the group membership prediction. Based on 5 groups and 6 trait variables, 2 canonical discriminant functions were high and significantly correlated for the prediction of genotype membership into salinity groupings. Canonical discriminant function 1 (Can1) and canonical discriminant function 2 (Can2) accounted for 81% and 12% of the variance in the traits, respectively (B.4). The loading of the variables to canonical discriminant functions showed that SIS, Chl_R, ShL_R, Ion_leak, and Sh_Na/K were positive and highly correlated to Can1, while Sh_K was negatively correlated (B.5). From the variance explained by Can1 and the loading of trait variables, it appeared that Can1 is a measure of the overall characteristics of salinity tolerance by the six parameters. In contrast, Can2 was positively correlated to Sh_K and Chl_R but negatively correlated to ShL_R and Ion_leak. Therefore, this result suggests that Can2 differentiates genotypes based on their K^+ and chlorophyll concentrations. In Can1, the maximum separation of group means was observed between HT and S (-3.96 vs 3.37) and mean separation between HS and T was 1.86 vs -1.55. Examination of Can2 showed separation of HT from the T group (1.17 vs -1.84) and separation of MT from the S group (-0.81 vs 0.90). All groups with negative mean values to Can1 had some tolerance to salinity (HT, T, and MT). In contrast, HS and S groups had positive mean values to Can1.

In the plot of salinity groups against Can1 and Can2, the MT group was placed in the center between the T and HS groups (Figure 2.2). The HT group had negative mean to Can1 (-3.96) and positive mean to Can2 (1.17), indicating that HT had low values in SIS, Ch_R, ShL_R, Ion_leak, and Sh_Na/K but with positive high K^+ concentration. The T group had both negative mean values to Can1 (-1.55) and Can2 (-1.84), indicating that the T group is like the HT group, but it has lower K^+ concentration as compared to HT group. Between T and MT, the T has higher negative mean values in both Can1 and Can2. The Sensitive (S) group had positive mean values

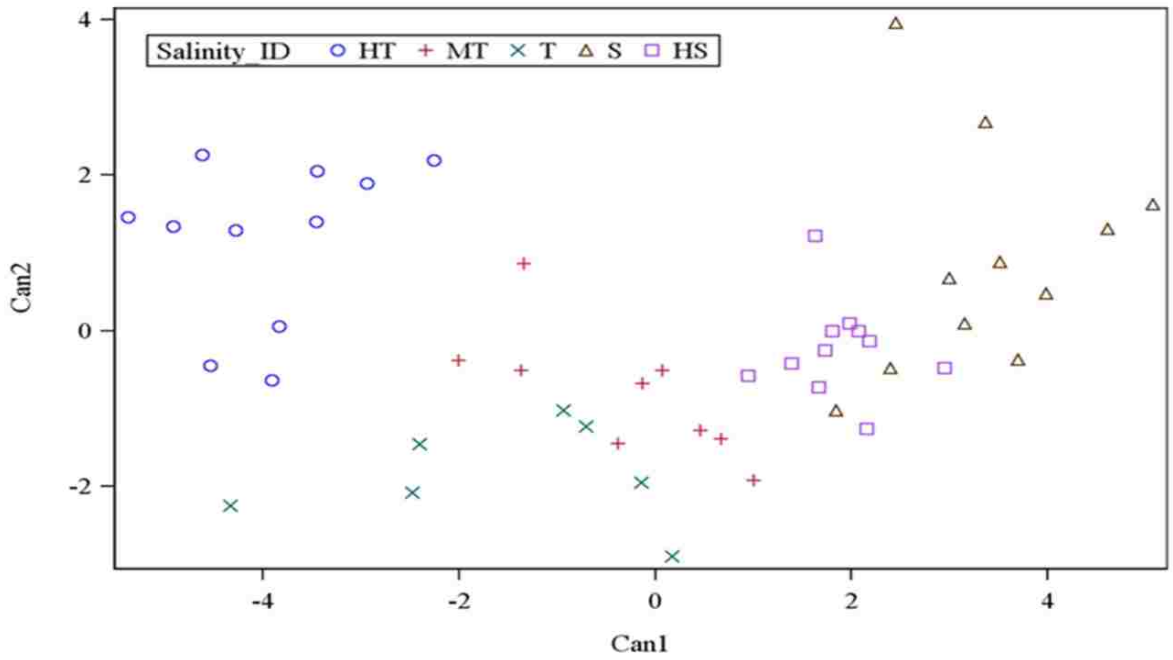

Figure 2.2. Population structure of 49 rice genotypes by canonical discriminant analysis of morphological and physiological trait responses to salt stress.

to Can1 (3.37) and Can2 (0.90), indicating higher mean values in all traits and low K^+ concentration. The highly sensitive (HS) group was the total opposite of HT group, with positive and negative mean values in Can1 (1.86) and Can2 (-0.23), respectively.

Further analysis by multivariate analysis of variance (MANOVA) for 6 variable traits across 5 groups indicated that the groups are significantly different. Moreover, LS means comparison for each trait between groups showed significant differences of HT from S and HS groups in all traits (Table 2.3, B.6). Conversely, the T group was significantly different from the HT group in ShL_R, ion leakage, and Sh_K, while a significant difference was observed only in Chl_R between T and MT. On the other hand, the S group was significantly different to MT in SIS, Chl_R, and ion leakage; and significantly different to HS in Chl_R alone. Nonetheless, overall pairwise contrasts between groups were highly significant in all comparisons, indicating the complete separation between groups based on the six quantitative traits.

Table 2.3. Least square (LS) means of salinity groups in six parameters.

Group	SIS	Chl_R	ShL_R	Ion_leak	Sh_K	Sh_Na/K
HT	4.27	32.84	39.58	32.98	1111.67	2.57
T	4.68	24.59	46.27	53.89	838.97	3.20
MT	5.82	42.18	48.93	44.57	878.65	3.60
S	7.37	68.63	47.59	55.68	797.46	3.92
HS	7.41	54.52	49.04	49.39	785.04	3.83

SIS=salt injury score; Chl_R=% reduction in chlorophyll; ShL_R=shoot length % reduction; Ion_leak=index of injury by ion leakage; Sht_K=shoot potassium concentration; Sh_Na/K=Na/K ratio in shoot; HT=highly tolerant; T=tolerant; MT=moderately tolerant; S=sensitive; HS=highly sensitive.

2.3.5 Genetic diversity of 49 rice genotypes

The genetic relationship among the genotypes was assessed to identify parental genotypes for the breeding program and to determine if the observed clustering of 49 genotypes based on salinity stress responses can be explained by their DNA profile. An unweighted neighbor-joining tree of 49 genotypes, based on 427 alleles using 146 SSR markers, separated the genotypes into two major groups of *indica* (clusters A, B) and *japonica* (clusters C, D) subspecies with two sub-clusters within a group (Figure 2.3).

Analysis of molecular variance showed significant genetic differences among the four populations (PhiPT= 0.505 at P (rand perm. 999) =0.001) with 49% and 51% variance within and among populations, respectively (Table 2.4). Differentiation of the clusters showed that USA varieties had fewer numbers of alleles, lower percentages of polymorphic loci and very few unique alleles compared to *indica* genotypes. Based on Shannon's information index, the donor genotypes (*indica* group) showed higher genetic diversity than the USA genotypes even with fewer sample sizes (Table 2.5). Similarly, Nei's genetic distance between the C and D clusters is only 0.093, indicating a narrow genetic diversity among the USA genotypes. The relationship

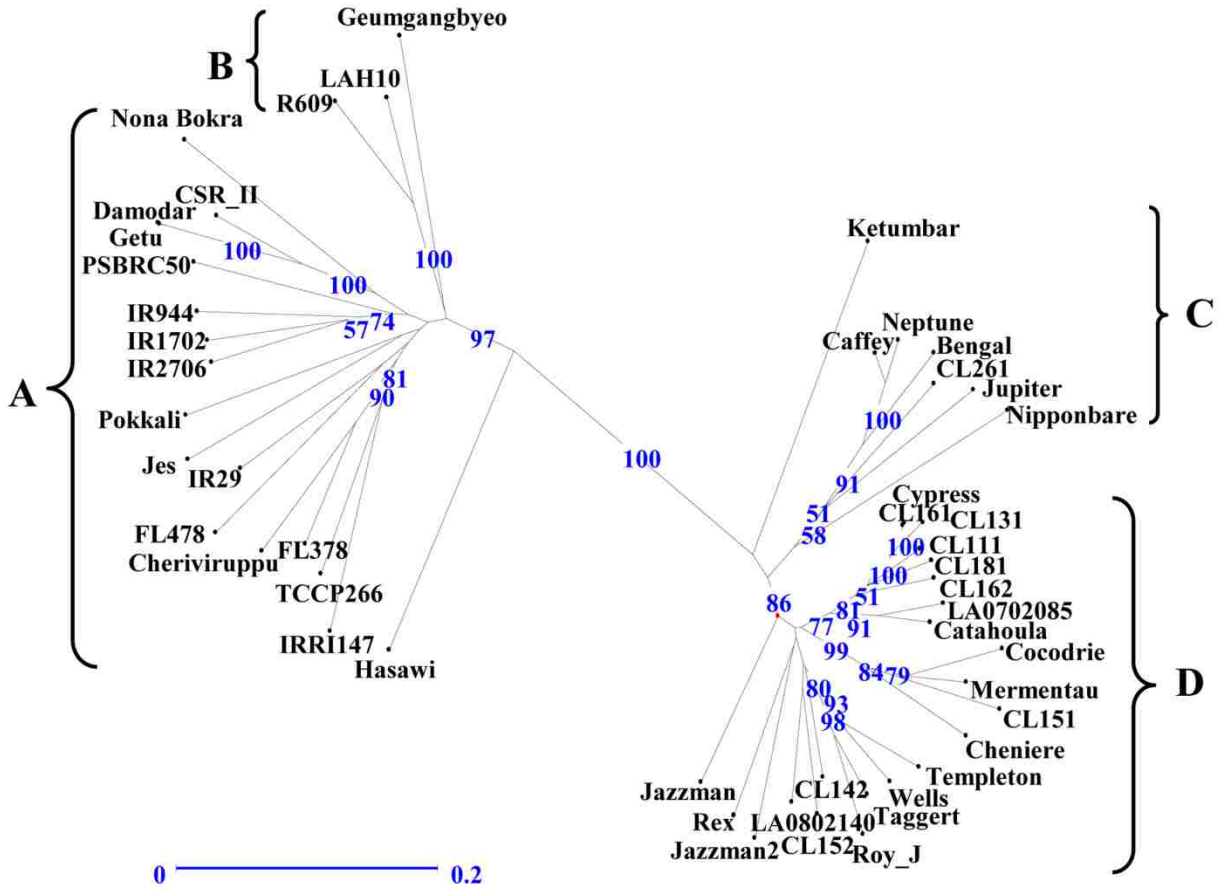

Figure 2.3. Genotypic clustering by unweighted neighbor-joining tree showing the genetic relationship among the 49 rice genotypes based on 146 SSR markers. Horizontal bar indicates distance by dice coefficient. Numbers on nodes are bootstrap values based on 100 iterations.

Table 2.4. Summary of analysis of molecular variance (AMOVA).

Source of variation		df	SS	MS	Est. Variance	% Variance
Among Populations		3	1630.032	543.344	46.600	51%
Within Populations		45	2054.132	45.647	45.647	49%
Total		48	3684.163		92.247	100%
PhiPT :	0.505					
P(rand perm. 999)	0.001					

Populations refer to the rice clusters (A, B, C, and D) in Figure 2.3. df =degree of freedom; SS=sum of squares; MS=mean square; Est. Variance=estimated variance; % Variance=percent variance; PhiPT=estimate of genetic distance among populations; P (rand perm.999)=significance of genetic distance at 999 random permutations.

Table 2.5. Genetic differentiation between population clusters of rice genotypes by 146 SSR markers. Population clusters (A, B, C, and D) are from the Figure 2.3.

Population cluster	A	B	C	D
Sample size	17	3	7	22
Mean No. of different alleles	1.618	0.883	1.199	1.164
Mean No. of effective Alleles = 1 / (p^2 + q^2)	1.378	1.237	1.291	1.264
Mean Expected Heterozygosity = 2 * p * q	0.227	0.133	0.173	0.157
Shannon's Information Index = -1* (p * Ln (p) + q * Ln(q)	0.35	0.194	0.263	0.239
No. of different bands	358	237	284	277
No. of bands unique to a single population	40	2	6	5
Percentage of polymorphic loci	78%	33%	53%	52%

between the subgroups among the USA varieties is the obvious separation of the medium grain (C) from the long grain varieties (D). Further examination of *indica* varieties showed that cluster A is a mixture of traditional and Pokkali-derived lines of medium and long grain cultivars. As expected, the aromatic rice variety 'Jes' (Anonymous, 2009), a long grain mutant of Khao Dawk Mali developed for temperate rice growing areas in the US, was grouped to cluster A. In contrast, Ketumbar, a short grain *indica* genotype from Indonesia (Negrão et al., 2011), was grouped into cluster C of medium grain *japonica* varieties. However, the grouping of tolerant Pokkali and susceptible IR29 in cluster A indicated that genetic profiling based on the SSR markers spanning the 12 chromosomes of rice cannot explain the varietal grouping based on salinity responses. Furthermore, the Mantel test of correlation between phenotypic and genetic distance matrices was low (r= 0.206) although significant at 999 permutation test. Therefore, the clustering suggests genetic similarity of genotypes based on subspecies and grain morphology.

2.4 Discussion

Crop breeding programs aim to make new varieties that will better cope with abiotic and biotic stresses. In developing salt tolerant cultivars, rice breeding programs are making efforts to evaluate diverse germplasm to enhance their utility (Ismail et al., 2007). Overall, the *indica*

cultivars are more tolerant to salinity than japonica cultivars because of their superior ability in excluding Na^+, absorbing K^+, and maintaining a low Na^+/K^+ ratio in shoots (Gregorio and Senadhira, 1993; Lee et al., 2003). Many traditional landraces that can withstand high levels of salinity are good candidates for breeding salt-tolerant cultivars. However, due to their undesirable agronomic traits, they are not used (Gregorio et al., 2002). In the USA, rice breeding programs in the Southeastern region have been successful in breeding high yielding varieties. However, none of these varieties have been evaluated for the level of tolerance to salinity stress. Here, we evaluated the genetic diversity, as well as the morphological and physiological responses, of 49 diverse rice genotypes that included rice cultivars of the Southeastern USA and several exotic donors and breeding lines with varying levels of tolerance to salinity stress. The six quantitative traits were used for objective varietal classification and delineation of the levels of salinity tolerance. The use of cluster analysis and validation by discriminant analysis was implemented for accurate classification for salinity tolerance.

Among the traits evaluated for salt stress response, genotypes varied significantly for shoot parameters, but not for root traits (B.1), suggesting that salinity tolerance is more likely controlled in the shoot. This possibly explained the higher occurrence of induced DNA methylation in shoots as compared to roots in some rice varieties tested for salinity response (Karan et al., 2012). Different trait parameters showed different ranking of genotypes in response to salinity stress, indicating wide natural phenotypic variation among the 49 rice genotypes. The correlation of all traits allowed us to identify relationships among traits that described salinity tolerance. Instead of considering only visual salt injury scores, other parameters, such as ion leakage, chlorophyll concentration, shoot length, shoot K^+ concentration, and shoot Na^+/K^+ ratio could be unbiased parameters for assessing salinity tolerance.

Previous studies suggested that the toxicity of salt stress could be due to Na^+ accumulation in the shoot (Lin et al., 2004). Our results, however, did not show that sodium accumulation was more in salt-sensitive varieties, which could lead to increased ion leakage due to injured plasma membranes (Lv et al., 2012). Instead, our results are similar to the findings of Yeo et al. (1990), in which there was no significant variation among rice genotypes in the shoot uptake of sodium. Likewise, we did not find a significant correlation of visual salt injury score and shoot sodium concentration (Table 2.2). These results suggested that salinity tolerance among the tolerant varieties is not a function of restricting sodium uptake, but more likely in the compartmentalization of sodium to alleviate its toxic effect (Blumwald, 2000). This finding is consistent with prior reports in rice cv. Pokkali (Kader and Linberg, 2005), *Salicornia europaea* (Lv et al., 2012), *Arabidopsis thaliana* (Apse et al., 1999), and *Saccharomyces cerevisiae* (Nass and Rao, 1988). Other donors for a high degree of salt tolerance as Pokkali were FL478, FL378, TCCP266, Nona Bokra, Hasawi, Damodar, and Cheriviruppu (Group I, Figure 2.1). The high positive correlation of shoot length reduction and % chlorophyll reduction to SIS indicated that the photosynthetic capacity of salt-sensitive plants became limited, leading to chlorosis and shoot growth reduction under salt stress (Apse et al., 1999; Lin et al., 2004; Munns and Tester, 2008). Among the donor cultivars, Hasawi and Pokkali had the least growth reduction and relatively low chlorophyll reduction. In addition, CSR II, FL478, TCCP 266, IR944, and Geumgangbyeo showed low chlorophyll reduction despite high shoot growth reduction.

Another obvious trait for the mechanism of tolerance among the donor cultivars is the high potassium uptake resulting in lower Na^+/K^+ ratio (Gregorio and Senadhira, 1993; Koyama et al., 2001; Bonilla et al., 2002; Ren et al., 2005; Pushparajan et al., 2011; Wang et al., 2012). In contrast, the USA varieties, with the exception of Jupiter and Jazzman, had shoot K^+

concentrations less than 900 mmolkg^{-1}. Previous studies by Ren et al. (2005) indicated that the *SKC1* gene from Nona Bokra maintains high shoot K^+ concentration, thereby regulating the Na^+/K^+ homeostasis under salt stress. Our results showed that, aside from Nona Bokra, other donor cultivars that can be used for improvement of salinity tolerance through high shoot K^+ concentration and low Na^+/K^+ ratio are FL378, Damodar, Hasawi, Ketumbar, PSBRC50, Cheriviruppu, and IR2706-11-2.

Previous attempts to characterize salt-tolerant rice varieties were done using agro-morphological traits (Caldo et al., 1996; Zeng et al., 2003; Sanni et al., 2012). In most breeding strategies, the simple visual salt injury scoring (Gregorio et al., 1997) is widely used for characterization because it reflects the overall plant's response to salt stress. However, the inherent subjectivity and the quantitative nature of salinity tolerance complicate the evaluation for salinity tolerance. Thus, other studies suggest the use of Na-Ca selectivity (Zeng et al., 2003), tiller number and Na-K selectivity (Zeng, 2005), and proline concentration (Kanawapee et al., 2012) as criteria for classification of rice varieties for salt tolerance. However, varietal differences showed that it is natural for varieties to be superior in one trait and inferior in others (Yeo et al., 1990). Instead of characterizing rice genotypes for traits one by one, we employed the multivariate cluster analysis using the six quantitative traits across the 49 genotypes. The five traits (ShL_R, Chl_R, ion leakage, Sh_K, and Sh_Na/K) showed significant and high correlation to SIS. Thus, they are unbiased estimate of a variety's performance in response to salinity stress. Our results demonstrated that the groupings were robust, and varietal assignment to the level of salinity tolerance was confirmed by discriminant analysis. As indicated by MANOVA and discriminant functions, the levels of salinity tolerance were significantly distinct against each other. The morphological responses of the HT group were least affected by salt stress due to high

K^+ uptake, resulting in low Na^+/K^+ ratios and possibly by effective compartmentalization of Na^+ in shoot. In contrast, higher shoot length reduction, higher ion leakage, and lower shoot K^+ concentration separated the T group from HT varieties. The T and MT groups had the same salt responses, but the ability to maintain lower chlorophyll reduction made T superior to MT. The HT group was significantly superior to the S and HS groups in all traits, while the T and MT groups were statistically superior to S and HS only in the overall visual score and chlorophyll reduction. Therefore, the genotypes in T and MT groups offered a novel source of tolerance and an apparent mechanism distinct from those found in the HT group. Between S and HS, trait responses were not significantly different, except in chlorophyll reduction. The S group had even higher chlorophyll reductions than HS group, suggesting that S and HS should be treated as one group (Table 2.3). While SIS offers a simple screening method and accounted for the overall performance of rice varieties under salt stress, our results emphasized the importance of five other traits (ShL_R, Chl_R, ion leakage, Sh_K, and Sh_Na/K) in objective varietal classification for salinity tolerance. Furthermore, our results demonstrated the power of multivariate analyses (clustering, MANOVA, and canonical and linear discriminant analyses) in confirmation and demarcation of levels of tolerance. Overall, the phenotypic clustering indicated the absence of highly tolerant USA varieties. However, LAH10, R609, and Cheniere exhibited some level of tolerance. LAH10 is a rice hybrid developed from R609. Thus, it is likely that the tolerance of LAH10 is inherited from R609.

Another important finding in this study is the information on genetic diversity. Numerous studies have classified rice varieties using DNA based markers such as RFLP (Zhang et al., 1992), AFLP (Subudhi et al., 1998), SSR (Ni et al., 2002), and SNP markers (McNally et al., 2009). Similar to previous differentiation studies using DNA markers (Zhang et al., 1992, Ni et

al., 2002, Thomson et al., 2007), the genotypic grouping mainly separated the genotypes into japonica or indica subspecies (Figure 2.3). Among the USA genotypes, LAH10 and R609 clustered into the indica group, thus confirming the absence of high tolerance among the USA japonica varieties. Overall, despite the use of 146 markers resulting to 427 scored alleles, genotypic clustering was independent of phenotypic clustering in response to salt stress. Our results were consistent with the findings of Zeng et al., (2004), who used only 25 SSR markers to evaluate genetic diversity among rice genotypes with different adaptations to saline soils. The genotypic clustering separated the *indica* from the *japonica* clades, but not on the basis of salinity response. Interestingly, the 49 genotypes were subdivided into either long grain or short grain. Therefore, our results suggested a limitation of whole genome scanning using SSR markers in differentiating the polymorphism between salt tolerant and sensitive lines. Since salinity tolerance is polygenic in nature, it is likely that the markers we used have little or no association at all to the genes controlling salt tolerance. Additionally, our phylogenetic tree indicates independent and multiple lineages of acquiring salinity tolerance probably by local environmental adaptation (Bromham, 2015). As genotyping by sequencing is becoming more accessible, it is likely the best way to increase the resolution of genetic differentiation that eventually can aid in genomic selection or development of markers linked to the physiological traits for salinity tolerance. Those markers will be useful in the marker-assisted breeding for pyramiding of physiological traits contributing to high tolerance (Yeo and Flowers, 1986). Nonetheless, the result of our DNA profiling indicated a narrow genetic diversity among USA varieties and therefore emphasized the need to expand the gene pool of USA rice germplasm, particularly for abiotic stress tolerance through the use of *indica* germplasm. Our results confirmed that exotic germplasm such as Nona Bokra, Hasawi, Cheriviruppu, Damodar,

Ketumbar, Pokkali, TCCP266, FL378, and FL478 (Cluster I) possess high salinity tolerance during the seedling stage. However, many of these genotypes are photosensitive. Our initial salinity screening during the reproductive stage (data not shown) showed high grain sterility among the non-photosensitive donor cultivars except the TCCP266 genotype. TCCP266 is a somaclonal variant of Pokkali with better agronomic traits and with white pericarp (Gregorio et al., 2002). In contrast, Geumgangbyeo, LAH10, and R609 (Cluster IV-tolerant group) showed less sterility and less grain weight reduction during reproductive stage screening. While access to genetic diversity is an important component to a successful breeding strategy (Negrão et al., 2011), our results showed that the USA varieties were genetically more distant to cluster B (Figure 2.3). Therefore, Geumgangbyeo, R609, and LAH10 can be used as novel sources of seedling and reproductive salinity tolerance. Geumgangbyeo is a semi-dwarf rice variety from South Korea, and it is listed as a salt tolerant cultivar during the seedling stage in the GRIN database (http://www.ars-grin.gov/cgi-bin/npgs/html/ob2_acc.pl?75019+5.04+5.6, accessed 2 October 2014). Our results showed that it has a SIS of 3.92, lower root length reduction, higher chlorophyll content, lower shoot Na^+ concentration, and lower Na/K ratio relative to Pokkali. LAH10 is a medium grain hybrid rice developed from R609 that is a restorer line used in hybrid rice breeding. Therefore, the use of R609 or LAH10 will enhance the prospect of developing salt tolerant hybrid rice.

2.5 Conclusion

Overall, this study demonstrated the use of several multivariate analyses in the classification and validation of the differences among rice genotypes for salinity tolerance. Effective identification and selection for high tolerance can be achieved by the accumulation of multiple favorable traits under salt stress. Thus, we propose the use of a linear combination of multiple

traits as a predictor of tolerance for unbiased classification. Finally, the rice genotypes identified here will provide novel sources of seedling stage salinity tolerance.

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CHAPTER 3. MOLECULAR DISSECTION OF SEEDLING SALINITY TOLERANCE OF RICE (*ORYZA SATIVA L.*) USING A HIGH-DENSITY GBS-BASED SNP LINKAGE MAP*

3.1 Introduction

Progress in breeding rice with salt tolerance is slow due to genetic complexity of salinity tolerance (Flowers and Flowers, 2005). Some germplasm sources with high salt tolerance are available. However, majority of these germplasm sources possess many undesirable traits that decrease their usefulness in a plant improvement program. Pokkali, Nona Bokra, and Hasawi, which are highly tolerant and often used as donors in breeding for salt tolerance, are tall, photosensitive, low yielding, and have red kernel. In addition, salt tolerance screening is difficult because the phenotypic response of rice to salt stress is highly affected by other confounding environmental factors (Gregorio and Senadhira, 1993; Flowers, 2004). Hence, the search for QTLs and DNA markers tightly linked to traits related to salt tolerance becomes a major objective in most breeding programs. It is assumed that molecular markers will facilitate a fast and cost-effective screening of large populations (Munns and James, 2003).

Since the advent of molecular markers, QTL analyses for salinity tolerance at seedling stage were conducted using RIL (Koyama et al., 2001; Gregorio et al., 2002; Wang et al., 2012), $F_{2:3}$ lines (Lin et al., 2004), and backcross populations (Thomson et al., 2010; Alam et al., 2011). QTLs for visual scoring, survival, shoot and root lengths, Na^+/K^+ ratio, Na^+ and K^+ concentrations in root and shoot at 100-120 mM salt stress were frequently investigated. Most of the QTL mapping studies have indicated polygenic nature of salinity tolerance. Among the QTLs for traits related to salt tolerance, only *qSKC1* was successfully isolated by map-based cloning (Ren et al., 2005). The *SKC1* gene from Nona Bokra encodes an HKT-type transporter that regulates the Na^+/K^+ homeostasis under salt stress. In earlier reports, the QTL designated as *Saltol* (Gregorio, 1997) and a gene '*SalT*' (Causse et al., 1994) for Na^+/K^+ ratio were located on

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chromosome 1. Numerous QTL mapping studies for salinity tolerance were based on linkage maps constructed using AFLP (Gregorio, 1997), RFLP (Koyama et al., 2001; Bonilla et al., 2002; Lin et al., 2004), and SSR markers (Thomson et al., 2010; Wang et al., 2012). The population size was usually small and the markers were sparse due to limited polymorphism between the parents. The rapid development in the sequencing technology makes single nucleotide polymorphism (SNP) to become the marker of choice for QTL mapping. Bimpong et al. (2013) used 194 polymorphic SNP markers for mapping QTLs related to salinity tolerance. More recently, Kumar et al. (2015) applied the genome-wide association mapping on 220 rice varieties using a custom-designed array containing 6000 SNPs. Major association of Na^+/K^+ ratio still co-localized to the *Saltol* locus with additional QTLs on chromosome 4, 6, and 7. Significant SNPs were identified and some candidate genes were suggested. However, tight association of candidate genes in or around a single variant still needs enrichment with more markers at a locus to avoid false association. Moreover, complete resequencing of the locus in tolerant and non-tolerant lines or in bi-parental population are needed to add credence to the robustness of GWAS using SNP array.

The introduction of genotyping-by-sequencing (GBS) and the availability of whole genome sequence of rice have accelerated the identification of millions of SNPs across the whole genome. To date, GBS is becoming popular for population studies, genetic diversity, QTL mapping, and genomic selection (He et al., 2014). GBS enabled the construction of high-density linkage map and QTL analysis in maize, wheat, barley (Poland et al., 2012; Chen et al., 2014), oat (Huang et al., 2014), and chickpea (Jaganathan et al., 2015). In rice, GBS has been applied in QTL mapping for leaf width and aluminum tolerance (Spindel et al., 2013), pericarp color and some agronomic traits (Arbelaez et al., 2015), and rice blast resistance (Liu et al., 2015). Several

QTL mapping studies for salinity tolerance have been reported. However, QTLs and markers flanking QTLs for salinity tolerance are not being utilized in breeding programs. The main reason for this is attributed to the large chromosome intervals delimited by those QTLs. Thus, identification of candidate genes and understanding of salinity tolerance mechanisms still remain a challenge.

In this study, recombinant inbred lines at F_6 generation were developed from a cross between Bengal and Pokkali. Bengal is a high yielding, early maturing; semi-dwarf medium grain cultivar developed from the cross of MARS//M201/MARS (Linscombe et al., 1993). It is sensitive to salinity stress (De Leon et al., 2015). Pokkali is a highly tolerant landrace often used as a donor for salinity tolerance. However, it is notable for many undesirable traits such as low-yield, tall, and highly susceptible to lodging. It is photoperiod-sensitive, awned, with red pericarp and poor cooking quality (Gregorio et al., 2002). We used GBS technique to construct a high-resolution genome-wide SNP genetic map for identification of additive and epistatic QTLs for salinity tolerance. Segregation distortion loci (SDLs) and QTLs for plant height were mapped to show the quality and accuracy of the genetic map and QTL mapping. Our ultra-high density map allowed us to map QTLs with high resolution and identify candidate genes that may play important role in salt tolerance mechanisms in rice. The candidate genes identified in this study will serve as useful targets for functional genomics, gene pyramiding, and for gene-based marker-assisted breeding for salinity tolerance.

3.2 Materials and methods

3.2.1 Plant materials and population development

A mapping population was developed by crossing Bengal and Pokkali as female and male parent, respectively. The resulting F_1 plants were selfed and advanced by single seed descent

method to generate 230 recombinant inbred lines (RILs) in F_6 generation. RILs grown in unsalinized condition were extracted for DNA and were genotyped by the Cornell Genomic Diversity Facility using the GBS method.

3.2.2 Phenotypic characterization and tissue collection

The phenotypic evaluation was conducted in the greenhouse with day time and night time temperature settings at 26-29^{o}C. The hydroponics system was used in the screening for seedling salt tolerance following the IRRI standard evaluation technique (Gregorio et al., 1997). The parental lines and 230 RILs were pre-germinated in a paper towel for two days and then transplanted to hydroponic set up containing 1g/L of Jack's Professional (20-20-20) (J.R. Peters, Inc.), supplemented with 300mg/L of ferrous sulfate. The pH of the solution was maintained at 5.0-5.1 and plants were allowed to grow for two weeks. The whole experiment was conducted in randomized complete block design replicated three times, with ten plants per line per replicate.

At 14th day after planting, the plants were subjected to 6dSm^{-1} for two days and then to 12dSm^{-1} salt stress. After six days of salt stress, the amount of chlorophyll content was measured on the mid-length of the second youngest leaf using a SPAD-502 chlorophyll meter (Spectrum Technologies, Inc.). Five plants per line of uniform growth were evaluated for traits related to salinity tolerance. On the 9th or 11th day, when the susceptible check plants were dead, lines were phenotyped for salt injury score, shoot length, and root length. A score of 1 was given to unaffected plants, score of 3 to healthy plants but stunted, score of 5 to plants showing green leaves and stem with some tip burning and leaf rolling, score of 7 to plants with green stem but all leaves are dead, and a score of 9 to completely dead plants. Shoot length and root length were measured in centimeter. Shoot length was measured from the base of the culm to the tip of the tallest leaf. Root length was measured from the base of the culm to the tip of the longest root.

Shoot length to root length ratio was derived by dividing the shoot length by the root length. For dry weight, five plants per line were collected and dried at 65^{o}C oven for five days prior to weighing.

3.2.3 Measurement of Na^+ and K^+ concentration in shoot

The amount of Na^+ and K^+ in the shoot was measured from 100 mg ground tissue taken from a pool of five plants per line. Briefly, the shoots of the plants were collected, rinsed with water, oven dried for 5 days and ground to fine powder. The tissue was digested with 5ml of nitric acid and 3 ml of 30% hydrogen peroxide at 152-155^{o}C heating block for 3 hours (Jones and Case, 1990). The digested tissue was diluted to a final volume of 125 ml. Flame photometer (model PFP7, Bibby Scientific Ltd, Staffordshire, UK) was used to quantify the Na^+ and K^+ concentrations in each sample. The final concentrations were computed from the derived standard curve of different dilutions of Na^+ and K^+ and the ratio of Na^+ and K^+ concentration (NaK) was calculated from these values.

3.2.4 Statistical analyses

The phenotypic data for each trait were analyzed by ANOVA and LS mean of each line was extracted using the GLIMMIX procedure. The RIL line was entered as a fixed effect and replication as a random effect. Broad sense heritability for each trait was computed by family mean basis following Holland et al. (2003). CORR procedure was implemented to determine the relationship among traits. All data analysis was conducted using Statistical Analysis System (SAS) software version 9.4 for Windows (SAS Institute, 2012). Frequency distribution for each trait was constructed in Microsoft Excel 2010.

3.2.5 Genotyping-by-sequencing of Bengal, Pokkali, and RIL population

Leaf tissues were collected from each of the parental lines and RIL. The DNA was extracted using the Qiagen DNeasy Plant Mini Kit following the manufacturer's protocol (Qiagen Inc., Valencia, CA, USA). Genomic DNA libraries were prepared as described by Elshire et al. (2011). Each DNA was cut by *ApeKI* enzyme and the adapters were ligated to barcode the DNA of each line. Pooled DNA from parents, 189 RILs, 94 other lines, and 3 blanks was sequenced in one lane with the Illumina HiSeq sequencer at Genomic Diversity Facility, Cornell University Institute of Biotechnology (http://www.biotech.cornell.edu/brc/genomic-diversity-facility). The Tassel GBS pipeline was used to process the data and SNP calling was based on the Nipponbare reference genome MSU release 7 (Kawahara et al., 2013).

3.2.6 Construction of linkage map and QTL analysis

Sequence alignment and SNP calling were done by the Genomic Diversity Facility, Cornell University. A total of 1,593,692 tags were sequenced, of which, 1,215,287 (76.3%) were aligned to unique positions, 134,210 (8.4%) had multiple alignments and 244,195 (15.3%) were not aligned. Upon processing and filtering of SNPs, the resulting SNPs markers were reduced to a total of 33,987, with an average individual depth of 5.5 or site depth of 4.6 and individual mean missingness of 0.28. Pokkali and two RILs were declared as failed samples for having less than 10% of the mean reads per sample. They were removed before further analysis, resulting in a total of 187 RILs for final analysis. The hapmap data file containing the filtered SNP calls were further analyzed prior to linkage map construction and QTL analysis. The Bengal parent was successfully sequenced, thus providing data for differentiation of alleles among RILs. To validate the GBS SNP calling, we amplify and re-sequenced thirty-eight positions of GBS SNP calls in Bengal and Pokkali. Allele differentiation and allele origin among RILs were confirmed

with Bengal and Pokkali re-sequenced data available in our laboratory. With the breeding scheme of the mapping population, only three possible genotypes may exist at polymorphic loci with bi-allelic SNP calling. The 2, 0, -1 coding numbers were then used to code for different alleles in the genotype data. SNP call for each marker across the population was coded as 2 if the allele was the same as Bengal. A code of 0 was given to the alternative allele and was assumed as the allele from Pokkali. Since our materials are F_6 RIL, most of the loci were homozygous and should be segregating into 1:1. However, with low read depth due to highly multiplexed nature of GBS, all heterozygous SNPs (Y=T|C, M=A|C, W=T|A, R=A|G, S=C|G, K=G|T) and missing SNP (N) calls were coded as -1. All SNP markers monomorphic across the 187 RILs were removed. Likewise, all SNP markers with more than 10% missing SNP calls were purged before further analysis. As a result, only 9,303 SNP markers were retained and used for linkage and QTL mapping. The order of SNP markers along the chromosome was fixed based on the physical position of SNPs in the MSU Rice Genome Annotation (Osa1) Release 7. Genetic distances of SNP markers based on recombination rates were converted using the Kosambi mapping function. To see if segregation distortion of markers occurs in the QTLs detected in this study, interval mapping of segregation distortion locus (SDL) was also conducted. Significant SDLs were declared for loci exceeding the 2.0 LOD threshold level.

Nine traits were used for QTL mapping. The mean of three replications was used as phenotypic score for each trait. Except for salt injury score, Na^+ concentration, K^+ concentration, Na^+/K^+ ratio, chlorophyll content, shoot length, root length, shoot dry weight, and shoot length to root length ratio showed normal distribution. Hence, the data were directly used for QTL mapping. For SIS, data were log transformed to improve the normality of RIL distribution prior to QTL mapping. Analysis of additive QTLs for traits related to salinity tolerance was performed

by interval mapping (IM-ADD), and inclusive composite interval mapping (ICIM-ADD) methods. By interval mapping method, parameters for QTL detection were set to a scanning window size of every 1cM with LOD threshold value set at 2.0 to declare significant QTLs. In ICIM-ADD, the parameters were set as follows: missing phenotype by mean replacement, stepwise regression method every 1cM window size with the probability levels of entering and removing variables set at 0.001, and a second step scanning by interval mapping for significant QTL detection at LOD threshold of 2.0. Epistatic QTLs were identified by interval mapping every 5 cM window with LOD threshold set at 3.0. The phenotypic variation explained by QTLs and their genetic effect were estimated. Confidence interval of each QTL was delimited by the flanking markers within the 1-LOD drop from the estimated QTL position. QTL interval size is computed from the distance between the physical positions of left and right flanking markers. Significant QTL for each trait was named with the trait followed by numbers indicating the chromosome location and megabase (Mb) position of the QTL. For example, *qK1.8* indicated the presence of a QTL for shoot K^+ concentration in chromosome 1 located at 8 Mb region. All linkage, SDL and QTL analyses were implemented in QTL IciMapping software version 4.0.6.0 (Meng et al., 2015).

3.2.7 Candidate gene prediction

To identify potential candidate genes within QTL intervals, the physical positions of SNP markers flanking the QTLs were searched in MSU Rice Genome Annotation (Osa1) Release 7. Genes contained within each QTL were listed (Supplementary Table S3.3, available upon request). To understand the roles of candidate genes in the mechanism of salinity tolerance, classification and annotation of candidate genes were inquired using the Panther Classification System (Mi et al., 2016).

3.3 Results

3.3.1 Phenotypic characterization under salt stress

The parents and RIL population were evaluated under salt stress for salt injury score (SIS), chlorophyll content (CHL), shoot length (SHL), root length (RTL), shoot length to root length ratio (SRR), dry shoot weight (DWT), shoot Na^+ and K^+ concentrations, and Na^+/K^+ ratio (NaK ratio). At 12dSm^{-1} salt stress, the RILs and parents showed varying levels of tolerance. Bengal and Pokkali showed significant contrasting response in SIS, SHL, RTL, DWT, and NaK ratio (Table 3.1). However, the differences in CHL, SRR, Na^+ and K^+ concentrations, were not statistically significant between parents. Pokkali showed consistently lower SIS, Na^+ concentration, NaK ratio, and higher K^+ concentration than Bengal. Among the RILs, all traits

Table 3.1. Phenotypic response of parents and F_6 RIL population for traits related to salt tolerance at seedling stage.

Trait Name	Bengal Mean	Pokkali Mean$^{\beta}$	RIL Mean	Std. Dev.	RIL Range	RIL Pr>F§	H$^{¥}$
Na^+ (mmolkg^{-1})	1700.00	1424.30^{ns}	1430.70	246.24	861.97-2733.35	<0.0001	0.98
K^+ (mmolkg^{-1})	420.00	591.00^{ns}	547.30	107.59	335.99-884.18	<0.0001	0.95
NaK (ratio)	4.07	2.38**	2.80	0.56	1.25-5.32	<0.0001	0.24
SIS	8.40	3.00***	4.70	0.72	3.00-8.73	<0.0001	0.44
CHL (SPAD unit)	20.56	19.54^{ns}	24.20	4.25	13.72-43.67	<0.0001	0.45
SHL (cm)	32.07	44.52***	40.70	3.21	22.60-59.73	<0.0001	0.90
RTL (cm)	6.73	10.08**	7.40	0.64	4.67-11.27	<0.0001	0.61
DWT (g)	0.06	0.11*	0.10	0.01	0.04-0.16	<0.0001	0.01
SRR (ratio)	4.98	4.53^{ns}	5.60	0.53	3.08-9.79	<0.0001	0.63

Na^+, shoot sodium concentration; K^+, shoot potassium concentration; NaK, ratio of the shoot sodium and shoot potassium content; SIS, salt injury score, CHL, chlorophyll content; SHL: shoot length; RTL, root length; DWT, shoot dry weight; SRR, shoot length to root length ratio.
$^{\beta}$Significant differences between Bengal and Pokkali, ^{ns}no significant differences, *significant at 0.05 probability level, **significant at 0.01 probability level, ***significant at 0.001 probability level.
§Genotypic differences among RIL.
$^{¥}$Broad sense heritability computed on family mean basis.

showed significant genotypic differences ($p < 0.0001$), indicating a wide range of variation. The RIL population had a mean value between the parental means for all traits except in CHL and SRR. Pokkali had an average SIS of 3; Bengal had 8.4, while the RILs had a mean SIS of 4.7. The RIL population had a mean Na^+ accumulation of 1430 $mmolkg^{-1}$ in shoot, which is much lower than Bengal (1700 $mmolkg^{-1}$), and marginally higher than Pokkali (1424 $mmolkg^{-1}$). In contrast, the mean K^+ accumulation was highest in Pokkali (591 $mmolkg^{-1}$), followed by RILs (547 $mmolkg^{-1}$) and lowest in Bengal (420 $mmolkg^{-1}$). The RIL population had mean chlorophyll content greater than either parent. As indicated in the frequency distribution (Figure 3.1) and the range of RIL values for each trait (Table 3.1), several lines were phenotypically superior to the parents. There were many transgressive segregants with much lower Na^+ than Bengal (Figure 3.1a), lower NaK ratio (Figure 3.1c) and SHL and higher CHL, DWT, RTL and SRR than Bengal or Pokkali (Figure 3.1e f g h). Similarly, some lines accumulated twice the K^+ concentration of Pokkali (Figure 3.1b). But there was no line that showed higher tolerance than Pokkali as judged by SIS (Figure 3.1d). There was wide variation for heritability values for traits. Heritabilities for Na^+, K^+ concentrations, and SHL were 0.98, 0.95, and 90, respectively. In contrast, NaK ratio, SIS, CHL, RTL, and SRR had moderate heritability of 0.24-0.63 while DWT has very low heritability.

3.3.2 Correlation of traits

Correlations among all traits (Table 3.2) revealed that SIS was highly significant and positively correlated to Na^+ concentration and NaK ratio. The SIS was highly significant and negatively correlated to CHL, SHL, RTL, DWT, and SRR, indicating the negative effect of salt stress on the overall growth and photosynthetic capability of plants. On the other hand, K^+

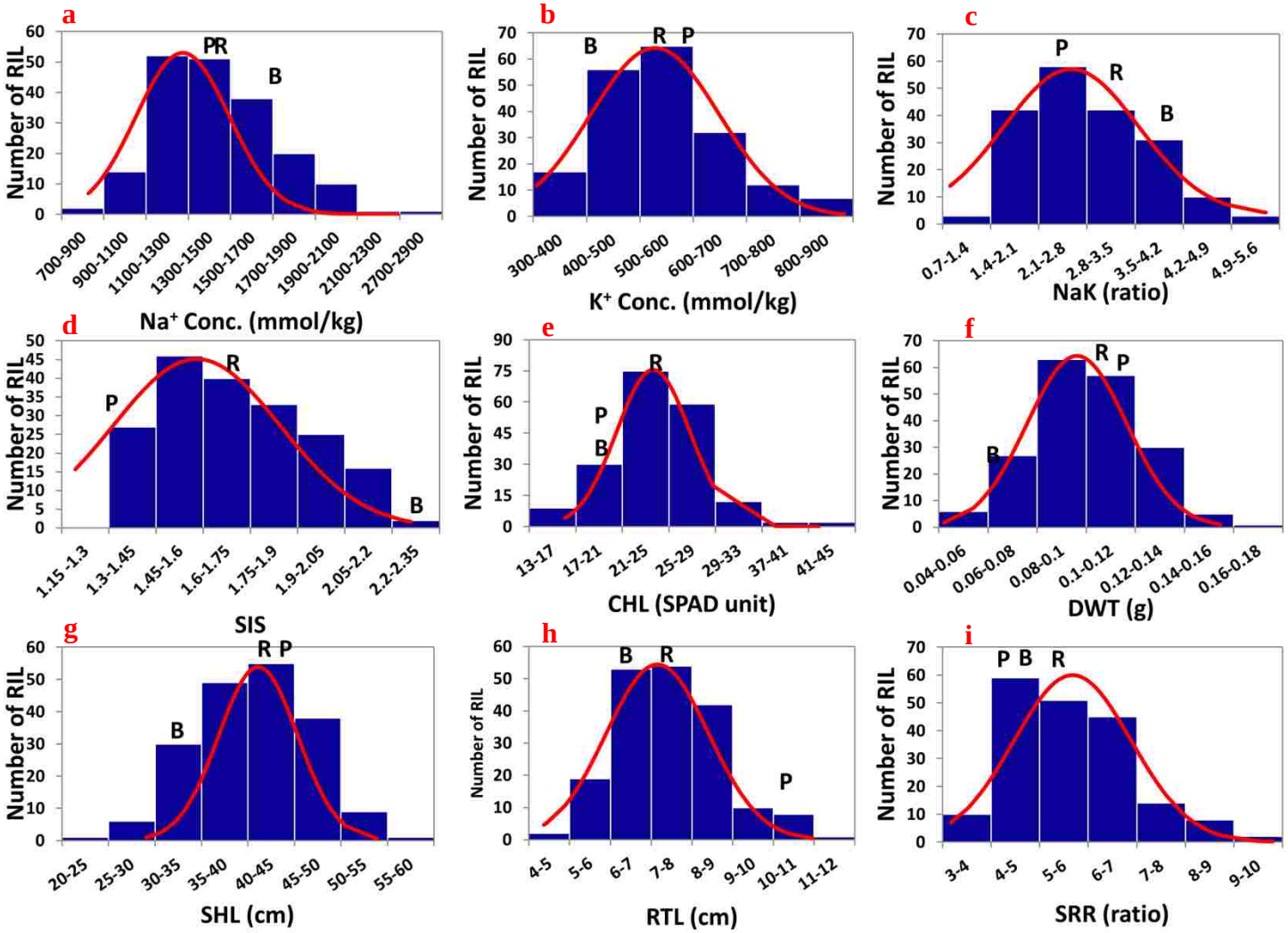

Figure 3.1. Frequency distribution of Bengal/Pokkali F_6 RIL population for traits related to seedling salinity tolerance. B, R, and P indicate the positions of the parents and mean of the RIL. (a) Na^+ Conc., Na^+ concentration; (b) K^+ conc., K^+ concentration; (c) NaK, Na^+/K^+ ratio; (d) SIS, log transformed salt injury score; (e) CHL, chlorophyll content measured by SPAD-502 unit; (f) DWT, dry weight; (g) SHL, shoot length; (h) RTL, root length; (i) SRR, Shoot length to root length ratio; B, Bengal; P, Pokkali; R, RIL.

Table 3.2. Pearson correlation matrix of traits measured in response to salt stress at 12dSm^{-1} in Bengal/Pokkali F_6 RIL population at seedling stage.

	Na^+	K^+	NaK	SIS	CHL	SHL	RTL	DWT	SRR
Na^+	1								
K^+	0.1271**	1							
NaK	0.594***	-0.649***	1						
SIS	0.337***	-0.129**	0.337***	1					
CHL	-0.128**	0.092*	-0.157***	-0.214***	1				
SHL	0.039	0.253***	-0.151***	-0.236***	0.221***	1			
RTL	0.057	-0.105*	0.095*	-0.109**	0.059	0.204***	1		
DWT	0.006	0.144***	-0.099*	-0.475***	0.177***	0.539***	0.279***	1	
SRR	-0.024	0.277***	-0.195***	-0.099*	0.111**	0.593***	-0.638***	0.173*	1

Na^+, shoot sodium concentration; K^+, shoot potassium concentration; NaK, ratio of the shoot sodium and shoot potassium content; SIS, salt injury score; CHL, chlorophyll content; SHL, shoot length; RTL, root length; DWT, dry weight; SRR, shoot length to root length ratio.

*significant at 0.05 probability level, **significant at 0.01 probability level, ***significant at 0.001 probability level.

concentration was positively correlated to Na^+ concentration, SHL, CHL, DWT, and SRR but negatively correlated to NaK ratio, SIS, and RTL. The relationships among traits in RIL population were consistent to the correlation of traits observed in the 30 US rice genotypes (De Leon et al., 2015), thus indicating reliability and reproducibility of our salt tolerance screening.

3.3.3 Linkage mapping

GBS generated a total of 33,987 SNP markers which were furtherly filtered for polymorphic markers and for markers with less than 10% missing data across the population. A total of 9,303 SNPs markers were retained and used in the linkage map construction (Figure 3.2, Supplementary Table S3.1, available upon request). On the average, about 775 SNP markers were placed per chromosome (Table 3.3). The final linkage map had a total length of 1650 cM with 2,817 recombination sites. The average distance between adjacent markers was 0.59 cM or 39,798 bp, with maximum resolution of 0.27 cM. The average marker density was 5.6 SNP markers per cM or 3.3 SNP markers per recombination point. The map was saturated with SNP markers across all chromosomes. However, twenty large gaps were observed on chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 11, and 12 that ranged between 5 cM to 13 cM. With 9,303 SNP markers, the linkage map had a physical to genetic map length ratio of 225 Kb/cM.

3.3.4 Identification of additive and di-genic epistatic QTLs for traits related to salinity tolerance

To detect novel additive and epistatic QTLs for traits related to salinity tolerance, the phenotype and GBS data were used in interval mapping (IM) and inclusive composite interval mapping (ICIM) methods.

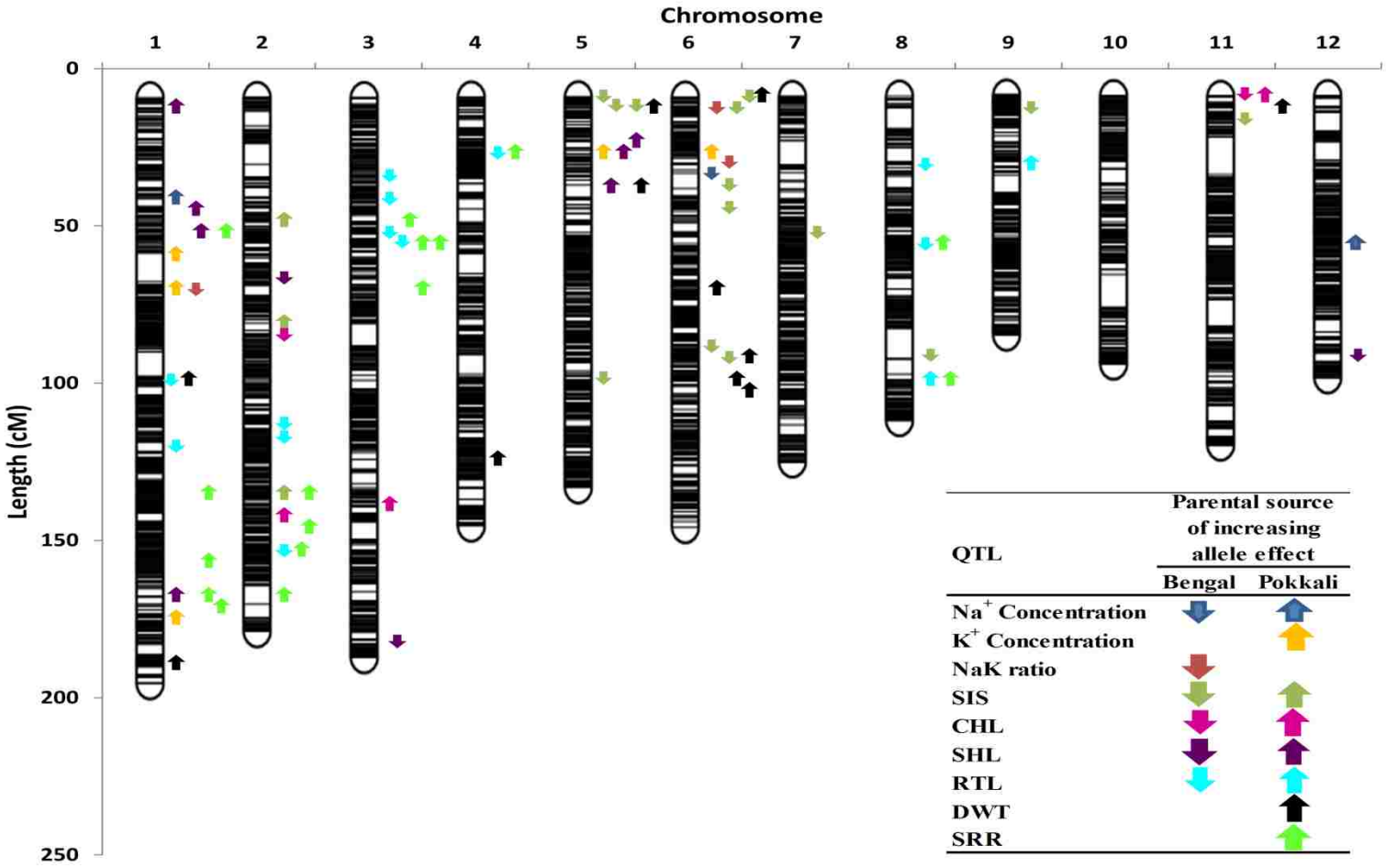

Figure 3.2. Molecular genetic map showing the positions of QTLs for nine traits investigated under salt stress. Linkage and QTL mapping were implemented in ICIM QTL Mapping 4.0 using 9,303 GBS-SNP markers in 187 Bengal/Pokkali F_6 RILs. Chromosome regions that are dark indicate the saturation of markers while regions that are white indicate the absence of marker placed in those segments. Genetic distance in centimorgan was determined by Kosambi map function. Each arrow represents a single QTL for a particular trait.

Table 3.3. Summary distribution, coverage, and intervals of SNP markers in the Bengal/Pokkali RIL linkage map.

Chromosome	No. of SNP markers used	Chromosome length coverage (Mb)	Genetic length (cM)	No. of recombination point	No. of SNP markers/ cM	No. of SNP markers/ unique position	Min. interval (cM)	Max. interval (cM)	Average Interval (cM)	No. of Gaps >5cM
1	1245	43,237,333	199.8	363	6.2	3.4	0.27	9.98	0.55	2
2	1001	35,875,736	182.9	324	5.5	3.1	0.27	7.19	0.56	2
3	1068	36,405,799	191.2	320	5.6	3.3	0.28	8.01	0.60	2
4	822	35,501,387	148.1	244	5.6	3.4	0.27	6.88	0.60	2
5	780	29,507,277	135.6	243	5.8	3.2	0.27	4.35	0.56	0
6	842	30,869,147	148.6	258	5.7	3.3	0.27	5.33	0.57	1
7	736	29,582,943	127.4	225	5.8	3.3	0.27	8.7	0.57	1
8	471	28,399,689	113.9	162	4.1	2.9	0.27	10.57	0.70	3
9	584	22,779,506	85.9	164	6.8	3.6	0.27	6.49	0.52	1
10	517	23,117,196	95.2	149	5.4	3.5	0.28	11.55	0.64	1
11	622	28,973,227	121.8	187	5.1	3.3	0.28	13.09	0.65	3
12	615	27,488,377	99.9	178	6.2	3.5	0.27	6.75	0.56	2
Total	9303	371,737,617	1650.2	2817	67.7	39.7	3.27	98.89	7.08	20
Average[§]	775.3	30,978,134.75	137.5	234.8	5.6	3.3	0.27	8.24	0.59	1.7

[§]Average value per chromosome

- QTLs for shoot Na^+ concentration

The IM and ICIM methods consistently detected three additive QTLs for shoot Na^+ concentration (Table 3.4). The QTLs were located on chromosomes 2, 6, and 12. Each additive QTL explained at least 5.5% of the phenotypic variation. Pokkali alleles of *qNa2.7* and *qNa12.18* had increasing effect while for *qNa6.5* Bengal allele had the increasing effect. Interval mapping of epistatic QTLs detected seven pairs of QTLs with significant contribution to the variation in Na^+ concentration (D.1). Four of the seven pairs of epistatic QTLs had large effect (PVE=11-16%) while the other three pairs had small effects (PVE=8-9%). Nine interacting QTLs with increasing effect were from Bengal alleles and five were from Pokkali. None of the additive QTLs co-localized with epistatic QTLs.

- QTLs for shoot K^+ concentration

The IM method detected five additive QTLs (*qK1.8, qK1.11, qK1.38, qK5.4,* and *qK6.4*) for shoot K^+ concentration. The *qK1.8* and *qK1.11* were large-effect QTLs, each accounting for at least 13% of the variation for shoot K^+. The other three QTLs had small effects (5-8% PVE) and were located on chromosomes 1, 5, and 6. The *qK1.11 and qK1.38* were also detected by ICIM with LOD values of 7.7 and 5.4, respectively. Both *qK1.11* and *qK1.38* were large effect QTLs in ICIM method with PVE of 16% and 10%. In contrast, *qK1.8, qK5.4,* and *qK6.4* were not detected in ICIM. All additive QTLs for K^+ concentration had increasing effect that originated from Pokkali, indicating the importance of Pokkali alleles for increased uptake of K^+ in the leaves. Five pairs of epistatic QTLs were detected for K^+ concentration (D.1). The *qK1.7* and the *qK2.3* pair had a PVE of 21% and LOD score of 3.5, with Pokkali allele contributing toward increased K^+ accumulation. The *qK1.7* also interacted with *qK12.17* and accounted for 9 % of the variation in K^+ accumulation. Additionally, *qK11.19* and *qK12.18* pair had a PVE of 10%

Table 3.4. Additive QTLs for traits related to seedling-stage salt tolerance in Bengal/Pokkali F_6 RIL population identified by IM and ICIM methods.

Phenotype	QTL	Chr[β]	Position (cM)	Left Marker	Right Marker	QTL Interval Size (bp)	LOD	PVE (%)	Add. Effect	Parental Source of Increasing Allele Effect[§]	No. of genes in QTL interval
Na^+ conc.-IM	*qNa2.7*	2	48	S2_7769844	S2_7939496	169,652	2.30	5.55	-66.59	P	24
	qNa6.5	6	34	S6_5269698	S6_5533752	264,054	2.40	5.97	69.15	B	34
	qNa12.18	12	60	S12_18687038	S12_18741493	54,455	2.25	5.51	-66.36	P	5
Na^+ conc.-ICIM	*qNa2.7*	2	48	S2_7769844	S2_7939496	169,652	2.30	5.55	-66.59	P	24
	qNa6.5	6	34	S6_5269698	S6_5533752	264,054	2.40	5.97	69.15	B	34
	qNa12.18	12	60	S12_18687038	S12_18741493	54,455	2.25	5.51	-66.36	P	5
K^+ conc.-IM	*qK1.8*	1	63	S1_8656025	S1_8901503	245,478	5.80	13.65	-46.34	P	33
	qK1.11	1	71	S1_11529325	S1_11581799	52,474	5.93	13.66	-45.13	P	6
	qK1.38	1	173	S1_38794029	S1_39047133	253,104	3.51	8.30	-33.32	P	40
	qK5.4	5	31	S5_4699921	S5_5326365	626,444	2.25	5.51	-27.00	P	86
	qK6.4	6	31	S6_4890290	S6_5269698	379,408	3.33	8.21	-32.92	P	61
K^+ conc.-ICIM	*qK1.11*	1	71	S1_11529325	S1_11581799	52,474	7.74	16.08	-48.95	P	6
	qK1.38	1	173	S1_38794029	S1_39047133	253,104	5.38	10.71	-37.86	P	40
NaK ratio-IM	*qNaK1.11*	1	71	S1_11529325	S1_11581799	52,474	4.15	9.83	0.29	B	6
	qNaK6.2	6	15	S6_2927160	S6_2962502	35,342	3.58	8.46	0.26	B	7
	qNaK6.5	6	33	S6_5269698	S6_5533752	264,054	5.12	13.21	0.32	B	34
NaK ratio-ICIM	*qNaK1.11*	1	71	S1_11529325	S1_11581799	52,474	2.64	5.66	0.22	B	6
	qNaK6.5	6	33	S6_5269698	S6_5533752	264,054	3.71	8.85	0.26	B	34
Salt injury score-IM	*qSIS2.8*	2	50	S2_8730258	S2_8927908	197,650	3.54	8.58	-0.06	P	25
	qSIS2.19	2	81	S2_19331684	S2_19454952	123,268	3.21	7.66	-0.06	P	14
	qSIS2.28	2	131	S2_28239596	S2_28274467	34,871	2.64	6.37	-0.05	P	8
	qSIS5.03	5	1	S5_312457	S5_329699	17,242	2.83	6.74	0.06	B	4
	qSIS5.1a	5	12	S5_1686924	S5_1707475	20,551	2.83	6.76	0.06	B	5

(Table 3.4 continued)

Phenotype	QTL	Chr[β]	Position (cM)	Left Marker	Right Marker	QTL Interval Size (bp)	LOD	PVE (%)	Add. Effect	Parental Source of Increasing Allele Effect[§]	No. of genes in QTL interval
Salt injury score-IM	*qSIS5.24*	5	106	S5_24057323	S5_24281632	224,309	3.13	7.51	0.06	B	39
	qSIS6.2	6	15	S6_2927160	S6_2962502	35,342	2.08	5.04	0.05	B	7
	qSIS6.5	6	37	S6_5848568	S6_5905669	57,101	3.04	7.23	0.06	B	11
	qSIS6.7	6	48	S6_7646442	S6_7661883	15,441	3.12	7.41	0.06	B	3
	qSIS6.20	6	90	S6_20929261	S6_20929283	22	3.96	9.44	0.07	B	1
	qSIS11.2	11	18	S11_2838776	S11_3716306	877,530	2.67	8.36	0.06	B	136
Salt injury score-ICIM	*qSIS5.1b*	5	11	S5_1441967	S5_1454837	12,870	9.71	13.33	0.08	B	2
	qSIS6.2b	6	9	S6_2123411	S6_2242943	119,532	3.59	4.46	0.05	B	23
	qSIS6.21	6	92	S6_21253244	S6_21256132	2,888	6.92	9.11	0.07	B	1
	qSIS7.14	7	57	S7_14598897	S7_14625841	26,944	3.62	4.50	0.05	B	7
	qSIS8.24	8	93	S8_24763939	S8_25110888	346,949	2.62	3.28	0.04	B	47
	qSIS9.8	9	13	S9_8608506	S9_9070610	462,104	7.09	9.19	0.07	B	51
	qSIS11.2	11	21	S11_2838776	S11_3716306	877,530	2.34	3.53	0.04	B	136
Chlorophyll content-IM	*qCHL11.1*	11	5	S11_1086712	S11_1293020	206,308	2.19	5.41	-1.00	P	34
	qCHL11.2	11	14	S11_2666525	S11_2724222	57,697	2.02	4.86	-0.95	P	7
Chlorophyll content-ICIM	*qCHL2.20*	2	86	S2_20258450	S2_20346560	88,110	3.69	7.44	1.18	B	7
	qCHL2.30	2	143	S2_30353435	S2_30402468	49,033	2.34	4.69	-0.94	P	7
	qCHL3.26	3	136	S3_26705619	S3_26709038	3,419	3.22	6.42	-1.10	P	1
Shoot length-IM	*qSHL1.1*	1	11	S1_1708228	S1_1747144	38,916	2.04	5.03	-1.42	P	7
	qSHL1.7a	1	48	S1_7259818	S1_7296346	36,528	3.93	9.26	-1.95	P	7
	qSHL1.38	1	168	S1_38286772	S1_38611845	325,073	25.35	48.03	-4.43	P	52

(Table 3.4 continued)

Phenotype	QTL	Chr[β]	Position (cM)	Left Marker	Right Marker	QTL Interval Size (bp)	LOD	PVE (%)	Add. Effect	Parental Source of Increasing Allele Effect[§]	No. of genes in QTL interval
	qSHL3.34	3	185	S3_34720589	S3_35060080	339,491	2.36	5.65	1.54	B	69
	qSHL5.4	5	29	S5_4565557	S5_4699921	134,364	2.32	5.64	-1.52	P	23
	qSHL1.38	1	168	S1_38286772	S1_38611845	325,073	36.91	51.64	-4.59	P	52
	qSHL2.18	2	77	S2_18806154	S2_18937362	131,208	3.01	2.71	1.04	B	25
	qSHL3.34	3	185	S3_34720589	S3_35060080	339,491	4.40	3.96	1.29	B	69
	qSHL5.3	5	25	S5_3353753	S5_3506138	152,385	7.08	6.79	-1.66	P	21
	qSHL12.25	12	93	S12_25709174	S12_25887173	177,999	2.25	2.05	0.91	B	30
Root length-IM	*qRTL1.26*	1	121	S1_26421289	S1_26447134	25,845	2.73	6.52	0.32	B	6
	qRTL2.24	2	114	S2_24961302	S2_24961342	40	4.14	9.72	0.39	B	0
	qRTL2.26	2	120	S2_26028043	S2_26070191	42,148	4.21	9.91	0.39	B	9
	qRTL2.33	2	160	S2_33573567	S2_33614297	40,730	3.94	9.50	0.39	B	7
	qRTL3.6	3	36	S3_6011601	S3_6027452	15,851	3.47	8.23	0.36	B	2
	qRTL3.7	3	44	S3_7130220	S3_7209963	79,743	4.47	10.70	0.41	B	15
	qRTL3.10	3	57	S3_10116591	S3_10132745	16,154	5.04	11.99	0.43	B	2
	qRTL4.10	4	24	S4_10625625	S4_10726368	100,743	2.01	4.88	0.28	B	14
	qRTL8.4	8	37	S8_4558562	S8_4858127	299,565	2.12	5.34	0.36	B	41
	qRTL8.19	8	59	S8_19884635	S8_19898432	13,797	3.27	7.75	0.41	B	2
	qRTL8.27	8	109	S8_27238050	S8_27304101	66,051	2.10	5.13	-0.28	P	9
	qRTL9.14	9	39	S9_14960521	S9_14976723	16,202	2.66	6.45	-0.36	P	3
Root length-ICIM	*qRTL1.22*	1	102	S1_22666852	S1_22677418	10,566	2.27	3.54	0.23	B	2
	qRTL1.26	1	121	S1_26421289	S1_26447134	25,845	2.18	3.41	0.23	B	6
	qRTL3.9	3	56	S3_9853159	S3_9891061	37,902	4.29	7.59	0.34	B	7
Dry weight- IM	*qDWT1.21*	1	97	S1_21707357	S1_21733437	26,080	2.34	5.60	-0.01	P	6
	qDWT4.32	4	126	S4_32367131	S4_32367159	28	2.39	5.73	-0.01	P	1

(Table 3.4 continued)

Phenotype	QTL	Chr[β]	Position (cM)	Left Marker	Right Marker	QTL Interval Size (bp)	LOD	PVE (%)	Add. Effect	Parental Source of Increasing Allele Effect[§]	No. of genes in QTL interval
	qDWT5.4	5	29	S5_4565557	S5_4699921	134,364	6.58	15.04	-0.01	P	23
	qDWT5.5	5	42	S5_5997340	S5_6196044	198,704	6.54	15.47	-0.01	P	32
	qDWT6.13	6	72	S6_13046472	S6_13097774	51,302	2.01	5.16	0.00	P	10
	qDWT6.20	6	90	S6_20929261	S6_20929283	22	3.75	8.95	-0.01	P	1
	qDWT6.23	6	102	S6_23812023	S6_24039384	227,361	3.71	8.91	-0.01	P	32
	qDWT11.2	11	10	S11_2379158	S11_2402109	22,951	2.44	6.03	-0.01	P	3
Dry weight-ICIM	*qDWT1.40*	1	185	S1_40372283	S1_40412316	40,033	2.07	3.13	0.00	P	6
	qDWT4.32	4	126	S4_32367131	S4_32367159	28	3.66	5.93	-0.01	P	1
	qDWT5.4	5	29	S5_4565557	S5_4699921	134,364	7.57	12.98	-0.01	P	23
	qDWT6.06	6	3	S6_692773	S6_782975	90,202	3.71	6.02	-0.01	P	13
	qDWT6.24	6	104	S6_24107596	S6_24228831	121,235	4.46	7.46	-0.01	P	19
Shoot-root ratio-IM	*qSRR1.7*	1	50	S1_7520182	S1_7569628	49,446	3.79	9.09	-0.38	P	5
	qSRR1.29	1	135	S1_29561423	S1_29568978	7,555	3.12	7.42	-0.33	P	2
	qSRR1.36	1	159	S1_36158467	S1_36189206	30,739	5.84	13.42	-0.45	P	5
	qSRR1.382	1	170	S1_38286772	S1_38611845	325,073	10.31	23.01	-0.59	P	52
	qSRR2.28	2	133	S2_28317911	S2_28375704	57,793	4.71	10.96	-0.41	P	7
	qSRR2.31	2	146	S2_31037977	S2_31043939	5,962	3.20	7.62	-0.34	P	1
	qSRR2.33	2	160	S2_33573567	S2_33614297	40,730	4.18	9.90	-0.39	P	7
	qSRR2.34	2	168	S2_34660774	S2_35085922	425,148	2.94	7.37	-0.33	P	68
	qSRR3.8	3	49	S3_8327882	S3_8353264	25,382	2.65	6.32	-0.31	P	6
	qSRR3.10	3	57	S3_10116591	S3_10132745	16,154	2.69	6.58	-0.31	P	2
	qSRR3.11	3	70	S3_11848358	S3_11865689	17,331	2.48	5.93	-0.30	P	1
	qSRR4.10	4	24	S4_10625625	S4_10726368	100,743	2.44	5.91	-0.30	P	14
	qSRR8.19	8	59	S8_19884635	S8_19898432	13,797	2.38	5.70	-0.35	P	2

(Table 3.4 continued)

Phenotype	QTL	Chr[β]	Position (cM)	Left Marker	Right Marker	QTL Interval Size (bp)	LOD	PVE (%)	Add. Effect	Parental Source of Increasing Allele Effect[§]	No. of genes in QTL interval
Shoot root ratio-ICIM	*qSRR1.7*	1	50	S1_7520182	S1_7569628	49,446	6.93	8.73	-0.37	P	5
	qSRR1.386	1	171	S1_38636497	S1_38768787	132,290	15.64	22.43	-0.59	P	22
	qSRR2.33	2	160	S2_33573567	S2_33614297	40,730	8.53	10.92	-0.41	P	7
	qSRR3.9	3	56	S3_9853159	S3_9891061	37,902	4.33	5.25	-0.28	P	7
	qSRR8.26	8	107	S8_26716230	S8_26744324	28,094	2.53	3.01	0.21	B	5

[β] Chromosome where the QTL was located.
[§] Parental source of increasing allele effect was either Pokkali (P) or Bengal (B).
Add, additive; conc, concentration.

while the remaining two pairs accounted for 9% of the phenotypic variation. Six and four interacting QTLs with increasing effect involved Pokkali and Bengal alleles, respectively. All additive QTL positions were independent of epistatic QTLs.

- QTLs for NaK ratio

For NaK ratio, three additive QTLs (*qNaK1.11, qNaK6.2, qNaK6.5*) were significant in IM method but only two of the additive QTLs (*qNaK1.11, qNaK6.5*) were detected in ICIM. The *qNaK 6.5* explained 13% of the phenotypic variation while *qNaK6.2* and *qNaK1.11* were small-effect QTLs. All NaK ratio QTLs had increasing effect due to Bengal alleles. Of the seven pairs of epistatic QTLs, two pairs were large effect QTLs (PVE=11% and 18%) and five pairs were minor QTLs with PVEs lower than 9%. There was no epistatic QTL found in the same chromosome intervals for additive QTLs for NaK ratio, K^+, or Na^+ concentrations. Most of the QTLs with increasing allele effects were from Bengal, although, four epistatic QTLs with increasing effect were from Pokkali (D.1).

- QTLs for SIS

A total of eleven chromosomal regions with significant additive effect were detected on chromosomes 2, 5, 6, and 11 by IM. All QTLs are having small effects of at least 5% but not more than 9% of the phenotypic variation. Three QTLs were mapped on chromosome 2 (*qSIS2.8, qSIS2.29*, and *qSIS2.28*) with increasing effects from Pokkali alleles. In contrast, ICIM detected seven QTLs. The additive QTLs were distributed on chromosomes 5, 6, 7, 8, 9, and 11. The *qSIS5.1b* was a major QTL, explaining about 13% of the phenotypic variation. However, *qSIS5.1b* had increasing salt sensitivity effect from Bengal allele. Except for QTLs on chromosome 2, all other additive QTLs had increasing effect from Bengal allele. Between the two mapping methods, all QTLs were different except for *qSIS11.2*. For epistatic QTLs, five

pairs of interacting QTLs were significant of which four pairs explained 11-15% of the SIS variation. Among the additive QTLs, *qSIS6.2* was significantly interacting with *qSIS6.30* and increased the PVE from 5% to 15% (D.1). All interacting QTLs had increasing effect from Bengal alleles except the *qSIS2.20*.

- QTLs for chlorophyll content

A total of five chromosome regions with additive effects were detected for chlorophyll content under salt stress. Two QTLs were detected on chromosome 11 by IM while ICIM detected two QTLs on chromosome 2 and one QTL on chromosome 3. All additive QTLs were minor-effect QTLs, with increasing CHL effects from Pokkali alleles except *qCHL2.20*. In contrast, epistatic QTL mapping detected ten significant pairs of interacting QTLs. Eight QTL pairs had large effect with PVE as high as 36%. All additive QTLs were independent of epistatic QTLs for CHL.

- QTLs for shoot length

Six additive QTLs were detected by IM and another six QTLs were detected by ICIM. The *qSHL1.38* and *qSHL3.34* were significant QTLs in both methods. The *qSHL1.38* was a major QTL with LOD value of 37 and accounted for 48-52% of the phenotypic variation. The additive effect of *qSHL1.38* had increasing effect from Pokkali allele. Other SHL QTLs were located on chromosome 2, 3, 5, and 12 with small effects. Seven pairs of QTLs were significant in epistatic QTL mapping. Five pairs had 11% PVE and the other two pairs had 9% PVE. There was no epistatic QTL that co-localized with additive QTL.

- QTLs for root length

Twelve additive QTLs were detected for root length by IM. In contrast, ICIM detected only three QTLs, with *qRTL1.26* common in both methods. Two large-effect QTLs on chromosome 3

(*qRTL3.7* and *qRTL3.10*) were highly significant and accounted for 10% and 12% of the phenotypic variation, respectively. Both QTLs had increasing effects from Bengal alleles. All other QTLs were minor-effect QTLs, with increasing allele effects originating from Bengal. Five significant pairs of interacting QTLs with PVE ranging between 9-17% were detected. None of the interacting QTLs were found similar or co-localizing to additive QTLs.

- QTLs for dry weight

For shoot dry weight, nine additive QTLs were significant by IM. Three QTLs located on chromosome 5 *(qDWT5.2, qDWT5.4* and *qDWT5.5*) were large-effect QTLs that accounted for 11%, 15%, and 15% of the phenotypic variation, respectively. Other QTLs were distributed on chromosomes 1, 4, 6, and 11, with PVE of at least 5%. In contrast, ICIM detected five significant QTLs for DWT. Two QTLs (*qDWT4.32* and *qDWT5.4*) were common in both methods. Among the five QTLs by ICIM, *qDWT5.4* had the largest effect (PVE=13%) with LOD score of 7.6. All DWT additive QTLs had increasing effects coming from Pokkali alleles. Analysis of epistatic QTLs detected six pairs of interacting QTLs. All pairs of interacting QTLs except *qDWT4.16* and *qDWT10.19* had large effect of at least 10% PVE. Intervals of all epistatic QTLs were independent of additive QTLs (D.1).

- QTLs for shoot-to-root ratio

Additive QTL mapping by IM detected three large-effect and two small-effect QTLs located on chromosomes 1 and 2. The *qSRR1.382, qSRR1.36* and *qSRR2.28* were highly significant and had PVE of 23%, 13%, and 11%, respectively. Conversely, ICIM method identified five significant additive QTLs. Among the QTLs, two were large effects QTLs (*qSRR1.386* and *qSRR2.33*) with PVE of 22% and 11%, respectively. Pokkali alleles had increasing effect in all additive QTLs for SRR. For interacting QTLs, five large-effect QTL pairs of Bengal and Pokkali

origin were detected. All interacting QTLs were mapped to chromosomal regions different from additive QTLs.

3.3.5 Quality and accuracy of QTL mapping

Segregation distortion is commonly observed in populations developed from crosses between *indica* and *japonica* rice varieties. We mapped the regions of segregation distortion to determine if significant SDLs co-localized to the QTLs detected in this study. Interval mapping for SDLs detected sixteen significant intervals that were skewed toward either parent (Table 3.5). For each chromosome, at least one SDL was mapped, except on chromosomes 2, 4, and 12. In most of the SDLs, Pokkali allele transmission was favored. In chromosome 11 alone, four significant intervals showed segregation distortion favoring inheritance of Pokkali alleles. The average interval size of SDLs was about 198Kb, with the smallest and largest interval size of 600 bp (*sdl11.26*) and 1.4Mb (*sdl9.12*), respectively. By comparing the positions of QTLs against the positions of SDLs, the additive QTL *qK1.8* and epistatic QTL *qCHL9.12* overlapped exactly with *sdl1.8* and *sdl9.12* intervals. Therefore, these two QTLs should be considered with caution as they deviate from the expected 1:1 segregation ratio in the RIL population. The Bengal allele was transmitted to progeny lines more frequently than the Pokkali allele in *sdl1.8*. In contrast, Pokkali allele was favorably inherited in *sdl9.12*. Overall, most additive and epistatic QTLs mapped in this study were in chromosomal regions not affected by segregation distortion.

Plant height is one of most frequently studied traits in QTL mapping. Several studies showed that plant height has high heritability and stable at different growth stages at different environments (Yan et al., 1998). In rice, 1,011 QTLs were reported for plant height (www.gramene.org). Among these QTLs, *sd1* is the main QTL that played a major role in the development of semi-dwarf varieties in rice (Khush, 1999). To assess the quality of our

Table 3.5. Interval mapping of segregation distortion loci (SDLs) in Bengal/Pokkali F_6 RIL population.

SDL	Chromosome	Position (cM)	Left Marker	Right Marker	Interval size (bp)	LOD	Segregation ratio	
							Bengal	Pokkali
sdl1.8	1	63	S1_8656025	S1_8901503	245,478	7.0103	1	0.419
sdl1.12	1	74	S1_12394007	S1_12414777	20,770	6.6211	1	0.4304
sdl3.29	3	153	S3_29855008	S3_30045852	190,844	4.0197	0.5244	1
sdl3.34	3	181	S3_34487907	S3_34521908	34,001	3.4639	0.5504	1
sdl5.22	5	96	S5_22077219	S5_22142421	65,202	3.2006	0.5639	1
sdl2.4	6	23	S6_4269744	S6_4327404	57,660	3.2649	0.5605	1
sdl6.9	6	57	S6_9246940	S6_9317830	70,890	3.0751	1	0.5706
sdl7.26	7	109	S7_26680214	S7_26796826	116,612	2.5927	0.5983	1
sdl8.7	8	43	S8_7488739	S8_7668333	179,594	29.5389	0.1136	1
sdl8.16	8	52	S8_16619372	S8_16941109	321,737	22.0004	0.1761	1
sdl9.12	9	29	S9_12915373	S9_14359383	1,444,010	13.3385	0.2847	1
sdl10.12	10	31	S10_12765359	S10_12968073	202,714	2.5777	0.5992	1
sdl11.17	11	61	S11_17286328	S11_17316420	30,092	3.5648	0.5455	1
sdl11.22	11	91	S11_22242895	S11_22274274	31,379	2.8801	0.5814	1
sdl11.23	11	101	S11_23708208	S11_23866022	157,814	2.9439	0.5778	1
sdl11.26	11	115	S11_26254930	S11_26255530	600	5.3304	0.4724	1

phenotypic data and the accuracy of our QTL mapping, we surveyed plant height QTLs in rice under normal or stress conditions and compared the positions of our SHL QTLs to see if we can detect any of the previously reported plant height QTLs. In both mapping methods, the green revolution gene *sd1* gene, LOC_Os01g66100 (Spielmeyer et al., 2002) was located within our major QTL designated as *qSHL1.38*, with LOD value as high as 36 and PVE of 51%. The *sd1* gene is about 95 Kb away from the left SNP marker and 226 Kb from the right SNP marker of *qSHL1.38*. Moreover, *qSHL12.25* was found within the region of *qPHT12-1* on chromosome 12 located between 23,603,156-26,017,884 bp region (Hemamalini et al., 2000). Also, *qSHL3.34* was covered within the interval of *QPh3c* located between 32,945,649-36,396,286 bp of chromosome 3 (Li et al., 2003). The minor QTL *qSHL1.7* was flanked within *ph1.2* located in 5,941,464-7,445,919 bp region on chromosome 1 (Marri et al., 2005); while *qSHL2.18* was found within the reported QTL on chromosome 2 at 17,484,665-33,939,159 bp region (Huang et al., 1996). Additionally, *qSHL5.6* was confirmed within the QTL region of chromosome 5 located in between 5,255, 880-6,700,408 bp region (Mei et al., 2003) and in *ph5* located between 6,132,767-18,875,558 bp region on chromosome 5 (Zhuang et al., 1997). In summary, the locations of six *SHL* QTLs matched with previously reported plant height QTLs. In addition, four new minor QTLs were mapped in this study, each contributing at least 5% of the plant height variation. Together with other QTLs for other traits, a total of eleven QTLs in this study were validated (Table 3.6). Therefore, our QTL mapping by IM and ICIM methods using ultra-high density genetic map is robust and informative.

3.3.6 Identification of candidate genes in the QTL regions

The saturation of SNP markers in our linkage map allowed us to detect QTLs at an interval size much shorter than previously reported QTLs. In this study, the average interval size of a

Table 3.6. Summary of additive QTLs co-localizing to previously reported QTLs.

Trait	QTL in this study	Previous QTL	Reference
K^+ concentration	*qK1.11*	*qSKC1*	Thomson et al. (2010)
	qK6.4	*QTL on chr. 6, at 30cM*	Koyama et al. (2001)
NaK ratio	*qNaK1.11*	*qSNK1*	Thomson et al. (2010)
		QTL on chr. 1, at 74cM	Koyama et al. (2001)
Salt injury score	*qSIS9.8*	*qSES9*	Thomson et al. (2010)
Plant height	*qSHL1.38*	*sd1*	Spielmeyer et al. (2002)
	qSHL1.7	*ph1.2*	Marri et al. (2005)
		qPH1.2	Bimpong et al. (2013)
	qSHL2.18	*QTL on chr. 2 at 17-33 Mb*	Huang et al. (1996)
	qSHL3.34	*QPh3c*	Li et al. (2003)
	qSHL5.6	*ph5*	Zhuang et al. (1997)
		QTL on chr. 5 at 5.2- 6.7 Mb	Mei et al. (2003)
	qSHL12.25	*qPHT12-1*	Hemamalini et al. (2000)
Shoot dry weight	*qDWT6.24*	*qDWT6.1*	Bimpong et al. (2013)

QTL was 132 Kb, with minimum and maximum interval size of 22 bp and 877 Kb, respectively (Table 3.4). For nine traits, IM and ICIM mapped 64 and 36 additive QTLs. Fifteen QTLs were commonly detected in both methods with a total of 85 QTLs. To identify candidate genes underlying fitness of rice under salt stress, we looked at all genes in the QTL region using flanking markers. For 36 additive QTLs by ICIM, a total of 704 genes were present within QTLs (Supplementary Table S3.3, available upon request), of which, 110 were annotated while the 594 genes were identified as expressed proteins, hypothetical proteins, transposon, and retrotransposon proteins. Similarly, for 64 additive QTLs identified by IM method, only 111 of 1046 genes were annotated. For the 1344 gene models in the 85 QTLs for nine traits, 79 genes were classified in 7 biological processes, 50 genes were classified into 7 molecular functions, and 49 genes were classified into 16 protein classes (Figure 3.3). A large portion of the candidate

genes was involved in metabolic processes and responses to stimuli. Candidate genes classified in biological regulation and localization (six transporters) were found within QTLs.

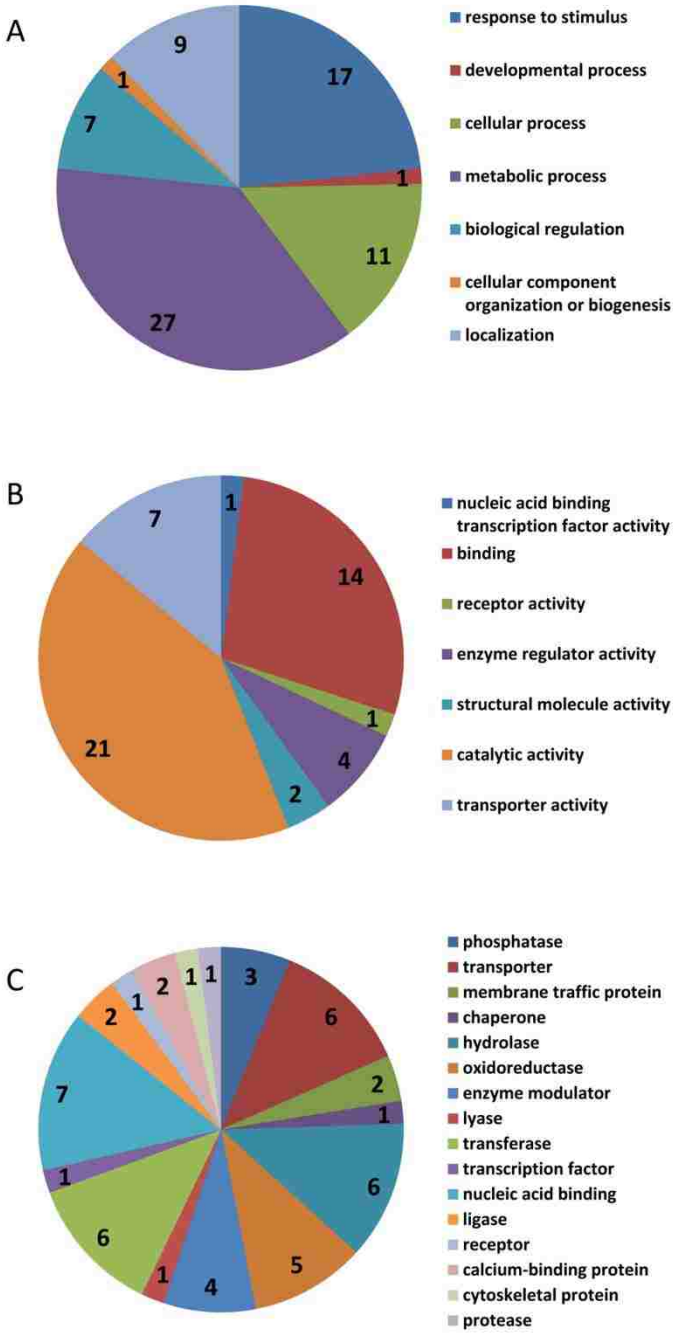

Figure 3.3 Functional classification of annotated candidate genes delimited by additive QTLs for salinity tolerance. (A) classification by biological class; (B) classification by molecular function; (C) classification by protein class.

3.4 Discussion

QTL mapping has been implemented in many breeding programs to discover genes underlying quantitative traits. However, many of these reported QTLs covered large chromosome intervals, thus, limiting the application of flanking markers in predicting the phenotype of the plant. A major constraint to previous QTL mapping studies is the number of available polymorphic markers. However, with reduction in DNA sequencing cost, high resolution QTL mapping is now possible using SNP markers. In this study, we utilized the GBS approach to develop an ultra-high-density genetic linkage map of rice for identification of QTLs for traits related to salinity tolerance. Thirty-eight SNP calls segregating in the RIL population were validated by re-sequencing the target region in both parents. Out of 38 SNP markers, only one SNP call in Bengal was not in agreement (D.2). Therefore, the GBS data have high quality SNP calls for linkage and QTL mapping. In spite of the large number of SNP markers placed on the linkage map, there were twenty gaps of about 5cM intervals. These gaps could be due to removal of SNP markers during filtering process. Due to multiplexing of large number of DNA samples in the GBS, representation of a SNP in all samples was greatly reduced resulting in removal of more than two-thirds of the GBS data. The linkage map closely resembled the rice genetic map of Harushima et al., (1998). Mapping of segregation distortion loci using this map indicated sixteen intervals showing segregation distortion (Table 3.5). Two SDLs co-localized to QTLs for salinity tolerance (*qK1.8* and *qCHL9.12*). Therefore, genetic variances contributed by these QTLs may not be accurate due to segregation distortion. In addition to availability of numerous SNP markers for linkage map construction, the quality of phenotypic estimates is equally important for QTL mapping. We assessed this by comparing our shoot length QTLs with reported plant height QTLs. Ten QTLs for SHL were detected (Table 3.4), of which, six QTLs

for plant height including the major *sd1* (*qSHL1.38*) co-localized to previously reported plant height QTLs. Validation of those QTLs suggests that our phenotypic and genotypic data for QTL mapping are of high quality (Table 3.6). With five to six markers per cM, the average QTL interval size was 132Kb. The maximum resolution of QTL was about 22 bp interval (*qSIS6.20*) and the largest QTL interval size was about 877Kb (*qSIS11.2)* (Table 3.4).

Previous QTL mapping studies for salinity tolerance mainly focused on detecting additive QTLs despite the complex nature of salinity tolerance. In this study, we also mapped interacting QTLs significantly contributing to the phenotypic variation of each trait under salt stress (D.1). Di-genic interval mapping for epistatic QTLs revealed interaction of alleles from Pokkali and Bengal. In general, interacting QTLs were located in chromosome intervals independent of additive QTLs. Likewise, the variance explained by epistatic QTL pair was higher than the variance explained by individual additive QTL. For example, additive QTLs for Na^+ concentration and CHL revealed only a few small-effect QTLs. In contrast, many of the epistatic QTL pairs for Na^+ and CHLs had larger PVE as high as 35%. Therefore, these findings indicated the importance of epistatic QTLs in salt stress response in rice. Many of the QTLs flanked small intervals with few candidate genes. Overall, the ultra-high density genetic map and the high-quality phenotypic data facilitated a high resolution QTL mapping for salinity tolerance. In addition, the genetic map will be useful in discovery of novel QTLs for other contrasting agronomic traits between Bengal and Pokkali.

Since the beginning of the search for QTLs underlying salinity tolerance, Na^+ concentration, K^+ concentration, NaK ratio, and salt injury score were often investigated. Similar to previous reports, Na^+ concentration was highly correlated to SIS or standard evaluation score (SES) and survival of rice plants under salt stress (Yeo et al., 1990; Platten et al., 2013). It had significant

positive correlation to NaK ratio and shoot K^+ concentration (Table 3.2). The Na^+ and K^+ relationship implies that as shoot Na^+ concentration increases, shoot K^+ concentration also increases. It is likely that during salt stress, many lines do not discriminate these cations, thus, suggesting possible accumulation of Na^+ and K^+ in the shoot through non-selective cation channels (Demidchik and Maathuis, 2007). This is evident in the high heritability of Na^+ and K^+ concentrations in the population (Table 3.1). In previous studies of QTLs for shoot Na^+ concentration, QTLs were mapped on chromosomes 1 (Thomson et al., 2010), 3, 9, 11, (Wang et al., 2012), 4 (Koyama et al., 2001), and 7 (Lin et al., 2004). None of our additive QTLs for Na^+ concentration co-localized to previous QTLs. But the epistatic QTL *qNa6.4* is possibly the same additive QTL in chromosome 6 at 24cM (Koyama et al., 2001). The effects of additive QTLs for Na^+ concentration were small. Surprisingly, four pairs of interacting intervals had significant large effects (11-15% PVE), suggesting that interactions among Na^+ QTLs were important in the accumulation of Na^+ in shoot. Alleles of Na^+ QTLs from both parents contributed to shoot Na^+ accumulation. In contrast, all alleles of additive QTLs for shoot K^+ concentration were from Pokkali (Table 3.4). Therefore, it is interesting to know the underlying genes for K^+ accumulation and their role in accumulation of other cations like Na^+. The presence of transgressive segregants exhibiting higher concentration of shoot K^+ and lower NaK ratio than Pokkali suggests the presence of positive alleles in both parents for selective cation transport during salt stress (Figure 3.1). In case of Pokkali, salt tolerance response could be due to maintenance of high K^+ concentration or low NaK ratio (Ren et al., 2005) and by compartmentalization of Na^+ ions into the shoot vacuoles (Kader and Lindberg, 2005). The strong relationship among Na^+, K^+, and SIS prompted us to look for the co-location of QTLs underlying these traits. Our result showed that *qNa6.5* and *qNaK6.5*, *qK1.11* and *qNaK1.11*, and

qSIS6.2 and *qNaK6.2* co-localized in the same intervals (Table 3.4). Therefore, it is possible that these traits shared the same underlying causal genes. The co-location of *qNa6.5* and *qNaK6.5* is more likely not coincidental because both alleles of the two QTLs came from Bengal and had increasing effect in the concentration of Na^+ ions. On the other hand, the co-location *of qK1.11* and *qNaK1.11* is consistent with co-location of shoot K^+ concentration, *SKC1* and shoot Na^+/K^+ ratio, *SNK1* (Thomson et al., 2010, Wang et al., 2012). Allele substitution of Bengal with Pokkali at *qK1.11* had increasing effect in the shoot K^+ concentration. In contrast, Bengal allele of *qNaK1.11* had increasing effect on NaK ratio, thus, corroborating the desirability of Pokkali allele at the locus for salt tolerance. In previous studies, *SKC1* was responsible for 10- 40% of the variation in shoot K^+ concentration (Koyama et al., 2001; Lin et al., 2004; Thomson et al., 2010; Wang et al., 2012). Here, the *qK1.11* accounts for only 16% of the variation. The discrepancy in the estimation of PVE is likely attributed to differences in population size and number of markers used in different studies. The q*K1.11* is covering a 52Kb interval between 11.52-11.58 Mb region on chromosome 1 with six genes. This interval is within the reported *SKC1* by Thomson et al., (2010), but, downstream of 11.46Mb region of the cloned *HKT1;5* (Ren et al., 2005). While Thomson et al. (2010) assumed *HKT1;5* (LOC_Os01g20160) as the underlying gene for *qSKC1* or *Saltol,* it is also possible that other genes contributing toward salt tolerance might be present in the *SKC1* region. This possibility is supported by the findings from a genome-wide association mapping study (Kumar et al., 2015), where twelve significant SNPs were located between 9.6 to 14.5 Mb region of chromosome 1. One of the twelve SNPs with high linkage disequilibrium (LD) at 11.6 Mb region (1:11608731) is 26Kb away from the right marker of *qK1.11*. Furthermore, *HKT1;5* allele mining in several rice cultivars showed a weak association of *HKT1;5* allele to low Na^+ concentration to account for salinity tolerance. The

HKT1;5 allele in aromatic group that included Pokkali showed low Na^+ concentration. However, several cultivars having different *HKT1;5* alleles (Aus, FL478, Hasawi, Daw, *Japonica* lines, and *O. glaberrima)* also showed low Na^+ concentration and high salt tolerance (Platten et al., 2013). Additionally, our genetic map data showed the availability of markers that flanked *HKT1;5* gene (Supplementary Table S3.1, at 70.2cM) and the absence of segregation distortions in these regions (Table 3.5), but the IM and ICIM methods both detected QTL for high shoot K^+ concentration downstream of *HKT1;5*. Interestingly, the *qK1.11* interval contained two transposons, three uncharacterized expressed proteins, and a CC-NBS-LRR-encoding gene (LOC_Os01g20720). NBS-LRR genes are the largest class of resistance genes implicated in the recognition of pathogen-derived avirulence protein. In rice, a gene encoding a CC-NBS-LRR, *Pb1,* provided a durable panicle blast resistance by interacting with WRKY45 transcription factor for the activation of signal transduction pathway (Inoue et al., 2013). On the other hand, overexpression of *ADR1* gene encoding a CC-NBS-LRR in *A. thaliana* showed enhanced drought tolerance (Chini et al., 2004). Therefore, the role of LOC_Os01g20720 gene in *qK1.11* in activation of the signal transduction pathway during salt stress to accumulate high K^+ ions in shoot should be investigated. Other QTLs for shoot K^+ concentration such as *qK1.8, qK1.38, qK5.4, qK6.4,* and *qK6.5* covered at least 250 kb intervals containing 33, 40, 86, 61, and 34 gene models, respectively. Candidate genes present in these QTL intervals include protein kinases, transcription factors, ethylene, auxin-responsive proteins, flavin-containing monooxygenases, and several expressed proteins of unknown function. In contrast, *qNa2.7* was saturated with transposons and retrotransposons except for a putative membrane lipid channel, scramblase protein (LOC_Os02g14290). The *qNa12.18* flanked four transposons and a hypothetical protein.

For NaK ratio QTLs, the co-location of *qSIS6.2* and *qNaK6.2* confirmed the significant correlation of NaK ratio to SIS. Both intervals had increasing effect from Bengal, indicating the undesirability of Bengal allele at this locus. Both QTLs delimited only seven genes including a WRKY113 transcription factor (LOC_Os06g06360). Whether WRKY113 is interacting with the CC-NBS-LRR in *qK1.11* or *qNaK1.11* like the *Pb1*, presents an interesting perspective to study gene interactions and salt tolerance. In contrast, the large-effect *qNaK6.5* (or *qNa6.5*) still covered a 264Kb interval and contained 34 gene models. Candidate genes in this interval are MYB transcription factor (LOC_Os06g10350), cyclic nucleotide-gated ion channel (LOC_Os06g10580), transcription elongation factor SPT5 (LOC_Os06g10620), and leaf senescence-related protein (LOC_Os06g10560). Among the NaK QTLs, *qNaK1.11* is likely the same QTL as *qSNK1* (Koyama et al. 2001; Thomson et al., 2010).

SIS reflects the overall plant's response to salt stress. Hence, we are particularly curious in finding QTLs to identify underlying genes for this trait. Among the additive QTLs, *qSIS5.1b* had PVE of 13% with increasing effect coming from Bengal allele. Therefore, in breeding for low SIS, the corresponding Pokkali allele at *qSIS5.1b* is desirable. The variance explained by *qSIS6.2* alone was only 5%, but, interaction to *qSIS6.30* increased the PVE to 15% (D.1). This result indicated the additive and epistatic effect of a locus and emphasized the importance of QTL interactions in understanding the complexity of SIS or salt tolerance. Among previously mapped QTLs for salt evaluation score (SES) or salt tolerance rating (STR), the *qSIS9.8* was located within the interval of *qSES9* (Thomson et al., 2010). The *qSIS2.8* interval contained twenty-five genes, one of which encoded a cyclic nucleotide-gated ion channel. In contrast, *qSIS5.1b* and *qSIS6.20* contained two and one gene, respectively. Both QTLs delimited a lectin protein kinase (LOC_Os06g35870, LOC_Os05g03450). In *A. thaliana*, lectin protein kinases were involved in

the protein-protein interactions for structural stability of plasma membrane and plant cell wall (Gouget et al., 2006). Therefore, it will be interesting to see if plasma membrane stability conferred by lectin protein kinase enhances salinity tolerance. Similarly, the *qSIS6.21* interval confined a single candidate gene that encodes a receptor-like protein kinase 5 precursor (LOC_Os06g36270). In *qSIS5.03*, a vacuolar ATP synthase (LOC_Os05g01560) is one of the four genes in the interval while a trehalose phosphatase is one of the five candidate genes in *qSIS5.1a*. In rice, transcript expression of a mitochondrial ATP synthase (RMtATP6) was induced in leaves by NaCl and $NaHCO_3$ treatments and overexpression of RMtATP6 in tobacco plants showed enhanced seedling salt tolerance (Zhang et al., 2006). On the other hand, overexpression of trehalose-6-phosphate phosphatase and trehalose-6-phosphate synthase increased tolerance to drought, salt, and cold in rice (Jang et al., 2003). Also, of great interest is the *qSIS6.7* interval that delimited only three genes including a pyrophosphate fructose-6-phosphate 1-phosphotransferase (LOC_Os06g13810) and a flavin monooxygenase in *qSIS7.14*. Pyrophosphate: fructose-6-phosphate 1-phosphotransferase was associated to seedling salt tolerance (Lim et al., 2014) while overexpression of a flavin monooxygenase designated as YUCCA enhanced drought tolerance of *A. thaliana* (Cha et al., 2015). Additionally, *qSIS8.24*, *qSIS9.8*, and *qSIS11.2* delimited genes involved in signal transduction pathway.

Plant vigor under salt stress is a good predictor of tolerance. In addition to common traits investigated under salt stress, CHL, and growth parameters such SHL, RTL, SRR, and DWT were also examined. In soybean, salinity tolerance was determined by a major QTL for chlorophyll content (Patil et al., 2016). In contrast, additive QTLs for CHL were all minor-effect QTLs while several pairs of epistatic QTLs had PVE as high as 35% (Table 3.4, 3.5). Comparison of CHL QTLs with earlier reported QTLs co-localized *qCHL2.20* and *qCHL3.26*

within the intervals of *qCHL2* and *qCHL3* (Thomson et al., 2010). All other CHL QTLs are novel, thus, offering new targets for further analysis. The *qCHL3.26* interval flanked a single unknown expressed protein (LOC_Os03g47190) while *qCHL2.20* contained six retrotransposons and one expressed protein. Aldehyde dehydrogenase (LOC_Os02g49720) and zinc-knuckle family protein (LOC_Os02g49670) were found in *qCHL2.30* interval. *Arabidopsis* plants overexpressing aldehyde dehydrogenase improved salinity tolerance of plants by reducing the accumulation of reactive oxygen species (Sunkar et al., 2003). Among the thirty-four genes in the *qCHL11.1*, a NAC transcription factor and a glutathione S transferase are promising candidate genes. In rice, overexpression of a NAC transcription factor showed increased tolerance to drought and salt stress (Zheng et al., 2009). Conversely, glutathione S-transferase had negative effect to drought and salt tolerance in *Arabidopsis* plants (Chen et al., 2012). The *qCHL11.2* interval contained seven genes, one of which encodes an HVA22. In barley and *Arabidopsis*, aleurone cells transformed with HVA22 inhibited the formation of GA-induced formation of vacuoles and programmed cell death (Gou and Ho, 2008). Since vacuoles are important storage of Na^+ for salt tolerance, HVA22 is a promising candidate gene for salt tolerance.

Among the SHL QTLs, *qSHL1.38* and *qSHL2.18* were congruent to *qPH1.2* (Bimpong et al., 2013) and *qPH2* (Thomson et al., 2010), respectively, for plant height QTLs investigated under salt stress. The SHL QTLs contained many candidate genes. In addition to the major *sd1* gene within *qSHL1.38*, other candidate genes were AP2 domain containing protein (LOC_Os01g04020) in *qSHL1.1*, KH domain containing protein (LOC_Os01g13100) in *qSHL1.7a*, auxin response factor1 in *qSHL1.7b*, potassium transporter (LOC_Os01g13520) in *qSHL2.18*, gibberellin 2-oxidase (LOC_Os05g06670) in *qSHL5.3*, gibberellin 3-beta-

dioxygenase (LOC_Os05g08540), cytokinin-O-glucosyltransferase (LOC_Os05g08480) and auxin OsIAA15 (LOC_Os05g08570) in *qSHL5.4*, OsMAD66 transcription factor (LOC_Os05g11380) in *qSHL5.6*, and OsSAUR57 in *qSHL12.25*. A putative RNA-binding protein containing a KH domain was reported to be important in *Arabidopsis* plants for heat stress tolerance (Guan et al., 2013). In other plants, AP2/ERF transcription factors were implicated in the control of metabolism, growth, and development, and in responses to environmental stress (Licausi et al., 2013).

The relationship of Na^+ concentration with SHL, RTL, DWT, and SRR were not significant. However, correlation of these traits to SIS indicated growth inhibition with increasing sensitivity to salt stress (Table 3.2). For RTL, large-effect additive QTLs were detected on chromosome 3 (*qRTL3.7* and *qRTL3.10*) while the rest were minor-effect QTLs located on chromosomes 1, 2, 3, 4, 8, and 9. The majority of root length variation was explained in the epistatic QTLs. Similarly, QTLs for DWT detected only three large-effect QTLs on chromosome 5 (*qDWT5.2, qDWT5.4,* and *qDWT5.5*) and all epistatic QTL pairs had PVE not lower than 10%. Five large-effect additive QTLs were mapped on chromosomes 1 and 2 for SRR. The *qSRR1.382* was located on the same interval of *qSHL1.38* and so, the same *sd1* gene determined the increased SRR. The fact that all DWT and SRR additive QTLs were contributed by Pokkali suggested the growth-increasing effect of Pokkali alleles under salt stress. On the other hand, the significant epistatic QTLs identified in all traits emphasized the importance of additive and epistatic effects for salinity tolerance.

The growth of roots during seedling stage under salt stress was not investigated before. All RTL QTLs in this study were new QTLs. A total of 117 gene models were delimited by fourteen QTLs. In *qRTL1.22,* only two gene models were present, a retrotransposon and an

uncharacterized expressed protein. Of particular interest is the VQ domain containing protein (LOC_Os01g46440) within *qRTL1.26*. In *Arabidopsis,* VQ-containing proteins interact with WRKY transcription factors and negatively regulate plant resistance to pathogen infection (Wang et al., 2015). Other candidate genes within RTL QTLs are aldehyde dehydrogenase (LOC_Os02g43194) and polyamine oxidase (LOC_Os02g43220) in *qRTL2.26*, ankyrin repeat-reach protein (LOC_Os02g54860) and trehalose-6-phosphate (LOC_Os02g54820) among seven genes contained in *qRTL2.33,* an integral membrane protein (LOC_Os03g11590) in *qRTL3.6,* MYB transcription factor (LOC_Os03g13310) and transporters (LOC_Os03g13240, LOC_Os03g13250, and LOC_Os03g17740) in *qRTL3.7* and *qRTL3*. An asparagine synthetase (LOC_Os03g18130) is within *qRTL3.10*, while a vacuolar protein sorting-associated protein 18 (LOC_Os08g08060), transporter (LOC_Os08g08070), and an RLK gene (LOC_Os08g08140) are delimited in *qRTL8.4*. The *qRTL9.14* contained only three genes, one of which was a WRKY gene (LOC_Os09g25060). The *qRTL8.27* contained a PDR ABC transporter gene (LOC_Os08g43120).

Koyama et al. (2001) detected one QTL for dry mass in chromosome 6 at 34cM region. A total of six DWT QTLs were mapped in chromosome 6 by IM and ICIM. However, none of our QTLs were localized at 34cM region. The *qDWT6.24*, however, validated the *qDWT6.1* detected by Bimpong et al. (2013). Notable candidate genes within DWT QTLs were transporters (LOC_Os01g38670, LOC_Os01g38680, LOC_Os05g04600, and LOC_Os05g08430) in the intervals of *qDWT1.2*, *qDWT5.2*, and *qDWT5.4*, calmodulin-binding transcription factors (LOC_Os01g69910 and LOC_Os05g10840) in *qDWT1.40*, a REX1 DNA repair gene (LOC_Os05g10980) in *qDWT5.5*, a MYB transcription factor (LOC_Os06g02250) in *qDWT6.06*, and a lectin protein kinase (LOC_Os06g35870) in *qDWT6.20*. In addition, a

calcium-binding mitochondrial carrier (LOC_Os06g40200) was present within *qDWT6.23* while an ABC-type transporter gene (LOC_Os06g40550) was in *qDWT6.24*.

SRR QTLs under salt stress were not investigated in previous QTL mapping studies. All QTLs for SRR were new QTLs for further understanding of plant's fitness under salinity stress. The large effect QTL *qSRR1.36* spanned five genes including a WRKY119 gene (LOC_Os01g62510). The *qSRR1.382* and *qSRR1.386* contained an amino acid transporter (LOC_Os01g66010) and several receptor-like protein kinases. In contrast, *qSRR2.31* delimited a single expressed protein. Again, a trehalose-6-phosphate (LOC_Os02g54820) and ankyrin repeat rich protein (LOC_Os02g54860) were two of the seven genes found in the *qSRR2.33* interval while a HEAT repeat protein was within *qSRR2.34* interval and another transporter was located in *qSRR3.9*. In addition to few candidate genes with known functions present within small-effect QTLs (*qSRR3.10, qSRR3.11, qSRR4.10, qSRR8.19*, and *qSRR8.26*), there were several uncharacterized expressed proteins.

Taken together, at least six transporter genes were located within six QTLs, of which, three transporter genes were found in QTLs for root length (LOC_Os3g11590 in *qRTL3.6*; LOC_Os3g17770 in *qRTL3.9*, and LOC_Os3g11590 in *qRTL3.7*), while one transporter gene was contained in *qSIS11.2* (LOC_Os11g06810), *qCHL11.2* (LOC_Os11g05800), and *qSHL3.34* (LOC_Os03g61290). In addition to transporters and genes for detoxification or osmotic adjustment (flavin monooxygenase and trahalose-6-phosphate), the prevalence of protein kinases suggest the role of signal transduction pathway and possible regulation of biological and cellular processes by transcription factors (Figure 3.3).

3.5 Conclusion

The availability of ultra-high density genetic map and robust phenotypic data enabled us to identify additive QTLs with high resolution and facilitated identification of candidate genes. Detection of significant epistatic QTLs in addition to additive QTLs validated the complex architecture of salinity tolerance, which is possibly determined by concerted interactions of several genes. While *Saltol* or *SKC1* may provide salinity tolerance and is already being introgressed into several rice varieties in Asia, it may not provide adequate tolerance to salt stress. Our result suggested the need to use of multiple QTLs, especially the genes for low salt injury score to enhance salinity tolerance. The candidate genes identified in this study will be useful targets for functional genomics, gene-pyramiding, and gene-based marker-assisted breeding. Our study demonstrated the power and application of GBS for QTL mapping of a complex genetic trait like salinity tolerance.

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CHAPTER 4. IDENTIFICATION AND VALIDATION OF STABLE QTLS FOR SEEDLING SALINITY TOLERANCE IN INTROGRESSION LINES (ILS) OF POKKALI IN BENGAL BACKGROUND

4.1 Introduction

Backcrossing is an established and efficient approach in introgression of both qualitative and quantitative traits from landraces and wild relatives to elite adapted varieties. The use of advanced backcross populations or introgression lines (ILs) has been widely employed in genetic studies to identify and validate the beneficial effect of QTLs from donor parents (Tanksley and Nelson, 1996). In tomato, ILs were useful in fine mapping of QTL for fruit mass (Eshed and Zamir, 1995). Likewise, ILs were developed and used in QTL mapping for fusarium head blight resistance in wheat (Buerstmayr et al., 2011), mineral accumulation in beans (Blair and Izquierdo, 2012), yield attributes in soybean (Kim et al., 2012), and nematode and fusarium wilt disease resistance in cotton (Ulloa et al, 2016). In rice, several introgression line populations were developed to transfer and map QTLs for agronomic and domestication traits (Furuta et al., 2014; Subudhi et al., 2015), yield and morphological traits (Thomson et al., 2003; Septiningsih et al., 2003; Tian et al., 2006), Zn and Fe content in grain (Xu et al., 2015), and photosynthesis parameters (Gu et al., 2012).

Among the abiotic stresses that negatively affect rice production, soil and water salinity is a major crop production constraint in the arid regions and coastal areas that heavily relied on irrigation. The genetics of salinity tolerance in rice has been investigated for many years. Several QTLs and genes for morphological and physiological traits associated to salinity tolerance were reported. However, development of salt tolerant rice varieties is still difficult and slow (Flowers and Flowers, 2005). Majority of QTLs detected so far in various mapping populations were small effect QTLs. Furthermore, the well-known and widely used tolerant donors, Pokkali and Nona

Bokra, are low yielding and possess many undesirable agronomic traits (Gregorio et al., 2002). To address the linkage drag associated with landraces, and for discovery of genes responsible for abiotic and biotic tolerance, IRRI had initiated a backcross breeding program in which 203 donor accessions were crossed to three high yielding varieties as recurrent parents (Ali et al., 2006). After 4 cycles of backcrossing, screening, and progeny testing, large number of introgression lines with significantly improved tolerance to biotic and abiotic stress were generated. Genotyping of selected 83 ILs using 160 SSR markers allowed the discovery and fine mapping of QTL for drought tolerance to a small region of ~3cM (Li et al., 2005). For salinity, backcross lines derived from Pokkali were evaluated to validate the *Saltol* QTL. However, further studies are needed because backcross lines containing *Saltol* and non-*Saltol* QTL showed same level of seedling salinity tolerance (Alam et al., 2011). Moreover, field stress evaluation of near isogenic lines containing *Saltol* locus did not show higher yield performance than the susceptible IR29 (Thomson et al., 2010).

The need for QTLs and perfect markers predictive of salinity tolerance is still a challenge. For these reasons, it is important to confirm the stability and contribution of QTLs toward salinity tolerance. Most of the QTL mapping studies were implemented in $F_{2:3}$ and RIL populations with limited number of genotypes and markers. In this study, we used ILs for QTL mapping of nine traits related to salinity tolerance using SSR and GBS-derived SNP markers. The QTLs identified in the ILs were compared to previously mapped QTLs in RIL population for confirmation. Also, we identified salinity tolerant lines that were near isogenic to Bengal, which would be useful as improved variety or resource materials in transferring salinity tolerance genes to other elite US varieties.

4.2 Materials and methods

4.2.1 Plant material and evaluation for salt tolerance

Introgression lines were developed from a cross between Pokkali and Bengal. Pokkali is highly tolerant to salinity stress (Gregorio et al., 2002) while Bengal is highly salt sensitive (De Leon et al., 2015). Bengal and Pokkali were used as recurrent and donor parent, respectively. Due to pollen sterility of F_1 plants, Bengal was used as pollen parent to generate BC_1 generation. However, in BC_1 and subsequent backcross generation Bengal was used as female to generate a BC_4F_1 population which was then self-pollinated repeatedly to finally produce BC_4F_4 lines by single seed descent method.

A total of 292 BC_4F_4 lines were screened for seedling salinity tolerance following the protocol described by De Leon et al. (2015). Briefly, ten plants per line per replication were grown for two weeks in nutrient solution containing 1g/L of Jack's Professional fertilizer 20-20-20 (J.R. Peters, Inc.) and 300mg/L ferrous sulfate. The seedlings were then placed at salt stress level of 6 dSm^{-1} for two days before subjecting to 12 dSm^{-1} salt stress. Only five plants of uniform growth were scored for morphological and physiological traits related to salinity tolerance. The whole experiment was conducted in randomized complete block design replicated three times. Chlorophyll content (CHL) was measured using a SPAD-502 chlorophyll meter (Spectrum Technologies, Inc.) four days after salt stress. When the susceptible parent Bengal showed the characteristic salt sensitivity reaction, plants were scored for visual salt injury score (SIS) of 1 to 9, with 9 as highly sensitive. The root and shoot lengths (RTL, SHL) were also measured at this time. The ratio of shoot length to root length (SRR) were computed while shoot dry weight (DWT) data were obtained from five plants per line that were oven-dried at 65^{o}C oven for five days prior to weighing. The concentrations of Na^+ and K^+ in the shoots were

estimated from 100 mg tissue taken from a pool of five oven-dried plants. The ground tissues were digested by nitric acid: hydrogen peroxide (5:3 ml) method at 152-155^{o}C heating block for 3 hours (Jones and Case, 1990). The amount of Na^+ and K^+ was measured by flame photometer (model PFP7, Bibby Scientific Ltd, Staffordshire, UK). The final concentrations of ions were estimated from the standard curve derived from different dilutions of Na^+ and K^+ ions.

4.2.2 Statistical analyses

Analysis of variance for each trait was computed by glimmix procedure where the line was entered as fixed effect and replication was entered as random effect. Least square means of each line was extracted for QTL analysis. Broad sense heritability was computed by family mean basis (Holland et al., 2003). To see the relationship among traits, correlation procedure was employed. All data analysis was conducted using Statistical Analysis System (SAS) software version 9.4 for Windows (SAS Institute, 2012). Histograms were constructed in Microsoft Excel 2010 to show the distribution of introgression lines for each trait.

4.2.3 Genotyping of ILs using SSR and SNP markers

Leaf tissues from 292 lines were collected from each BC_4F_4 line grown in non-saline nutrient solution. The tissues were ground and genomic DNAs were isolated following the CTAB method (Chen and Ronald, 1999). The concentration of each DNA was estimated by a ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and was adjusted to 25ng/ul for PCR amplification. SSR primers were used for initial genotyping. For each PCR reaction, the mixture contained 12.8µl water, 2.5µl 10X PCR buffer, 2.5µl 25mM $MgCl_2$, 2.5µl 2mM dNTPs, 1.25µl reverse and forward primers (50ng/µl), 1U Taq polymerase (Promega Corporation, Madison, USA) and 50 ng of DNA. The PCR amplification was conducted with the following settings: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 45 sec, 55°C for 45 sec,

72°C for 1 min and a final extension at 72°C for 5 min. The PCR products were run in 4.5% SFR agarose gel electrophoresis and alleles of each line were scored according to the banding pattern of the parents. From the 292 lines, a subset of 88 lines with varying levels of salinity response based on SIS and introgressions were randomly selected for genotyping-by-sequencing (GBS). The DNAs of 88 ILs were extracted using the Qiagen DNeasy Plant Mini Kit following the manufacturer's protocol (Qiagen Inc., Valencia, CA, USA). The Genomic Diversity Facility, Cornell University Institute of Biotechnology (http://www.biotech.cornell.edu/brc/genomic-diversity-facility) provided the GBS service that included the genomic DNA library construction following the method of Elshire et al. (2011), 288-plex sequencing using the Illumina HiSeq sequencer, and SNP calling based on the Nipponbare reference genome MSU release 7 (Kawahara et al., 2013). The resultant GBS data were filtered for QTL analysis. Each SNP call at a particular coordinate was treated as a marker. Due to low read depth in GBS, all heterozygous SNP calls were treated as missing data. All non-polymorphic SNP markers across the 88 introgression lines were removed. Likewise, all SNP markers having more than 10% missing data or N calls were discarded before further analysis. The SNP calls for each line were scored as either Pokkali or Bengal allele.

4.2.4 Estimation of genome composition and QTL analysis for traits related to salinity tolerance

The genotypic data using SSR and GBS-SNP markers were used separately to estimate the genome composition of each line. The physical position of SSR markers along the chromosomes were obtained from gramene.org while SNP markers were ordered based on their physical positions in the rice genome (MSU release 7). The relative distance between markers was computed by subtracting the physical position of the first marker to the next marker. If two neighboring markers showed alleles of Pokkali, the relative distances of two markers were added

to represent the length of the segment. For single marker introgression, the distance to the next marker was considered the length of the segment. Genotypes were selected to represent a set of chromosome segment substitution lines (CSSL) or ILs using CSSL finder v. 0.9.7.2.2 (Lorieux, 2005). Percent genome composition and Pokkali introgressed segments of each IL were also computed from the CSSL analysis.

The phenotypic and genotypic data were combined and used in the CSSL QTL mapping function of QTL IciMapping software v. 4.1 (Wang et al., 2016) . By single marker analysis (SMA) and stepwise regression-based likelihood ratio test (LRT) methods, significant QTLs were identified at LOD threshold set at 2.0. The position and information on the effect of QTLs were estimated. To validate the effect and significance of QTLs for each trait, the positions of QTLs detected in ILs were compared to those QTLs detected in Bengal x Pokkali F_6 RIL population (De Leon et al., 2016). Introgression lines with high salt tolerance were selected for further evaluation of genomic composition, phenotypic attributes, and QTLs they contained for inquiry of possible tolerance mechanism.

4.3 Results

4.3.1 Phenotypic evaluations

The trait responses of ILs and the parents under salt stress were summarized in Table 4.1. There were significant differences between Bengal and Pokkali for NaK, SIS, SHL, RTL, DWT, and SRR. However, the difference in Na^+, K^+ concentrations, and CHL were not statistically significant. In the 292 ILs, significant phenotypic differences were observed for all traits except CHL. The spread of trait means indicated the presence of transgressive segregants. The Na^+, K^+, and SHL showed high heritability while moderate heritability was recorded for SIS and RTL. However, NaK, CHL, SRR, and DWT had very low heritability. In all traits, the distributions of

Table 4.1. Mean phenotypic response of parents and 292 ILs ($BC_4F_{4)}$ in traits under salt stress.

Trait Name	Bengal Mean	Pokkali Mean[€]	ILs Mean	ILs Std. Dev.	ILs Pr>F[β]	ILs Range	Heritability[#]
Na^+ (mmolkg^{-1})	1232.57	940.82[ns]	1277.47	295.95	0.2804	859.34-1821.31	0.65
K^+ (mmolkg^{-1})	548.08	590.19[ns]	575.48	159.75	<.0001	320.93 - 836.66	0.95
NaK (ratio)	2.29	1.59*	2.31	0.57	<.0001	1.62 - 3.92	0.08
SIS	7.8	3.00***	6.60	1.21	<.0001	3.22 - 9.00	0.28
CHL (SPAD unit)	18.99	16.05[ns]	22.17	6.61	0.2136	15.85 - 45.32	0.05
SHL (cm)	31.67	47.20**	33.11	5.39	<.0001	22.4 - 55.8	0.54
RTL (cm)	8.68	9.97*	8.77	1.00	<.0001	4.05 - 10.37	0.25
DWT (g)	0.07	0.14**	0.08	0.02	<.0001	0.048 - 0.133	0.00
SRR (ratio)	3.66	4.75**	3.81	0.70	<.0001	2.91 - 6.80	0.07

[€]t-test between Bengal and Pokkali Means; [ns]not significantly different; *significant at α=0.05, **significant at α=0.01, ***significant at α=0.0001. [β] Genotypic differences among backcross lines. [#]Broad sense heritability by family mean basis. Na^+, shoot sodium concentration; K^+, shoot potassium concentration; NaK, ratio of the shoot sodium and shoot potassium content; SIS, salt injury score; CHL, chlorophyll content; SHL, shoot length; RTL, root length; DWT, shoot dry weight; SRR, shoot length to root length ratio.

ILs were continuous and close to normal distribution (Figure 4.1). The pattern of correlation among traits (Table 4.2) showed consistency with our previous study in Bengal x Pokkali RIL population (De Leon et al., 2016). The shoot Na^+ concentration was significant and positively correlated to shoot K^+ concentration, NaK, and SIS. Surprisingly, Na^+ was also positively correlated to CHL, which could be due to the lack of significant differences among ILs. On the other hand, SIS was significant and negatively correlated to SHL, RTL, SRR, and DWT.

4.3.2 Evaluation of genome composition and construction of ILs by SSR markers

A total of 136 SSR markers distributed across the 12 chromosomes of rice were selected for polymorphism survey and only 107 markers were polymorphic between parents. These polymorphic SSR markers were used to inquire the genetic make-up of ILs and for QTL mapping (E.1). The SSR markers were distributed over the rice genome every 3.7 Mb or every 15 cM, with an average of 9 markers per chromosome (Table 4.3). Out of 292 BP BC_4F_4 lines, only 276 lines had complete genotypic data for 107 SSR markers. Using the CSSL finder, the genome composition and Pokkali segments were evaluated in ILs and 72 ILs were selected by the program to cover the 12 chromosomes of rice (Figure 4.2). A total of 216 segments covering about 77% of Pokkali genome were transmitted to the ILs. Each chromosome contained an average of 6 segments accounting to an average size of 5.3 Mb. Segments of Pokkali genome were fully represented in chromosomes 2 and 9. But chromosomes 8 and 12 had 50% and 57% coverage, respectively while the other chromosomes had 66-88% coverage (Table 4.3). On average, the genome composition of each IL had 95% Bengal, with minimum and maximum of 86% and 99%, respectively. In contrast, each IL contained an average introgression of 4.7%, with minimum of 0.8% and maximum of 14% Pokkali (E.3). The majority of ILs had 1-2% Pokkali segment with 3-5 Mb length (Figure 4.3. A).

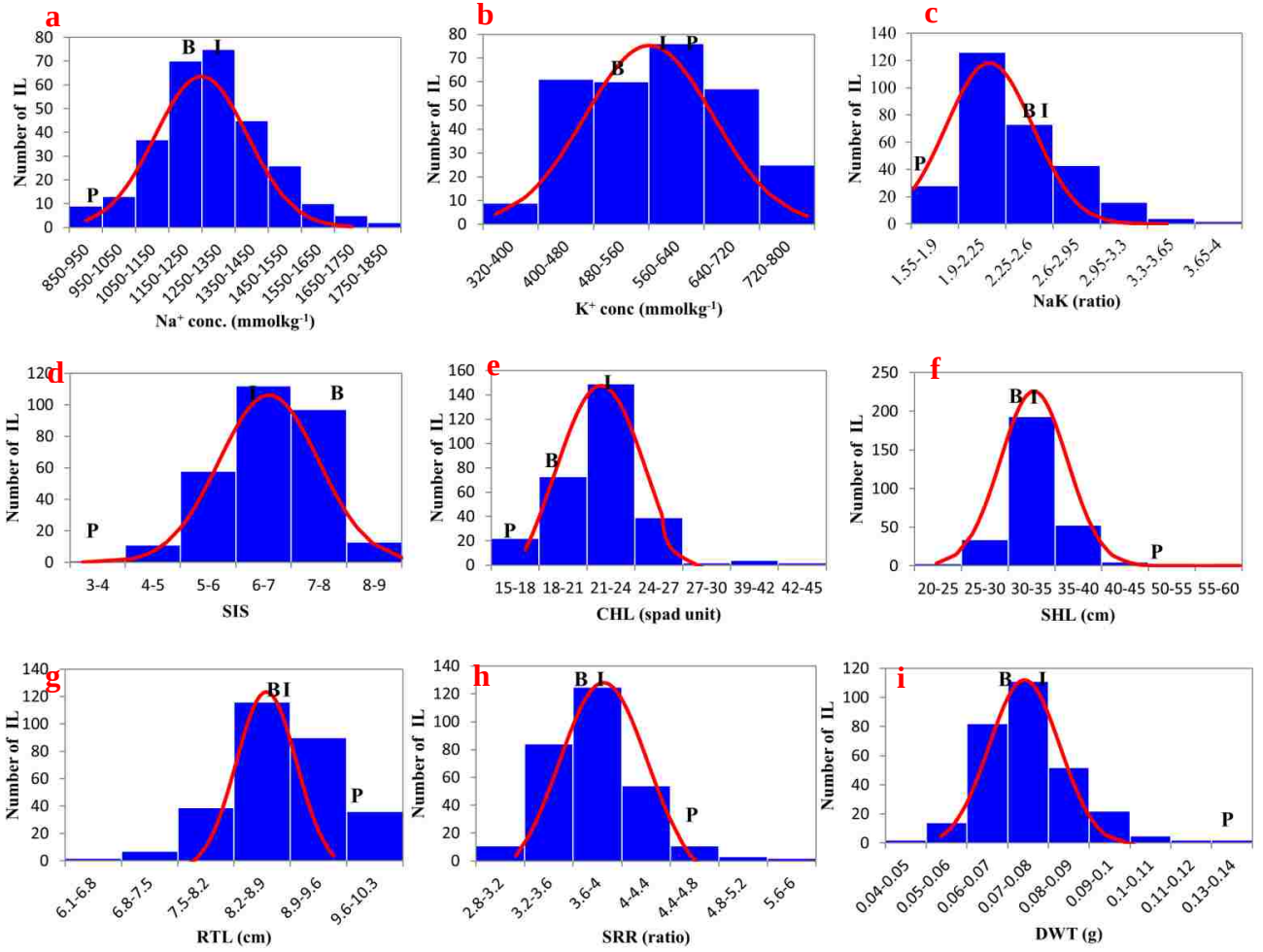

Figure 4.1. Frequency distribution of 292 ILs (BP BC_4F_4) for nine traits investigated under salt stress (EC=12dSm-1). B, R, and P indicate the positions of the parents and mean of the RIL. (a) Na^+ conc., Na^+ concentration; (b) K^+ conc., K^+ concentration; (c) NaK, Na^+/K^+ ratio; (d) SIS, log transformed salt injury score; (e) CHL, chlorophyll content measured by SPAD-502 unit; (f) SHL, shoot length; (g) RTL, root length; (h) SRR, shoot length to root length ratio; (i) DWT, dry weight; B, Bengal mean; P, Pokkali mean; I, introgression lines mean.

Table 4.2. Pearson correlation matrix of traits under seedling salinity stress in 292 ILs.

	Na^+	K^+	NaK	SIS	CHL	SHL	RTL	SRR	DWT
Na^+	1								
K^+	0.592***	1							
NaK	0.129*	-0.698***	1						
SIS	0.173**	0.134*	-0.088	1					
CHL	0.144*	0.197***	-0.117*	0.040	1				
SHL	-0.019	-0.050	0.083	-0.381***	-0.060	1			
RTL	0.077	0.143*	-0.107	`-0.118*	0.123*	0.337***	1		
SRR	-0.084	-0.146*	0.139*	-0.266***	-0.146*	0.680***	-0.435***	1	
DWT	-0.110	-0.185**	0.188**	-0.636***	0.029	0.564***	0.314***	0.291***	1

Na^+, shoot sodium concentration; K^+, shoot potassium concentration; NaK, ratio of the shoot sodium and shoot potassium concentration; SIS, salt injury score; CHL, chlorophyll content; SHL, shoot length; RTL, root length; DWT, shoot dry weight; SRR, shoot length to root length ratio.

Table 4.3. Basic statistics of Pokkali segments in introgression lines using SSR and SNP markers.

	SSR Marker information			Introgression Lines[a]				SNP Marker Information			Introgression Lines[b]			
Chr.	No. of markers used	Marker coverage (Mb)	Ave. marker interval (Mb)	No. of segments	Ave. % donor segment	Ave. donor segment size (Mb)	% Pokkali genome coverage[c]	No. of markers used	Marker coverage (Kb-Mb)	Ave. marker interval (Kb)	No. of segments	Ave. % donor segment	Ave. segment size (Mb)	% Pokkali genome coverage[c]
1	13	1-43	3.50	22	4.69	4.97	82.0	927	15-43	46.6	567	3.41	0.17	100
2	10	1-3	4.21	34	4.43	6.43	100	513	50-35.6	69.9	166	3.03	0.38	100
3	13	0.8-36	2.95	24	4.52	5.39	88.6	853	263- 36.4	42.7	394	4.78	0.19	100
4	8	4-33	4.03	18	3.40	4.98	52.3	508	310- 35.5	69.9	297	2.86	0.35	100
5	7	0.4 - 27	3.84	20	4.98	5.20	84.8	615	87-29.4	47.9	216	3.48	0.36	100
6	11	1.8 - 30	3.18	11	5.47	5.32	66.3	521	139-30.5	58.6	149	2.37	0.34	100
7	8	1-29	4.01	19	6.11	4.73	70.3	587	19-29.5	50.3	259	6.38	0.46	100
8	7	0.38 - 28	4.57	15	4.78	4.37	50.7	623	51-28.4	45.6	505	2.79	0.12	100
9	7	0.30 -23	3.73	21	3.39	6.13	100	446	244-22.7	51.1	263	8.00	0.59	100
10	6	3-20	3.38	15	6.75	6.78	86.9	408	49-23.1	56.6	140	4.20	0.49	100
11	9	0-27	3.35	12	3.58	5.21	93.0	486	124-28.9	59.6	170	6.74	0.68	100
12	8	1-26	3.50	5	3.54	4.10	57.0	310	279-27.4	88.6	72	3.10	0.59	100
Sum	107	361		216		63.61	932	6797	370.4		3198		4.72	
Ave.	8.92	1-30	3.69	6	4.64	5.30	77.6	566	135 - 31	57.3	266	4.26	0.39	100

[a] computed from 72 ILs genotyped by SSR markers. [b] computed from 88 ILs genotyped by SNP markers. [c] computed from the proportion of homozygous Pokkali chromosome segment (in Mb). Chr, Chromosome; Ave, Average.

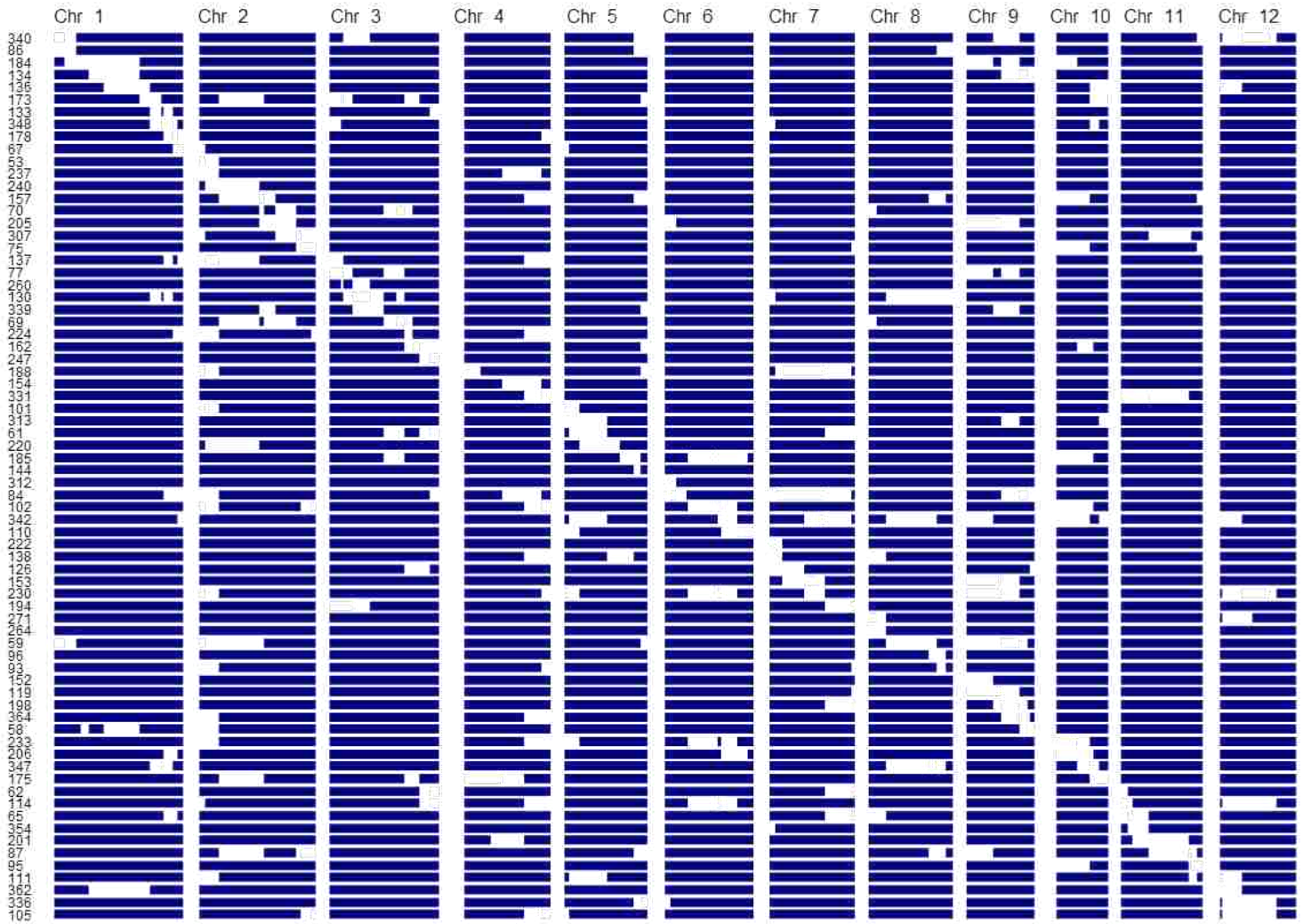

Figure 4.2. Graphical genotypes of 72 ILs developed from Bengal x Pokkali cross. Each row denotes a line selected for a chromosomal segment. Blue and white segments represent Bengal and Pokkali segments, respectively. Lines were genotyped using 107 SSR markers.

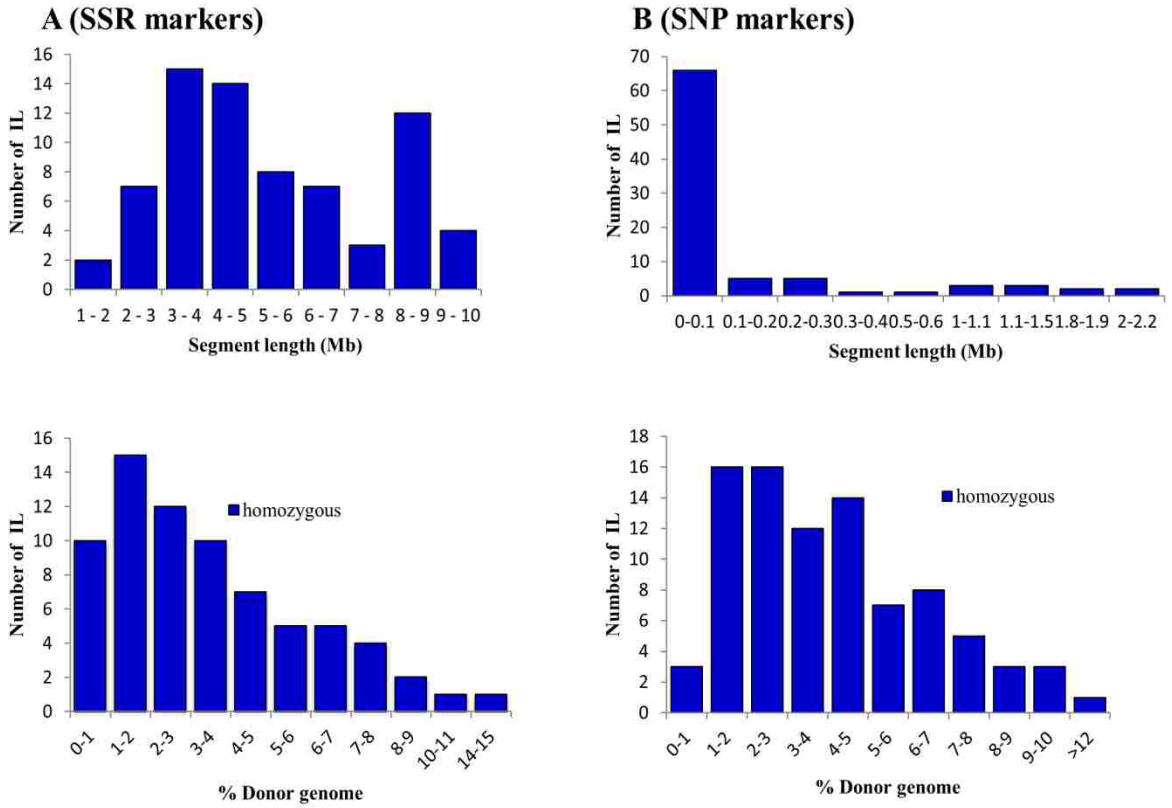

Figure 4.3. Frequency distribution of introgressed Pokkali segments in the selected introgression lines using SSR (A) and SNP (B) markers.

4.3.3 Evaluation of genome composition and construction of ILs by GBS-SNP markers

After filtering of the GBS data for the 88 ILs, a total of 6,797 SNP markers were retained and used for inquiry of genome composition and QTL mapping in ILs (Suppl. Table S4.2, available upon request). An average of 566 SNP markers was placed in each chromosome with an average interval of 57.3 Kb between markers. The genome compositions of 88 ILs were summarized in E.4. On average, the genome of an IL was 95.8% Bengal and 4.1% Pokkali. Among ILs, the number of Pokkali segments ranged from 6-143 segments that were distributed from one to twelve chromosomes of rice. Collectively, a total of 3,198 Pokkali segments were detected by

SNP markers in the 88 ILs with 266 segments per chromosome or an average size of 390Kb segment per chromosome (Table 4.3). The high frequency of SNP markers per chromosome indicated whole genome coverage of Pokkali among ILs. Chromosome 1 and 12 contained the highest and lowest number of Pokkali segments, respectively. The majority of the ILs was carrying 1-3% Pokkali genome with segments having a size of 100Kb (Figure 4.3. B).

4.3.4 QTL analysis for traits related to salinity tolerance

QTL analyses for nine traits were conducted separately in ILs genotyped by SSR and SNP markers. Single marker analysis (SMA) and stepwise-regression likelihood ratio test (LRT) methods were employed to see the consistency of detected QTLs. For QTL mapping in 72 ILs using SSR markers, a total of 18 QTLs were detected by SMA for five traits (Table 4.4) and 8 of these QTLs were significant by LRT-RSTEP. There were no significant QTLs detected for shoot Na^+, K^+ concentrations, NaK, and CHL. In contrast, QTL mapping in 88 ILs using SNP markers detected a total of 32 QTLs for 8 traits (Table 4.5) and 10 QTLs were common and significant by SMA and LRT. Because of the differences in density and positions of SSR and SNP markers, only *qDWT7.17* was congruent in QTL analysis using SSR and SNP markers (Figure 4.4).

- QTLs for shoot Na^+ concentration

There were no significant QTLs detected for Na^+ concentration using SSR markers (Table 4.4). In contrast, QTL mapping using SNP markers detected a single minor QTL located on chromosome 11. The *qNa11.5* accounted for 10% of the phenotypic variation in Na^+ concentration. The Bengal allele at the locus had increasing effect in the shoot Na^+ ion concentration (Table 4.5). Therefore, Pokkali allele at this QTL was desirable. Except for IL262, lines containing this QTL with Pokkali allele showed some tolerance despite higher Na^+ concentration than Pokkali (E.2).

Table 4.4. QTLs detected in ILs by SMA and LRT using SSR markers.

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Line containing Pokkali allele at QTL	Phenotype (mean value)
SIS_SMA	*qSIS1.39*	1	39.50	RM3810	2.275	6.456	-0.644	B	IL84	SIS(3.2)
									IL178	SIS(5.4)
									IL348	SIS(6.2)
	qSIS2.3	2	3.00	RM211	2.326	6.590	-0.442	B	IL84	SIS(3.2)
									IL188	SIS(5.7)
									IL137	SIS(5.8)
									IL53	SIS(6.1)
									IL101	SIS(6.2)
									IL224	SIS(6.5)
									IL230	SIS(4.1)
									IL233	SIS(5.3)
									IL59	SIS(5.3)
	qSIS6.5	6	5.40	RM253	2.498	7.040	-1.589	B	IL84	SIS(3.2)
	qSIS7.12	7	12.80	RM214	2.081	5.942	-0.855	B	IL84	SIS(3.2)
									IL153	SIS(5.2)
									IL188	SIS(5.7)
	qSIS7.17	7	17.50	RM5793	2.935	8.162	-0.788	B	IL84	SIS(3.2)
									IL153	SIS(5.2)
									IL188	SIS(5.7)
									IL230	SIS(4.1)
									IL342	SIS(6.2)
SIS_LRT	*qSIS1.39*	1	39.50	RM3810	2.055	10.154	-0.560	B	IL84	SIS(3.2)
									IL178	SIS(5.4)
									IL348	SIS(6.2)

(Table 4.4 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Line containing Pokkali allele at QTL	Phenotype (mean value)
	qSIS7.17	7	17.50	RM5793	2.935	16.989	-0.788	B	IL84	SIS(3.2)
									IL153	SIS(5.2)
									IL188	SIS(5.7)
									IL230	SIS(4.1)
									IL342	SIS(6.2)
SHL_SMA	*qSHL1.39*	1	39.50	RM3810	2.556	8.188	2.927	P	IL84	SHL(50 cm)
									IL178	SHL(39cm)
									IL348	SHL(36cm)
	qSHL1.41	1	41.10	RM5362	2.091	6.795	2.899	P	IL178	SHL(39cm)
									IL67	SHL(35cm)
									IL137	SHL(36cm)
									IL224	SHL(34cm)
									IL8	SHL(50cm)
	qSHL2.3	2	3.00	RM211	2.031	6.614	1.788	P	IL84	SHL(50cm)
									IL188	SHL(36cm)
									IL137	SHL(36CM)
									IL53	SHL(35cm)
									IL101	SHL(35cm)
									IL224	SHL(34cm)
									IL230	SHL(38cm)
									IL233	SHL(36cm)
									IL59	SHL(34cm)
	qSHL5.04	5	0.40	RM17749	2.674	8.535	3.606	P	IL67	SHL(35cm)
									IL101	SHL(35cm)
									IL230	SHL(38cm)
									IL105	SHL(53cm)
	qSHL6.5	6	5.40	RM253	4.152	12.670	8.603	P	IL84	SHL(50cm)

(Table 4.4 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Line containing Pokkali allele at QTL	Phenotype (mean value)
SHL_LRT	*qSHL5.04*	5	0.40	RM17749	3.861	16.175	3.734	P	IL67	SHL(35cm)
									IL101	SHL(35cm)
									IL230	SHL(38cm)
									IL105	SHL(53cm)
	qSHL6.5	6	5.40	RM253	5.340	23.491	8.810	P	IL84	SHL(50cm)
RTL_SMA	*qRTL2.20*	2	20.70	RM341	2.203	12.976	-0.431	B	IL173	RTL(7.8cm)
									IL157	RTL(8.3cm)
									IL59	RTL(8.3cm)
									IL175	RTL(7.7cm)
									IL87	RTL(6.8cm)
RTL_LRT	*qRTL2.20*	2	20.70	RM341	2.203	12.976	-0.431	B	IL173	RTL(7.8cm)
									IL157	RTL(8.3cm)
									IL59	RTL(8.3cm)
									IL175	RTL(7.7cm)
									IL87	RTL(6.8cm)
SRR_SMA	*qSRR2.37*	2	37.60	RM266	3.057	18.445	0.409	P	IL102	SRR(4.26)
									IL87	SRR(4.47)
									IL105	SRR(5.83)
	qSRR7.12	7	12.80	RM214	2.034	12.665	0.389	P	IL84	SRR(5.14)
									IL153	SRR(4.00)
									IL188	SRR(4.70)
SRR_LRT	*qSRR2.37*	2	37.60	RM266	3.882	18.174	0.427	P	IL102	SRR(4.26)
									IL87	SRR(4.47)
									IL105	SRR(5.83)

(Table 4.4 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Line containing Pokkali allele at QTL	Phenotype (mean value)
	qSRR7.12	7	12.80	RM214	2.857	12.936	0.413	P	IL84	SRR(5.14)
									IL153	SRR(4.00)
									IL188	SRR(4.70)
DWT_SMA	*qDWT2.3*	2	3.00	RM211	5.192	14.881	0.010	P	IL84	DWT(0.133)
									IL188	DWT(0.096)
									IL137	DWT(0.094)
									IL53	DWT(0.081)
									IL101	DWT(0.091)
									IL224	DWT(0.077)
									IL230	DWT(0.111)
									IL233	DWT(0.090)
									IL59	DWT(0.082)
	qDWT6.5	6	5.40	RM253	2.921	8.966	0.028	P	IL84	DWT(0.133)
	qDWT7.12	7	12.80	RM214	2.357	7.362	0.015	P	IL84	DWT(0.133)
									IL153	DWT(0.081)
									IL188	DWT(0.096)
	qDWT7.17	7	17.50	RM5793	2.324	7.266	0.012	P	IL84	DWT(0.133)
									IL153	DWT(0.081)
									IL188	DWT(0.096)
									IL230	DWT(0.111)
									IL342	DWT(0.068)

(Table 4.4 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Line containing Pokkali allele at QTL	Phenotype (mean value)
DWT_LRT	*qDWT2.3*	2	3.00	RM211	5.192	27.930	0.010	P	IL84	DWT(0.133)
									IL188	DWT(0.096)
									IL137	DWT(0.094)
									IL53	DWT(0.081)
									IL101	DWT(0.091)
									IL224	DWT(0.077)
									IL230	DWT(0.111)
									IL233	DWT(0.090)
									IL59	DWT(0.082)

[¥]Parental source of increasing allele: B, Bengal; P, Pokkali.

Table 4.5. QTLs detected in ILs by SMA and LRT using 6797 SNP markers.

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Lines containing Pokkali allele at QTL	Phenotype (mean value)
Na_LRT	*qNa11.5*	11	5.61	S11_5610372	2.04	9.68	-82.38	B	IL186	Na(938)
									IL263	Na(1032)
									IL262	Na(1080)
									IL353	Na(1011)
									IL57	Na(1111)
									IL65	Na(1198)
									IL89	Na(1253)
									IL91	Na(1240)
K_SMA	*qK1.3863*	1	38.63	S1_38636497	2.23	10.66	69.26	P	IL178	K(753)
									IL303	K(768)
									IL323	K(748)
									IL348	K(736)
									IL51	K(518)
									IL84	K(599)
K_LRT	*qK1.3863*	1	38.63	S1_38636497	2.23	10.66	69.26	P	IL178	K(753)
									IL303	K(768)
									IL323	K(748)
									IL348	K(736)
									IL51	K(518)
									IL84	K(599)
NaK_SMA	*qNaK3.32*	3	32	S3_32078967	2.31	11.27	0.33	P	IL51	NaK(2.34)
									IL52	NaK(3.64)
									IL61	NaK(2.30)
									IL98	NaK(2.83)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Lines containing Pokkali allele at QTL	Phenotype (mean value)
SIS_SMA	*qSIS5.034*	5	0.34	S5_340482	2.79	7.14	-0.05	B	IL230	SIS(4.10)
									IL313	SIS(5.13)
									IL67	SIS(5.0)
									IL68	SIS(4.47)
									IL83	SIS(5.53)
									IL91	SIS4.33
									IL99	SIS(4.37)
	qSIS5.1	5	1.47	S5_1473882	2.36	5.53	-0.07	B	IL230	SIS(4.10)
									IL313	SIS(5.13)
									IL91	SIS(4.33)
									IL99	SIS(4.37)
	qSIS5.2	5	2.83	S5_2831482	2.07	4.97	-0.05	B	IL230	SIS(4.10)
									IL313	SIS(5.13)
									IL61	SIS(5.07)
									IL91	SIS(4.33)
									IL99	SIS(4.37)
	qSIS9.8	9	8.6	S9_8608506	2.17	5.35	-0.05	B	IL116	SIS(4.40)
									IL119	SIS(4.33)
									IL230	SIS(4.10)
									IL99	SIS(4.37)
	qSIS9.14	9	14.6	S9_14600108	2.18	5.25	-0.04	B	IL116	SIS(4.40)
									IL119	SIS(4.33)
									IL178	SIS(5.40)
									IL63	SIS(5.53)
									IL74	SIS(5.40)
									IL84	SIS(3.22)
									IL98	SIS(5.53)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Lines containing Pokkali allele at QTL	Phenotype (mean value)
SIS_LRT	*qSIS1.41*	1	41.81	S1_41818521	2.05	6.17	-0.05	B	IL178	SIS(5.40)
									IL323	SIS(5.65)
									IL52	SIS(5.40)
									IL67	SIS(5.00)
									IL84	SIS(3.22)
	qSIS1.42	1	42.31	S1_42310908	2.27	6.80	-0.07	B	IL178	SIS(5.40)
									IL67	SIS(5.00)
									IL84	SIS(3.22)
	qSIS5.034	5	0.34	S5_340482	2.50	8.14	-0.05	B	IL230	SIS(4.10)
									IL313	SIS(5.13)
									IL67	SIS(5.0)
									IL68	SIS(4.47)
									IL83	SIS(5.53)
									IL91	SIS4.33
									IL99	SIS(4.37)
	qSIS9.8	9	8.6	S9_8608506	2.17	7.79	-0.05	B	IL116	SIS(4.40)
									IL119	SIS(4.33)
									IL230	SIS(4.10)
									IL99	SIS(4.37)
CHL_SMA	*qCHL11.2*	11	2.32	S11_2322899	6.31	15.19	11.73	P	IL350	CHL(45.32)
CHL_LRT	*qCHL3.6*	3	6.96	S3_6962390	2.28	4.43	4.87	P	IL219	CHL(22.79)
									IL92	CHL(19.13)
									IL94	CHL(18.03)
									IL98	CHL(44.77)
	qCHL3.25	3	25.64	S3_25640338	2.48	4.86	4.22	P	IL162	CHL(23.67)
									IL198	CHL(21.31)
									IL98	CHL(44.77)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qCHL3.26	3	26.97	S3_26978157	2.06	3.65	2.95	P	IL198	CHL(21.31)
									IL253	CHL(25.62)
									IL336	CHL(21.63)
									IL340	CHL(23.26)
									IL98	CHL(44.77)
	qCHL11.2	11	2.32	S11_2322899	6.31	12.40	11.73	P	IL350	CHL(45.32)
SHL_SMA	*qSHL1.3810*	1	38.1	S1_38108856	2.17	2.64	3.09	P	IL178	SHL(39.27)
									IL323	SHL(44.93)
									IL348	SHL(36.88)
									IL51	SHL(40.27)
									IL89	SHL(38.67)
	qSHL1.3818	1	38.18	S1_38181791	3.33	5.31	4.34	P	IL178	SHL(39.27)
									IL323	SHL(44.93)
									IL348	SHL(36.88)
									IL51	SHL(40.27)
									IL84	SHL(50.33)
	qSHL1.3863	1	38.63	S1_38636497	4.40	5.93	4.37	P	IL178	SHL(39.27)
									IL303	SHL(42.05)
									IL323	SHL(44.93)
									IL348	SHL(36.88)
									IL51	SHL(40.27)
									IL84	SHL(50.33)
	qSHL1.3876	1	38.76	S1_38768787	2.99	4.20	3.63	P	IL137	SHL(35.73)
									IL178	SHL(39.27)
									IL303	SHL(42.05)
									IL348	SHL(36.88)
									IL51	SHL(40.27)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qSHL1.40	1	40	S1_40013502	2.81	3.89	2.83	P	IL137	SHL(35.73)
									IL178	SHL(39.27)
									IL206	SHL(33.20)
									IL303	SHL(42.05)
									IL323	SHL(44.93)
									IL348	SHL(36.88)
									IL51	SHL(40.27)
									IL52	SHL(32.40)
									IL65	SHL(37.02)
									IL84	SHL(50.33)
SHL_LRT	*qSHL1.3863*	1	38.63	S1_38636497	5.37	21.69	4.59	P	IL178	SHL(39.27)
									IL303	SHL(42.05)
									IL323	SHL(44.93)
									IL348	SHL(36.88)
									IL51	SHL(40.27)
									IL84	SHL(50.33)
	qSHL8.4	8	4.74	S8_4747595	2.47	9.03	2.98	P	IL106	SHL(36.47)
									IL138	SHL(36.62)
									IL199	SHL(34.03)
									IL271	SHL(34.47)
									IL65	SHL(37.0)
									IL76	SHL(55.80)
SRR_SMA	*qSRR1.3818*	1	38.18	S1_38181791	2.08	9.00	0.39	P	IL178	SRR(4.74)
									IL323	SRR(4.72)
									IL348	SRR(4.35)
									IL51	SRR(4.43)
									IL84	SRR(5.14)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qSRR1.3863	1	38.63	S1_38636497	3.04	11.54	0.42	P	IL178	SRR(4.74)
									IL303	SRR(4.89)
									IL323	SRR(4.72)
									IL348	SRR(4.35)
									IL51	SRR(4.43)
									IL84	SRR(5.14)
	qSRR8.5	8	5.34	S8_5341936	2.22	8.65	0.44	P	IL199	SRR(4.35)
									IL271	SRR(3.98)
									IL65	SRR(4.01)
									IL76	SRR(6.79)
SRR_LRT	*qSRR1.27*	1	27.95	S1_27956396	2.54	6.18	0.24	P	IL107	SRR(5.63)
									IL153	SRR(4.00)
									IL160	SRR(3.75)
									IL188	SRR(4.70)
									IL232	SRR(4.04)
									IL238	SRR(4.18)
									IL340	SRR(3.87)
									IL57	SRR(4.33)
									IL70	SRR(4.46)
									IL86	SRR(3.96)
									IL89	SRR(4.83)
									IL93	SRR(3.88)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qSRR1.2851	1	28.51	S1_28513474	2.64	6.26	0.28	P	IL107	SRR(5.63)
									IL153	SRR(4.00)
									IL160	SRR(3.75)
									IL188	SRR(4.70)
									IL232	SRR(4.04)
									IL238	SRR(4.18)
									IL340	SRR(3.87)
									IL57	SRR(4.33)
									IL70	SRR(4.46)
									IL51	SRR(4.43)
									IL93	SRR(3.88)
	qSRR1.2853	1	28.53	S1_28535873	2.07	5.01	0.21	P	IL107	SRR(5.63)
									IL153	SRR(4.00)
									IL160	SRR(3.75)
									IL188	SRR(4.70)
									IL232	SRR(4.04)
									IL238	SRR(4.18)
									IL340	SRR(3.87)
									IL57	SRR(4.33)
									IL70	SRR(4.46)
									IL86	SRR(3.96)
									IL89	SRR(4.83)
									IL93	SRR(3.88)
									IL51	SRR(4.43)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qSRR1.3863	1	38.63	S1_38636497	3.91	10.54	0.44	P	IL178	SRR(4.74)
									IL303	SRR(4.89)
									IL323	SRR(4.72)
									IL348	SRR(4.35)
									IL51	SRR(4.43)
									IL84	SRR(5.14)
	qSRR8.5	8	5.34	S8_5341936	3.16	8.27	0.48	P	IL199	SRR(4.35)
									IL271	SRR(3.98)
									IL65	SRR(4.01)
									IL76	SRR(6.79)
DWT_SMA	*qDWT1.41*	1	41.81	S1_41818521	2.07	4.67	0.01	P	IL178	DWT(0.078)
									IL323	DWT(0.087)
									IL52	DWT(0.087)
									IL67	DWT(0.102)
									IL84	DWT(0.133)
	qDWT1.42	1	42.31	S1_42310908	2.37	5.29	0.01	P	IL178	DWT(0.078)
									IL67	DWT(0.102)
									IL84	DWT(0.133)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele¥	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qDWT7.17	7	17.57	S7_17569558	2.84	6.49	0.01	P	IL153	DWT(0.091)
									IL166	DWT(0.069)
									IL186	DWT(0.082)
									IL188	DWT(0.096)
									IL190	DWT(0.079)
									IL230	DWT(0.111)
									IL303	DWT(0.081)
									IL52	DWT(0.087)
									IL65	DWT(0.102)
									IL84	DWT(0.133)
									IL92	DWT(0.089)
	qDWT7.18	7	18.8	S7_18801087	2.05	3.98	0.01	P	IL186	DWT(0.082)
									IL188	DWT(0.096)
									IL190	DWT(0.079)
									IL230	DWT(0.111)
									IL232	DWT(0.075)
									IL65	DWT(0.102)
									IL92	DWT(0.089)
	qDWT7.20	7	20.08	S7_20085299	3.14	7.39	0.01	P	IL186	DWT(0.082)
									IL188	DWT(0.096)
									IL65	DWT(0.102)
									IL84	DWT(0.133)
DWT_LRT	*qDWT1.42*	1	42.31	S1_42310908	2.13	7.28	0.01	P	IL178	DWT(0.078)
									IL67	DWT(0.102)
									IL84	DWT(0.133)

(Table 4.5 continued)

Trait	QTL	CHR	Position (Mb)	Marker	LOD	PVE (%)	Add Effect	Parental source of increasing allele[¥]	Lines containing Pokkali allele at QTL	Phenotype (mean value)
	qDWT5.034	5	0.34	S5_340482	2.26	8.46	0.01	P	IL219	DWT(0.069)
									IL230	DWT(0.111)
									IL313	DWT(0.091)
									IL67	DWT(0.102)
									IL68	DWT(0.096)
									IL83	DWT(0.095)
									IL91	DWT(0.097)
									IL99	DWT(0.082)
	qDWT7.17	7	17.57	S7_17569558	2.84	11.54	0.01	P	IL153	DWT(0.091)
									IL166	DWT(0.069)
									IL186	DWT(0.082)
									IL188	DWT(0.096)
									IL190	DWT(0.079)
									IL230	DWT(0.111)
									IL303	DWT(0.081)
									IL52	DWT(0.087)
									IL65	DWT(0.102)
									IL84	DWT(0.133)
									IL92	DWT(0.089)

[¥]Parental source of increasing allele: B, Bengal; P, Pokkali.

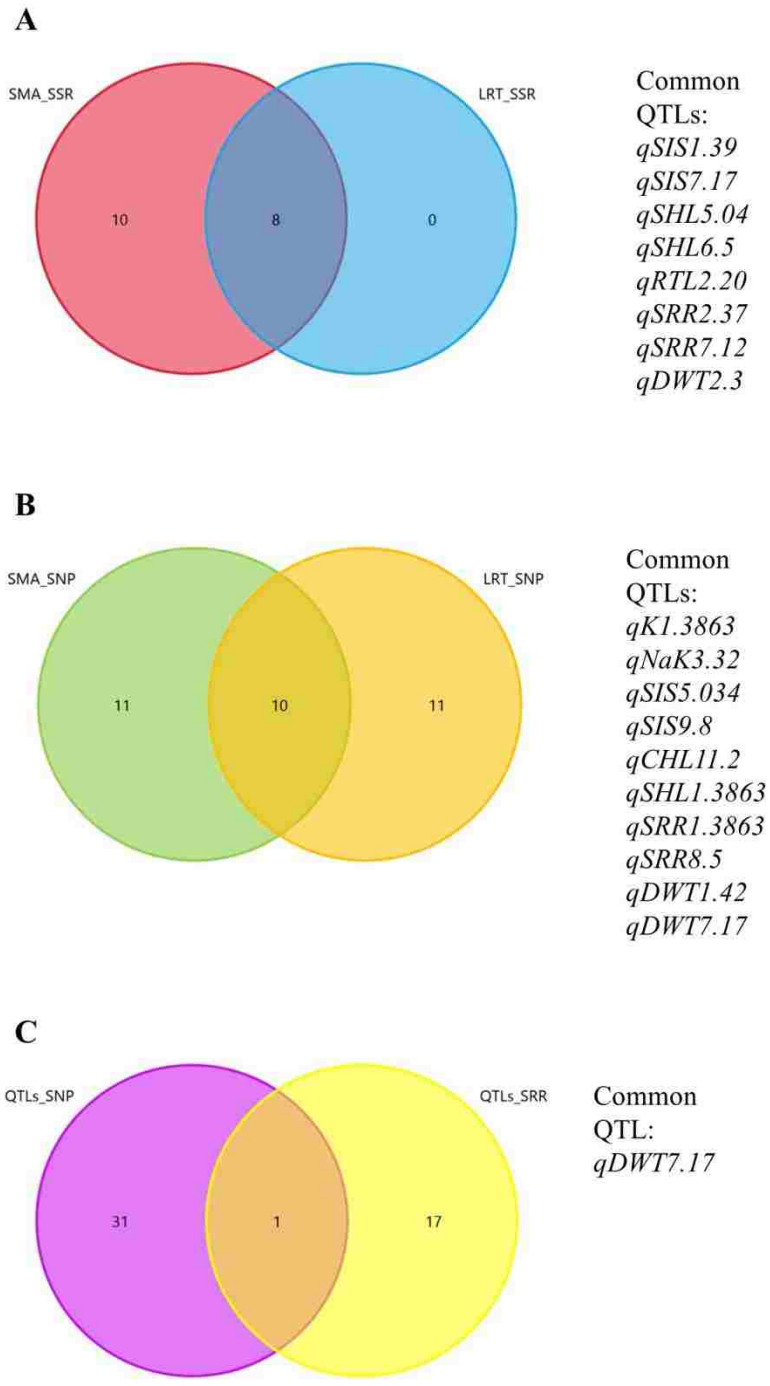

Figure 4.4. Comparison of salinity-tolerance QTLs detected in Pokkali introgression lines by SMA and LRT using SSR markers (A), SNP markers (B), and common QTLs identified by SSR and SNP markers (C).

- QTLs for K^+ concentration

The SMA and LRT methods using SNP markers detected a single QTL for K^+ concentration. The QTL *qK1.3863* was mapped on chromosome 1 and was responsible for 11% of the variation in K^+ concentration. Allele substitution of Bengal by Pokkali allele had increasing effect of 69.26 $mmolkg^{-1}$ at the locus (Table 4.5). In contrast, there were no significant QTLs for K^+ concentration by SSR markers. Except for IL51, lines containing Pokkali allele at *qK1.3863* had higher shoot K^+ concentration than Pokkali.

- QTLs for NaK

A single QTL for NaK was detected significant by SMA and LRT. The *qNaK3.32* was mapped on chromosome 3 at 32 Mb region. This QTL explained 11% of the variation in NaK. The Pokkali allele at this QTL had increasing effect. ILs containing this QTL had even higher NaK ratio than Bengal indicating the undesirable effect of Pokkali allele at the locus. On the other hand, there were no QTLs significant in both mapping methods using SSR markers.

- QTL for SIS

Using SSR markers, the SMA and LRT detected five QTLs for SIS on chromosomes, 1, 2, 6, and 7. Three of the QTLs had minor effects (*qSIS2.3, qSIS6.5, qSIS7.12*) and two had large-effects (*qSIS1.39* and *qSIS7.17*) with a contribution of 10-16% toward phenotypic variation for SIS. In contrast, mapping of QTLs by SNP markers detected five significant QTLs for SIS on chromosome 1, 5, and 9. The *qSIS5.034* and *qSIS9.8* were significant QTLs in both LRT and SMA methods. However, all QTLs had minor effects, and accounted for only 5-8% of SIS variation. Bengal alleles had increasing SIS effects in all of the QTLs. Pokkali alleles at SIS QTLs were therefore desirable. Using SSR markers, ILs containing introgressed Pokkali segments at SIS QTLs showed mean SIS of 3.2 to 6.5. In contrast, SIS QTLs by SNP markers

included only ILs with mean SIS of 3.2 to 5.7. Interestingly, ILs containing *qSIS9.8* had high tolerance and SIS value not more than 4.37.

- QTLs for CHL

There was no significant CHL QTL among ILs using SSR markers. However, mapping in ILs by SNP markers detected four QTLs on chromosomes 3 and 11. One of the QTLs (*qCHL11.2*) was highly significant with LOD value of 6.3 and was responsible for 12-15% of the phenotypic variation in CHL content. Introgression of Pokkali alleles had increasing CHL effects at QTLs.

- QTLs for SHL

Six QTLs were detected for SHL by SSR and another six QTLs were detected by SNP markers. The QTLs were located on chromosomes 1, 2, 5, 6, 7, and 8. Using SSR markers, two QTLs were detected on chromosome 1 while SNP markers detected five QTL in the 38-41 Mb regions. The *qSHL5.04*, *qSHL6.5*, and *qSHL1.3863* were highly significant and accounted for 16%, 23%, and 22% of the SHL variation, respectively. The Pokkali alleles at these QTLs had increasing effect for SHL.

- QTLs for RTL

A single QTL for RTL was significant by SSR markers on chromosome 2. Conversely, there were no QTLs detected by SNP markers in both SMA and LRT methods. The *qRTL2.20* accounted for 13% of the RTL variation. Bengal allele at the locus had increasing RTL effect. ILs containing Pokkali allele at *qRTL2.20* had shorter root length under salt stress.

- QTLs for SRR

Two QTLs located on chromosomes 2 and 7 were significant for SRR using SSR markers while SNP markers detected six significant QTLs on chromosomes 1 and 8. The two significant

QTLs in SSR mapping (*qSRR2.37* and *qSRR7.12*) were significant by SMA and LRT. Both QTLs had increasing effect from Pokkali alleles and accounted for 13-18% of the SRR variation. In contrast, the QTLs detected by SNP markers were minor-effect QTLs except for *qSRR1.3863* which explained for 10-12% of the SRR variation. The presence of Pokkali alleles at QTLs had increasing effect on SRR.

- QTLs for DWT

Four and six significant QTLs were detected by SSR and SNP markers, respectively. The QTLs were mapped on chromosomes 1, 2, 5, 6, and 7. The *qDWT2.3* was significant by SMA and LRT and was responsible for 15-28% of the DWT variation. Additionally, the *qDWT7.17* accounted for 6-12% of the phenotypic variation while other DWT QTLs had minor effects. Overall, the Pokkali alleles had positive effect in increasing the DWT.

4.3.5 Comparison of QTLs in ILs and RILs

QTL mapping for seedling salinity tolerance was previously conducted in F_6 RIL population developed from a cross between Bengal and Pokkali. Table 3.4 summarized the additive QTLs detected for the nine traits investigated under salt stress in the RIL population. To validate the QTLs for seedling stage-salinity tolerance, the QTLs detected in RIL and IL populations were compared. Among the 85 QTLs for nine traits mapped in RIL population, 25 QTLs in ILs co-localized or mapped adjacent to 14 QTLs in RIL population (Table 4.6). For Na^+ concentration and NaK ratio, there were no significant QTLs detected in the IL population that co-localized to QTLs in RIL. For K^+ concentration, the *qK1.3863* was near the *qK1.38* in RIL. For SIS, a total of five QTLs identified in RIL were detected in the ILs including the large-effect *qSIS5.1b* which was responsible for 13% of SIS variation in RIL population. For CHL QTLs, both *qCHL3.26,* and *qCHL11.2* were detected in the IL and RIL populations. For SHL, six QTLs were significant

Table 4.6. List of significant QTLs detected in Bengal x Pokkali IL (BC_4F_4) and F_6 RIL populations.

Trait	QTLs in IL	Chr.	Position (Mb)	QTLs in RIL	Interval Position (Mb)
Na^+ concentration	-				
K^+ concentration	*qK1.3863*	1	38.63	*qK1.38*	38.79 - 39.04
NaK	-				
SIS	*qSIS6.5*	6	5.40	*qSIS6.5*	5.84 - 5.90
	qSIS7.12	7	12.80	*qSIS7.14*	14.59 - 14.62
	qSIS7.17	7	17.50	*qSIS7.14*	14.59 - 14.62
	qSIS5.034	5	0.34	*qSIS5.03*	0.31 - 0.33
	qSIS5.1	5	1.47	*qSIS5.1b*	1.44-1.47
	qSIS9.8	9	8.60	*qSIS9.8*	8.60 - 9.07
CHL	*qCHL3.25*	3	25.64	*qCHL3.26*	26.705 - 26.709
	qSHL3.26	3	26.97	*qCHL3.26*	26.705 - 26.709
	qCHL11.2	11	2.32	*qCHL11.2*	2.66 - 2.72
SHL	*qSHL1.3810*	1	38.10	*qSHL1.38*	38.28 - 38.61
	qSHL1.3818	1	38.18	*qSHL1.38*	38.28 - 38.61
	qSHL1.3863	1	38.63	*qSHL1.38*	38.28 - 38.61
	qSHL1.3876	1	38.76	*qSHL1.38*	38.28 - 38.61
	qSHL1.39	1	39.50	*qSHL1.38*	38.28 - 38.61
	qSHL1.40	1	40.00	*qSHL1.38*	38.28 - 38.61
RTL	*qRTL2.20*	2	20.70	*qRTL2.24*	24.961 - 24.963
SRR	*qSRR1.27*	1	27.95	*qSRR1.29*	29.561 - 29.568
	qSRR1.2851	1	28.51	*qSRR1.29*	29.561 - 29.568
	qsRR1.2853	1	28.53	*qSRR1.29*	29.561 - 29.568
	qSRR1.3818	1	38.18	*qSRR1.382*	38.28 - 38.61
	qSRR1.3863	1	38.63	*qSRR1.382*	38.28 - 38.61
	qSRR2.37	2	37.60	*qSRR2.34*	34.66 - 35.08
DWT	*qDWT1.41*	1	41.81	*qDWT1.40*	40.37 - 40.41
	qDWT1.42	1	42.31	*qDWT1.40*	40.37 - 40.41

in ILs and localized near *qSHL1.38* that contained the major *sd1* gene for plant height. For RTL, *qRTL2.20* was mapped near the region of *qRTL2.2*. Six QTLs for SRR identified in IL population were mapped in close proximity of three QTLs detected in RIL population. Additionally, two QTLs of IL population for DWT were located near *qDWT1.40* identified in the RIL population.

4.3.6 Analysis of tolerant ILs

The IL population showed normal distribution for the SIS values (Figure 4.1). Among 292 lines, only sixteen lines with SIS score of less than or equal to 5.2 were significantly different to the susceptible Bengal parent at α=0.05. E.5 summarized the phenotype, and genotype of tolerant ILs. IL84 was the most tolerant line with an average SIS of 3.2 and had low NaK ratio like Pokkali. However, IL84 was morphologically similar to Pokkali in terms of SHL, SRR, and DWT. Among the lines with mean SIS between 4.0-4.8, IL230 had high shoot K^+ concentration, low NaK ratio, high CHL, and morphologically intermediate between parents in SHL, RTL, SRR, and DWT. In contrast, IL119 and IL91 were tolerant lines with SHL similar to Bengal. Other tolerant lines that showed a SIS of 5.0 to 5.2 had phenotypic attributes intermediate between Bengal and Pokkali under salt stress.

The number of Pokkali segments detected in each IL ranged between 1-8 segments by SSR or 19-70 segments by SNP markers. On the average, the number of Pokkali segments was 6-12 times higher in case of SNP markers than in SSR. Moreover, the difference in the genome composition of each line by SSR and SNP markers ranged between 1-6%, with higher detection of recurrent genome using SNP markers. Among the sixteen lines, the most tolerant IL84 had the highest number of Pokkali segments and had a genome composition of 86% Bengal and 14% Pokkali by SSR or 91% Bengal and 9% Pokkali by SNP markers. Conversely, IL119 had the lowest number of Pokkali segments and had about 96% Bengal and 4% Pokkali genome composition. Other lines had 1-7 or 19-56 Pokkali segments by SSR or SNP markers, respectively. Many of the tolerant lines had 85-99% of Bengal genome and 1-14% of Pokkali genome.

Lines containing Pokkali alleles at QTLs were indicated in Tables 4.4 and 4.5. For simplicity, Table 4.7 summarized the QTLs contained in each tolerant IL. The IL84 contained three SIS QTLs on chromosomes 1 and 9. Additionally, IL84 was the only line with Pokkali segment at *qK1.3863* and it contained Pokkali segments at SHL, SRR, and DWT QTLs on chromosome 1. IL230, on the other hand, had four SIS QTLs on chromosomes 5 and 9 and *qDWT7.17* for shoot dry weight. The presence of Pokkali segments at *qSIS9.8* and *qSIS9.14* increased salinity tolerance of lines IL119, IL99, and IL116. However, additional SIS QTLs (*qSIS5.034, qSIS5.1, qSIS5.2*) in IL99 showed no corresponding decrease in SIS when compared to IL119 and IL116. Except for *qSIS5.034* in IL230, IL91, IL99, IL68, IL67, and IL313, there was no other SIS QTL that overlapped among the sixteen ILs. Surprisingly, despite the absence of significant QTLs for nine traits in IL129, IL78, and IL130, these lines showed some tolerance with an average SIS of 4.7 to 5.2. For shoot Na^+ concentration, Pokkali allele at *qNa11.5* had decreasing effect and had positive effect on salinity tolerance of IL91 compared to IL313 that also contained the same SIS QTLs. The Pokkali allele at *qNaK3.32* had increasing effect in NaK ratio. Except for IL6, all other ILs had no introgressed segment at *qNaK3.32*. Similarly, all of the 16 tolerant ILs had no Pokkali segments at QTLs for CHL. For SHL and SRR, the large increasing effect of QTL on chromosome 1 at 38 Mb region was evident in IL84. Likewise, Pokkali segments for DWT QTLs on chromosomes 1 and 7 increased DWT in IL84, IL230, IL 67, and IL65.

4.4 Discussion

In the present study, ILs were developed from a cross between *japonica* and *indica* rice genotypes. The tolerant donor parent Pokkali is tall, susceptible to lodging, and low yielding landrace belonging to *indica* subspecies. It is photosensitive and the grains are awned with red pericarp (Gregorio et al., 2002). In contrast, Bengal is a high yielding, medium grain, and early

Table 4.7. List of tolerant ILs and the QTLs in each IL.

	QTLs detected by SNP markers							
BP IL	SIS	Na^+	K^+	NaK	CHL	SHL	SRR	DWT
84	*qSIS9.14* *qSIS1.41* *qSIS1.42* *qSIS6.5*		*qK1.3863*			*qSHL1.3818* *qSHL1.3863* *qSHL1.3876* *qSHL1.40*	*qSRR1.3818* *qSRR1.3863*	*qDWT1.41* *qDWT1.42* *qDWT7.17*
230	*qSIS5.034* *qSIS5.1* *qSIS5.2* *qSIS9.8*							*qDWT7.17*
119	*qSIS9.8* *qSIS9.14*							
91	*qSIS5.034* *qSIS5.1* *qSIS5.2*	*qNa11.5*						
99	*qSIS5.034* *qSIS5.1* *qSIS5.2* *qSIS9.8* *qSIS9.14*							
116	*qSIS9.8* *qSIS9.14*							
68	*qSIS5.034*							
93							*qSRR1.27* *qSRR1.2851* *qSRR1.2853*	
129								
78								
67	*qSIS5.034* *qSIS1.41* *qSIS1.42*	*qNa11.5*						*qDWT1.41* *qDWT1.42*
61	*qSIS5.2*			*qNaK 3.32*				
313	*qSIS5.034* *qSIS5.1* *qSIS5.2*							
65						*qSHL1.40* *qSHL8.4*	*qSRR8.5*	*qDWT7.17*
130								
57		*qNa11.5*					*qSRR1.27* *qSRR1.2851* *qSRR1.2853*	

maturing *japonica* rice variety (Linscombe et al., 1993) but highly sensitive to salinity stress (De Leon et al., 2015). To transfer salinity tolerance without compromising the high yielding performance of Bengal, we developed introgression lines of Pokkali in Bengal background. We characterized the ILs for nine traits under salinity stress and we conducted the PCR-based genotyping and GBS to inquire their genome composition. With phenotype and genotype data, QTL mapping for nine traits was conducted to validate the QTLs detected earlier in Bengal x Pokkali RIL population.

4.4.1 Phenotypic response of ILs under salinity stress

The ILs showed variation and continuous distribution for the traits, indicating the quantitative nature of salinity tolerance. The lines displayed varying levels of tolerance and several ILs showed transgressive phenotype, suggesting favorable and unfavorable gene combinations between Bengal and Pokkali (Figure 4.1). For shoot Na^+ and K^+ concentrations, many ILs accumulated higher Na^+ ions than Bengal and higher K^+ ions than Pokkali. However, very few lines have low NaK ratio. Based on SIS, ILs were skewed toward the Bengal parent and only 16 ILs with mean SIS values less than or equal to 5.2 were significantly different to Bengal (Table 4. 7). Similar to the findings in RILs (De Leon et al., 2016), we did not find a line with higher tolerance than Pokkali in term of SIS. In all SIS QTLs, Pokkali alleles were desirable and had decreasing effects at QTLs. Consistent to the growth response of RIL population to salinity stress, SHL, RTL, SRR, and DWT were significant and negatively correlated to SIS indicating the negative effect of salt stress on plant's growth. In contrast, SIS was positively correlated to Na^+ and K^+ concentrations in IL population. The general relationship among traits in both RIL and IL populations indicated reliable phenotyping between populations. The consistent high broad-sense heritability of Na^+, K^+ concentrations, and SHL suggested that phenotypic variations

among ILs were largely due to genetic variation, thus, supporting meaningful estimation of QTL effect for each trait. The SIS and RTL had moderate heritability while NaK, CHL, DWT, and SRR had very low heritability, indicating large non-genetic influence on these traits. Since the ILs were isogenic to Bengal, the phenotypic deviation of an IL from Bengal could be attributed to the presence of Pokkali segments for traits with high to moderate heritability.

4.4.2 Genome composition of ILs by SSR and SNP markers

The ILs were genotyped using SSR and GBS derived SNP markers to assess the genomic composition of each IL. Our results showed that 77.6% of Pokkali genome was transmitted in 72 ILs using SSR markers (Table 4.3). On average, each line contained three donor segments with 95% Bengal and 5% Pokkali genome (E.3). Our result was similar to the results by Tian et al. (2006) in which the use of SSR markers detected only 67.5% of the donor *O. rufipogon* genome in 159 BC_4F_4 lines developed in *O. sativa* background. In contrast, GBS-SNP markers indicated 100% transmission of Pokkali genome among 88 ILs. On average, each IL contains 36 Pokkali segments with 96% and 4% Bengal and Pokkali genome, respectively (E.4). The use of SSR markers detected 216 introgressed segments while SNP markers detected 3198 segments, a resolution that is fourteen times higher than using SSR markers (Table 4.3). Majority of the ILs carried 1-2% Pokkali genome with 3-5Mb length based on SSR markers (Figure 4.3). On the other hand, SNP markers showed that most of the ILs carried 1-3% Pokkali genome of about 100 Kb in length. Furthermore, a total of 18 QTLs were detected for five traits using SSR markers, while 32 QTLs for eight traits were detected by SNP markers (Figure 4.4). Using SNP markers, at least one QTL was detected for Na^+, K^+ concentrations, NaK ratio, and CHL. These results indicated increased efficiency of donor segment detection and higher resolution of mapped QTLs using SNP markers. In fact, thousands of SSR markers were available for rice (McCouch et al.,

2002). However, previous QTL mapping studies only involved less than 200 markers due to low number of polymorphic markers between parents. Therefore, the low density and sparsity of SSR markers provides incomplete and less precise information of donor segments and QTLs controlling a trait (Yu et al., 2011). With the prevalence of SNPs across the rice genome, the increased density of markers proved to be more informative and useful in identifying donor segments and QTLs that were undetected by SSR markers (Arbelaez et al., 2015). Nonetheless, both SSR and SNP markers indicated the same average estimate of percent recurrent genome of ILs (95%), which is very close to the 96% theoretical percent recurrent genome of a BC_4 line.

4.4.3 QTLs for traits related to seedling salinity tolerance

Introgression lines are a set of plants containing donor segments in the genetic background of a recurrent parent. The QTLs introgressed in ILs can be considered gain-of- function alleles making it suitable for QTL discovery and verification of previously mapped QTLs (Li et al., 2005). In spite of numerous QTLs detected for traits related to salinity tolerance in rice, firm conclusion on QTLs is still lacking (Negrão et al., 2011). This is because QTLs are dependent on specific crosses (Flowers and Flowers, 2005). Moreover, reliable phenotyping and genotyping are needed. Therefore, validation of QTLs should be employed to confirm the utility of QTLs and markers for rice breeding program. Based on our QTL mapping results for salinity tolerance in RIL and IL populations, Pokkali alleles had decreasing effects at SIS QTLs (Tables 4.4; 4.5; 3.4). Five SIS QTLs detected in RILs were significant in IL population (Table 4.6). The Pokkali allele at *qSIS6.5* with PVE of 7% could lower the SIS by 1.6 and the most tolerant IL84 carried introgression at this QTL. The large-effect *qSIS5.1b* in RIL population clearly contributed to salinity tolerance as indicated by ILs containing this QTL (*qSIS5.1*, Table 4.5). Furthermore, the presence of Pokkali allele at *qSIS9.8* in selected ILs showed high tolerance (lines with SIS value

of 4 only). Therefore, QTLs for SIS are stable and consistent between populations, and thus the SIS QTLs will be useful for improving salinity tolerance. In RILs, *qSIS5.1b* was narrowed down to two genes, of which, a gene encoding a lectin protein kinase (LOC_Os05g03450) is a promising candidate. In *A. thaliana*, a lectin protein kinase gene was implicated in structural stability of plasma membrane and plant cell wall (Gouget et al., 2006). For *qSIS5.034* or *qSIS5.03*, a vacuolar ATP synthase (LOC_Os05g01560) was a potential candidate gene (De Leon et al., 2016).

Very few QTLs were detected for Na^+, K^+, and NaK ratio in IL population. The main reason for this is likely due to limited number of ILs included in our QTL mapping. Although we phenotyped 292 ILs, only 72 and 88 lines were actually used in QTL mapping by SSR and SNP markers, respectively. It is possible that some lines carrying introgressions for those QTLs were excluded during optimization of CSSL selection. Nevertheless, we identified novel QTLs for Na^+ (*qNa11.5*) and NaK ratio (*qNaK3.32*) that were not detected in RIL population. The *qNa11*.5 is likely the same as *qSNC11* detected on chromosome 11 by Wang et al. (2012). However, the *qNaK3.32* is new and has not been reported in earlier studies. For shoot K^+ concentraation, *qK1.3863* is the same as the *qK1.38* detected in RIL population. Pokkali allele at this locus had increasing effect on shoot K^+ concentration as indicated by increased phenotypic means of the ILs containing this QTL. Lee et al. (2007) detected salinity tolerance QTL (*qST1*) around 38 Mb of chromosome 1. The *qST1* was responsible for 26-27% of the variation in salinity tolerance by visual scoring. Consistent to our RIL-QTL mapping, this locus was responsible for about 10 percent of the phenotypic variation for shoot K^+ concentration. Close to this QTL was a *SNAC2* gene (LOC_Os01g66120) located at 38.39 to 38.40 Mb region of chromosome 1. Transgenic rice

overexpressing *SNAC2* gene showed higher germination and growth rate than wild type plants under cold and salinity stress (Hu et al., 2008).

Previous study indicated the importance of a major QTL for shoot K^+ concentration on chromosome 1 named *qSKC1* (Lin et al., 2004). Fine mapping of *qSKC1* led to the cloning of *HKT1;5* gene located at 11.46 Mb region. The gene was implicated in regulating Na^+/K^+ homeostasis by unloading Na^+ ions from xylem for salinity tolerance (Ren et al., 2005). In a separate RIL mapping population, *Saltol* QTL for low NaK ratio was identified flanking the region of *qSKC1* (Gregorio, 1997; Bonilla et al., 2002). Further study on *Saltol* QTL assumed that the same *HKT1;5* gene was responsible for salinity tolerance (Thomson et al., 2010). Following these results, *Saltol* QTLwas introgressed to local elite varieties in Asia, West Africa, and Russia by marker-assisted backcrossing (Huyen et al., 2012; Singh et al., 2016; Bimpong et al., 2016; Usatov et al., 2015). In our RIL-QTL mapping, QTL for high shoot K^+ concentration (*qK1.11*) co-localized with low NaK ratio QTL (*qNaK1.11*) at 11.52-11.58 Mb region of chromosome 1(Table 3.4). The position of *qK1.11* or *qNaK1.11* however, is 60Kb downstream of HKT1;5. We did not detect significant QTL near or around *Saltol* or *qSKC1* region in spite of SSR and SNP markers availability at the locus. Among the ILs we genotyped, IL172 had Pokkali introgressed segment at 10.59-11.62 Mb region flanking the *SKC1/HKT1;5/ Saltol/qK1.11* locus (Supplementary Table S4.7, available upon request). However, IL172 was very sensitive and had a mean SIS of 8.1. Based on SNP markers, IL172 had 94% Bengal and 6 % Pokkali genome composition with 31 introgressed segments distributed in 8 chromosomes (E.4). While the *Saltol* provided some sort of seedling salinity tolerance, the effect of *Saltol* was not validated in our study. Among the 16 most tolerant lines, (Table 4.7, Supplementary Table S4.7, available upon request) there was no IL with introgression at *qSCK1/Saltol/ qK1.11/qNaK1.11* locus. Consistent

with the results by Thomson et al. (2010), screening of 39 BC_3F_5 lines identified tolerant lines without the Pokkali allele at *Saltol* locus. *Non-Saltol* lines showed minimal differences to *Saltol*-containing lines in salt injury score, NaK ratio, and chlorophyll content. Similarly, Alam et al. (2011) did not find significant differences in salinity tolerance (SES) between *Saltol* and *non-Saltol* QTL-containing backcross lines, thus raising question on the reliability of *Saltol* to protect rice during seedling salinity stress. Taken together, our results emphasized the importance of other QTLs in the development of salt tolerant rice. Therefore, breeding programs aiming to transfer salinity tolerance to elite local varieties should not be limited to selection of the *Saltol* QTL. Pyramiding of multiple QTLs in addition to *Saltol* may provide better chance of developing salt tolerant varieties.

As indicated by QTLs for SHL, SRR, and DWT, Pokkali allele on chromosome 1 at 38-42 regions have increasing growth effect and could be one of the mechanisms of salinity tolerance. Therefore, seedling vigor under salt stress should also be considered. IL84 had multiple QTLs between 38-42 Mb of chromosome 1. Additionally, IL93, IL65, and IL 57 contained QTLs for SHL, SRR, and DWT and all showed tolerance despite the absence of Pokkali derived-QTLs for SIS, NaK or K^+ concentration (Table 4.7). The *qSHL1.38* mapped in RIL was responsible for 52% of the variation in SHL and the Pokkali allele at this QTL had an additive effect of 4.5 cm (Table 3.4). The stability and increasing effect of *qSHL1.38* was confirmed in ILs containing introgression at this region (Table 4.5). In RIL-QTL mapping study, shoot K^+ concentration had significant positive relationships to SHL, SRR, and DWT (De Leon et al., 2016). Here, IL-QTL mapping results confirmed those relationships by co-localization of *qK1.3863* to *qSHL1.3863* and *qSRR1.3863*. In addition, *qDWT1.41* and *qDWT1.42* which are near to *qDWT1.40* in RIL population also co-localized with *qSIS1.41* and *qSIS1.42*, respectively. The co-location of

different QTLs indicated simultaneous improvement of rice for those traits. For example, introgression of *qK1.3863* may increase salinity tolerance. However, this locus will also increase the height and SRR of plant, which can make the resulting line susceptible to lodging. Therefore, care should be taken in selecting QTLs for breeding. Overall, fourteen QTLs detected in RIL for K^+, SIS, CHL, SHL, RTL, SRR, and DWT were validated in IL populations.

4.4.4 Important QTLs and ILs

Among the tolerant ILs, the most tolerant IL84 behaved like the tolerant Pokkali by accumulating high K^+ in the shoot and relatively less Na^+, resulting in low NaK ratio. Likewise, IL84 was tall, thus, sharing the possible mechanism of diluting Na^+ concentration in leaves. Additionally, IL84 had medium grain and red pericarp similar to Pokkali. Inspection of genotypic profile of IL84 (Suppl. Table S4.7) indicated the presence of Pokkali allele for the red pericarp gene (LOC_Os07g11020) on chromosome 7 at 6 Mb region (Furukawa et al., 2006). Therefore, despite the high tolerance in IL84, additional backcrossing will be needed to remove these undesirable traits. Alternatively, the remaining tolerant lines offered salinity tolerance different from Na^+/K^+ homeostasis. The 15 ILs had white pericarp and plant height nearly similar to Bengal (E.5). As indicated by high Na^+ and low K^+ concentrations in their shoot, the ILs could tolerate high Na^+ concentrations. This form of salinity tolerance is not by Na^+ exclusion but more likely by compartmentation of Na^+ ions in vacuoles and by synthesis of compatible solutes for osmotic adjustment (Munns and Tester, 2008). At this point, the exact mechanism of salinity tolerance is difficult to ascertain. However, based on salinity response (SIS), physiological traits and QTLs contained by tolerant ILs, our results suggest the importance of SIS QTLs in addition to *qSKC1/Saltol/qK1.11* in transferring salinity tolerance to other elite varieties. IL119 is a promising breeding line with similar morphological attributes like Bengal with high salt

tolerance and least number of Pokkali introgression. This line demonstrated the importance of at least two SIS QTLs (*qSIS9.8* and *qSIS9.14*) contributing to seedling stage salinity tolerance. The IL230 is another breeding line with additional SIS QTLs on chromosome 5. Overall, the fifteen lines offered potential for selection of high yielding version of Bengal with salinity tolerance.

4.5 Conclusion

Consistent to previous studies, our results have indicated the complexity and polygenic nature of salinity tolerance. Breeding rice by introgression of salinity tolerance to elite rice varieties should consider incorporating SIS QTLs in addition to *Saltol* or *qSKC1*. Due to near-isogenic nature, the tolerant lines identified in this study may serve as improved varieties or donor breeding lines to transfer salinity tolerance to other US varieties. Additionally, the tolerant lines will be useful in fine mapping and positional cloning of genes for salinity tolerance. The SNP markers flanking the QTLs can easily be converted to PCR-based markers for use in marker-assisted breeding.

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CHAPTER 5. CONCLUSIONS

In an effort to develop salinity tolerant variety adapted to Southern region of the United States, thirty US rice genotypes along with nineteen exotic donor genotypes were characterized under salt stress. Based on clustering method using morphological and physiological trait responses, the genotypes were classified into highly tolerant, tolerant, moderately tolerant, sensitive, and highly sensitive groups. The donor genotypes such as Nona Bokra, Pokkali, and breeding lines derived from Pokkali showed the highest tolerance based on linear combination of salt injury score, ion leakage, chlorophyll content, shoot length reduction, shoot K^+ concentration, and shoot Na^+/K^+ ratio. Among the US genotypes, LAH10, R609, and Cheniere were identified tolerant while CL162, Jupiter, Jazzman, Templeton, Cypress, Neptune, and Caffey showed moderate salinity tolerance. The classifications of genotypes were confirmed by discriminant analysis. MANOVA and canonical correlations indicated the differences between groups. The genotypes in tolerant group had higher shoot length reduction, ion leakage, and lower shoot K^+ concentration than the highly tolerant group. In contrast, the moderately tolerant group had significantly lower chlorophyll content than the tolerant group. The sensitive group, however, had significantly higher salt injury score, chlorophyll reduction, and ion leakage than the moderately tolerant group. Significant difference in chlorophyll reduction differentiated the sensitive group from the highly sensitive group. Therefore, our study demonstrated the use of multiple traits in understanding the variation in the levels of salinity tolerance among the US rice varieties and exotic donor lines.

Earlier studies classified rice varieties based on DNA profiles. To see if the varietal grouping based on phenotypic responses to salt stress can be explained by the similarity in their DNA sequence, we assessed the genetic relatedness of rice varieties using SSR markers. Based on our

results, the forty-nine rice genotypes were grouped into either *japonica* or *indica* subspecies. Further subgrouping indicated the separation of long and short grain rice varieties within the main group. However, the genotypic grouping based on DNA profiles did not correspond to varietal grouping based on salinity trait responses. Our findings suggest a limitation on the use of SSR markers in differentiating salt sensitive and tolerant rice varieties. It is probable that the SSR markers used in this study were not associated with the genes controlling salinity tolerance. Overall, the absence of high salinity tolerance and low genetic polymorphism among US varieties emphasized the need to expand the genetic pool of US rice germplasm for development of salt tolerant varieties.

Previous studies detected several QTLs associated with salinity tolerance. However, the QTLs and markers identified were not being utilized in breeding programs due to large chromosomal intervals covered by QTLs and limited number of polymorphic markers. Based on our results, the use of GBS-derived SNP markers increased the density of markers in our genetic map resulting in increased resolution of mapped QTLs and identification of candidate genes likely involved in the fitness of rice plants under salinity stress at seedling stage. For the nine traits we investigated, 85 QTLs of large and small-effects were detected. Of which, 11 QTLs validated the importance of 14 QTLs previously reported. Identification of several epistatic QTLs confirmed the genetic complexity associated with salinity tolerance. Based on the candidate genes delimited by the QTLs, our results suggest the importance of transporters, osmotic regulators, transcription factors, and signal transduction pathway genes. The genes identified in this study will be useful targets for functional genomics, gene pyramiding, and gene-based marker-assisted breeding.

In addition to QTL mapping using RIL population, we also conducted QTL mapping for traits related to salinity tolerance using an IL population from the same Bengal x Pokkali cross to validate the stability and effects of QTLs. A total of sixteen ILs were identified tolerant and significantly different compared to Bengal. However, none of these ILs had introgression at the *Saltol/SKC1/qK1.11* QTL, indicating the importance of other QTLs in conferring salinity tolerance. The QTLs for K^+ concentration, SIS, CHL, SHL RTL, SRR, and DWT were consistent in both RIL and IL populations. The importance and effect of SIS QTLs were evident in tolerant ILs containing Pokkali segments at QTL loci. Likewise, the presence of QTLs for SHL, SRR, and DWT indicated the role of seedling vigor in salinity tolerance. Additionally, based on tolerant ILs and the introgressed Pokkali segments at QTL loci, the probable mechanism of salinity tolerance could be Na^+ dilution in leaves, compartmentation of Na^+ ions, and synthesis of compatible solutes. The sixteen tolerant ILs identified in this study may serve as improved varieties or donor breeding lines for introgression of salinity tolerance to other rice varieties. The ILs will also be useful in fine mapping and positional cloning of genes controlling seedling stage salinity tolerance. Additionally, the SNP markers flanking the QTLs can be converted to PCR-based markers for marker-assisted breeding.

In summary, US rice varieties lack high degree of seedling stage salinity tolerance. The use of Pokkali as a donor was demonstrated in improving a US rice variety 'Bengal'. The QTLs detected in this study indicated the importance of pyramiding several QTLs to incorporate high level of seedling-salinity tolerance. Furthermore, the use of markers flanking significant QTLs will help in selection for salinity tolerance, and therefore, may accelerate the process of transferring salinity tolerance to other elite US rice varieties.

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APPENDIX B: SUPPORTING TABLES TO CHAPTER 2

B.1 Mean trait values of rice genotypes in salinity characterization.

Genotype	SIS	Ion leakage ($uScm^{-1}$)		Ion_ leak	Chlorophyll content (SPAD unit)		Chl_R	Root Length (cm)		RtL_R	Shoot Length (cm)		ShL_R	Rt_Na	Sh_Na	Rt_K	Sh_K	Rt_ Na/K	Sh_ Na/K
		Ro	Rt	(%)	Ctr	Sal	(%)	Ctr	Sal	(%)	Ctr	Sal	(%)	(mmol/ kg)	(mmol/ kg)	(mmol/ kg)	(mmol/ kg)	(ratio)	
Bengal	7.1	0.06	0.74	72	34	17	52	14	7	48	53	30	44	1444	2408	118	688	11	4.6
Caffey	6.2*	0.05	0.53	50	31	19	39	14	8	43	49	27	44	1737	2676	123	831	14	3.8
Catahoula	7.4	0.06	0.52	49	32	14	56	13	8	38	48	24	49	1721	2897	104	822	16	3.9
Cheniere	6.2*	0.06	0.71	69	31	23	27	12	8	38	46	23	51	1384	2582	103	785	12	3.5
Cheriviruppu	4.4***	0.04	0.36	33*	32	22	31	16	8	51	65	40	38	1576	2596	124	1120	13	2.3
CL 152	6.6	0.1	0.6	55	32	12	62	14	7	49	49	23	53^{+}	1402	2846	116	897	25	3.2
CL111	7.5	0.04	0.47	44	35	15	56	15	8	49	53	25	53^{+}	1144	2478	128	770	8	3.6
CL131	7.0	0.04	0.53	51	35	17	50	15	8	43	47	25	48	1710	2735	242	792	12	3.6
CL142	8.1	0.05	0.71	69	33	16	51	15	8	45	45	26	43	1863	2643	111	706	17	3.9
CL151	8.1	0.07	0.53	49	32	11	67	16	8	46	46	24	48	1892	2844	112	725	17	4.8
CL161	8.0	0.08	0.52	48	31	14	56	14	6	56	49	22	55^{++}	1536	2614	130	738	12	3.7
CL162	6.0*	0.03	0.35	33*	34	20	42	15	7	52	48	24	49	1422	2702	123	894	11	3.1
CL181	7.6	0.07	0.46	42	34	16	53	13	8	41	39	21	46	1322	2714	108	716	11	4.3
CL261	8.7	0.13	0.47	40	32	6	80	15	9	38	51	28	45	1526	3158	147	840	10	3.8
Cocodrie	7.2	0.08	0.62	59	34	7	78	15	7	51	53	25	53^{+}	1472	2534	116	860	11	3.0
CSR II (IRGC 83240)	3.8***	0.07	0.46	42	35	34	4***	14	9	37	41	23	43	1686	2517	150	861	12	3.0
Cypress	5.1***	0.04	0.43	40	32	21	36	15	7	53	51	26	48	1530	2493	130	731	12	3.7
Damodar (IRGC 17038)	5.0***	0.08	0.43	38	32	21	35	16	8	49	49	28	42	1702	2007	122	1333	15	2.1*
FL378	3.8***	0.04	0.33	30*	36	25	32	16	9	45	45	27	40	1623	3671	139	1336	12	2.8
FL478	3.0***	0.04	0.36	33*	33	27	18***	14	8	45	50	30	40	1299	2608	95	974	14	2.7
Getu (IRGC 17041)	3.9***	0.08	0.46	41	33	19	42	16	9	45	54	28	49	1806	3153	101	1032	18	3.0
Geumgangbyeo	3.9***	0.08	0.56	52	34	25	26	15	8	47	37	22	40	1545	2367	141	912	11	2.6
Hasawi (IRGC 16817)	4.0***	0.07	0.33	28**	30	21	31	15	9	41	62	41	34	1747	3203	141	1158	13	2.7
IR 1702-74-3-2	5.7***	0.06	0.5	51	32	18	42	15	9	42	44	24	45	1937	2958	151	1031	13	2.9
IR 2706-11-2	6.8	0.06	0.56	40	35	2	94	16	10	41	44	24	46	1572	3494	130	1103	12	3.2
IR 944-102-2-3-2	4.2***	0.11	0.46	47	33	27	19***	12	6	46	35	18	47	1246	2640	134	837	8	3.2

(B.1 continued)

Genotype	SIS	Ion leakage ($uScm^{-1}$)		Ion_ leak	Chlorophyll content (SPAD unit)		Chl_R	Root Length (cm)		RtL_R	Shoot Length (cm)		ShL_R	Rt_Na	Sh_Na	Rt_K	Sh_K	Rt_ Na/K	Sh_ Na/K
		Ro	Rt	(%)	Ctr	Sal	(%)	Ctr	Sal	(%)	Ctr	Sal	(%)	(mmol/ kg)	(mmol/ kg)	(mmol/ kg)	(mmol/ kg)	(ratio)	
IR29	7.7	0.08	0.55	52	35	17	52	15	8	49	41	24	42	1751	3226	140	821	13	4.0
IRRI 147	6.1**	0.04	0.37	34	33	19	43	15	7	52	43	27	37	1532	2423	131	1002	12	2.4
Jazzman	6.9	0.07	0.54	51	31	15	52	12	6	51	52	22	58^{+++}	1637	3362	112	921	15	3.9
Jazzman-2	7.5	0.04	0.45	42	32	14	56	13	7	46	47	24	50	1368	2158	113	614	11	3.5
Jes	7.2	0.04	0.48	46	30	16	46	12	7	44	36	21	42	1363	2702	106	629	12	4.7
Jupiter	6.2*	0.06	0.49	45	35	17	51	12	7	38	44	20	55^{++}	1426	3297	154	916	8	3.7
Ketumbar (IRGC 13516)	5.8**	0.05	0.31	27**	31	19	39	18	12	34	49	29	42	1734	3574	156	1156	11	3.1
LA 0702085	8.6	0.04	0.65	63	32	4	89	13	6	52	51	24	52^{+}	1894	2808	115	867	17	3.5
LA 0802140	7.6	0.05	0.52	50	32	12	64	15	8	45	50	23	54^{+}	1471	2655	110	781	11	4.1
LAH 10	4.4***	0.04	0.58	56	35	23	33	14	8	47	48	26	46	1391	2269	135	794	10	2.9
Mermentau	7.1	0.09	0.58	54	34	7	80	14	8	44	53	27	49	1638	2776	140	745	11	4.1
Neptune	6.3*	0.05	0.51	48	34	21	37	13	8	40	50	26	47	1503	2935	119	765	12	4.5
Nipponbare	5.8***	0.08	0.56	53	35	24	32	15	7	52	41	22	46	1665	3241	138	909	12	3.6
Nona Bokra(IRGC 01231)	4.0***	0.04	0.37	34	32	19	40	16	9	42	70	44	37	1809	2832	129	1059	14	2.7
Pokkali (IRGC 108921)	2.9***	0.04	0.33	30**	33	21	35	16	7	54	70	46	34	1585	2702	109	995	15	2.7
PSBRC50 (IRGC 99706)	4.9***	0.06	0.41	38	36	24	32	14	8	42	43	23	45	2067	2702	171	1129	13	2.4
R609 (MG)	4.4***	0.07	0.61	58	34	24	31	12	6	48	45	24	47	1151	2673	108	774	10	3.7
Rex	7	0.05	0.52	49	33	17	48	17	8	51	49	25	49	1696	2570	110	791	15	3.4
Roy J	6.2*	0.06	0.75	73	35	19	47	13	7	44	46	24	48	1423	2949	116	863	10	3.7
Taggert	5.9**	0.05	0.53	51	34	14	60	13	8	40	45	25	44	1710	3235	111	761	16	5.1
TCCP-266-1-38-13-1-3	3.0***	0.05	0.41	38	33	25	24*	15	8	48	55	31	43	1566	2199	183	966	9	2.3
Templeton	6.0*	0.05	0.45	41	31	19	39	14	9	37	50	27	47	1657	2549	120	786	14	3.8
Wells	7.9	0.08	0.61	57	33	14	58	15	8	46	50	25	50	1762	2961	127	878	14	3.7
Genotypic effect (Pr>F)	<.0001			<.0001			<.0001			0.993			<.0001	0.847	0.086	0.376	0.049	0.262	0.016

SIS= salt injury score; Ion_leak=index of injury by ion leakage; Ro=ion leakage in control treatment; Rt=ion leakage in saline treatment; Ctr=control; Sal=saline treatment; Chl_R=% Chlorophyll reduction; ShL_R=% shoot length reduction; RtL_R=% root length reduction; Rt_Na= Sodium concentration in root, Rt_Na/K=Na/K ratio in root; Sh_Na=Shoot sodium concentration (mmol/kg); Sh_K=shoot potassium concentration (mmol/kg); Sh_Na/K=Na/K ratio in shoot. *Significantly different to IR29 at the 0.05, ** at the 0.01, *** at the 0.001 probability levels.
$^{+}$Significantly different to Pokkali at the 0.05, $^{++}$ at the 0.01, $^{+++}$ at the 0.001 probability levels

B.2 List of SSR markers and allele variations across 49 rice genotypes

SSR marker	Chromosome	Map Position (Mb)[1]	No. of Allele	PIC value
RM220	1	4.4	2	0.498
RM283	1	4.8	2	0.245
RM6277	1	5.7	2	0.209
RM10483	1	7.7	2	0.495
RM1287	1	10.8	4	0.653
RM8094	1	11.2	5	0.609
RM3412	1	11.5	5	0.399
RM10748	1	11.7	5	0.656
RM140	1	12.3	2	0.040
RM10793	1	12.5	4	0.519
RM493	1	12.8	4	0.603
RM10825	1	13.3	2	0.390
RM10852	1	13.9	2	0.040
RM10864	1	14.2	3	0.566
RM562	1	14.6	5	0.429
RM10890	1	14.7	2	0.266
RM7075	1	15.1	5	0.604
RM6711	1	16.1	3	-0.090
RM466	1	17.2	3	-0.027
RM9	1	23.3	3	0.521
RM5	1	23.9	3	0.452
RM2318	1	24.1	3	0.919
RM8129	1	25.0	3	0.612
RM3143	1	26.8	3	-0.517
RM1297	1	28.6	2	0.475
RM5389	1	35.7	3	0.561
RM5781	1	35.7	2	0.300
RM8278	1	36.6	4	0.686
RM315	1	36.7	2	0.425
RM5362	1	41.0	2	0.039
RM3362	1	43.0	2	0.490
RM84	1	na	2	0.307
RM23	1	na	4	-0.392
RM154	2	1.1	8	-1.434
RM262	2	20.7	3	0.599
RM263	2	25.8	3	0.466
RM221	2	27.6	2	0.498

(B.2 continued)

SSR marker	Chromosome	Map Position (Mb)[1]	No. of Allele	PIC value
RM250	2	32.7	3	0.533
RM29	2	na	2	0.479
RM266	2	na	2	0.483
RM3203	3	0.8	3	0.556
RM5474	3	3.8	2	0.483
RM5819	3	4.2	3	0.566
RM5480	3	5.3	2	0.475
RM5513	3	6.2	2	0.348
RM282	3	12.4	2	0.398
RM6080	3	13.9	2	0.483
RM8208	3	22.4	5	0.556
RM3525	3	30.4	4	0.670
RM3564	3	33.4	2	0.459
RM6084	3	33.5	3	0.263
RM7389	3	36.1	3	0.338
RM7	3	na	3	0.716
RM571	3	na	3	0.591
RM5633	4	13.1	3	0.598
RM3742	4	19.7	2	0.425
RM5979	4	20.8	2	0.300
RM3866	4	23.2	3	0.522
RM3839	4	23.9	3	0.348
RM1388	4	25.0	4	0.633
RM3288	4	27.4	3	0.566
RM317	4	29.0	2	0.498
RM5503	4	30.1	2	0.408
RM3836	4	31.6	3	0.979
RM348	4	32.6	2	0.479
RM5506	4	33.3	3	0.533
RM127	4	34.5	4	0.386
RM5579	5	0.5	3	0.608
RM5361	5	0.5	2	0.384
RM159	5	0.8	6	-0.143
RM1366	5	2.9	4	0.336
RM3419	5	5.3	3	0.892
RM289	5	7.8	3	0.426

(B.2 continued)

SSR marker	Chromosome	Map Position (Mb)[1]	No. of Allele	PIC value
RM6645	5	15.0	2	0.179
RM5454	5	17.8	3	0.541
RM146	5	18.0	4	0.639
RM3663	5	21.3	2	0.150
RM3616	5	26.8	2	0.440
RM161	5	na	2	0.475
RM469	6	0.6	2	0.476
RM190	6	1.7	2	0.490
RM225	6	3.4	3	0.679
RM276	6	6.2	3	0.616
RM4924	6	18.5	4	0.449
RM7193	6	20.2	3	0.489
RM6298	6	23.7	2	0.396
RM5371	6	25.8	2	0.459
RM6782	6	26.0	2	0.372
RM295	7	0.4	5	0.287
RM6663	7	2.1	2	0.319
RM6574	7	4.6	2	0.449
RM11	7	19.2	2	0.313
RM11	7	19.2	4	0.601
RM5508	7	23.5	3	0.379
RM351	7	23.9	2	0.466
RM134	7	26.6	4	0.671
RM3555	7	27.9	3	0.603
RM248	7	29.3	4	0.697
RM10	7	na	2	0.439
RM346	7	na	3	0.561
RM6810	7	na	4	0.644
RM408	8	0.1	4	0.634
RM6863	8	2.0	2	0.506
RM1376	8	3.2	3	0.573
RM515	8	20.3	4	0.514
RM195	8	21.4	3	0.433
RM150	8	25.2	2	0.500
RM3496	8	27.8	3	0.525
RM8219	9	1.5	1	0.673
RM566	9	14.7	2	0.594

(B.2 continued)

SSR marker	Chromosome	Map Position (Mb)[1]	No. of Allele	PIC value
RM3700	9	15.4	3	0.628
RM257	9	17.7	3	0.399
RM160	9	19.8	2	0.475
RM107	9	20.0	2	0.475
RM6707	9	22.3	3	-0.517
RM205	9	22.7	1	0.833
RM285	9	na	2	0.425
RM219	9	na	3	0.646
RM216	10	5.0	3	0.619
RM8201	10	13.7	2	0.466
RM258	10	18.0	2	0.480
RM3451	10	21.5	2	0.499
RM228	10	22.2	3	0.622
RM333	10	22.3	3	0.766
RM244	10	na	1	0.880
RM269	10	na	3	0.106
RM167	11	4.1	2	0.307
RM202	11	9.0	3	0.553
RM229	11	18.4	3	0.680
RM1341	11	19.7	4	-0.098
RM206	11	22.0	4	0.681
RM224	11	26.8	4	0.698
RM21	11	na	2	0.408
RM254	11	na	3	0.491
RM3483	12	1.6	3	0.538
RM1302	12	2.6	3	0.441
RM7619	12	4.8	3	0.161
RM101	12	8.8	3	0.669
RM3331	12	23.5	4	0.678
RM6947	12	24.0	2	0.467
RM235	12	26.2	4	0.727
RM313	12	na	2	0.632
RM19	12	na	2	0.425
RM8250	na	na	3	0.563
RM1208	na	na	2	0.437

[1]Map position from Gramene Annotated Nipponbare Sequence 2009. na = not available.

B.3 Posterior probability of membership in salinity groupings by linear discriminant analysis

Genotype	From Salinity Group[$]	Classified into Salinity Group	HS	HT	MT	S	T
Hasawi	HT	HT	0	1	0	0	0
Cherivieruppu	HT	HT	0	0.9999	0	0	0.0001
Pokkali	HT	HT	0	0.9995	0.0001	0	0.0004
NonaBokra	HT	HT	0	0.9981	0.0009	0	0.0011
FL478	HT	HT	0	0.9824	0.0004	0	0.0173
FL378	HT	HT	0	1	0	0	0
TCCP266	HT	HT	0	0.7921	0.0025	0	0.2054
IRRI147	HT	HT	0.0002	0.9968	0.0020	0	0.0011
Ketumbar	HT	HT	0	0.9977	0.0022	0	0.0001
Damodar	HT	HT	0	1	0	0	0
Getu	MT	MT	0.0002	0.0608	0.6837	0	0.2554
CSR	T	T	0	0.1753	0.0031	0	0.8216
PSBRC50	HT	HT	0	0.9900	0.0025	0	0.0075
IR1702	MT	T[#]	0.0123	0.2863	0.2720	0.0002	0.4293
IR944	T	T	0	0.0028	0.0364	0	0.9607
IR2706	S	S	0.0042	0	0	0.9958	0
Nipponbare	T	MT[#]	0.0113	0.0011	0.5314	0	0.4562
Geumgangbyeo	T	T	0	0.0045	0.0089	0	0.9865
IR29	HS	HS	0.7660	0	0.0079	0.2259	0.0001
Cocodrie	S	S	0.0167	0	0	0.9833	0
R609(MG)	T	T	0.0033	0	0.1668	0.0001	0.8299
LAH10	T	T	0.0011	0.0001	0.0300	0	0.9688
LA0802140	HS	HS	0.7771	0	0.0117	0.2112	0
Cheniere	T	T	0.0108	0	0.0562	0	0.9330
Bengal	S	S	0.0201	0	0	0.9799	0
CL152	HS	HS	0.6536	0	0.1040	0.2407	0.0017
RoyJ	S	S	0.2727	0	0.0519	0.6317	0.0437
Rey	HS	HS	0.8067	0	0.1787	0.0074	0.0071
CL142	S	S	0.1774	0	0.0001	0.8226	0
Mermentau	S	S	0.0016	0	0	0.9984	0
Jupiter	MT	MT	0.0576	0	0.9388	0.0002	0.0034
Wells	HS	HS	0.8667	0	0.0163	0.1169	0.0001

(B.3 continued)

Genotype	From Salinity Group[$]	Classified into Salinity Group	HS	HT	MT	S	T
Catahoula	HS	HS	0.8801	0	0.0548	0.0650	0.0001
CL151	S	S	0.2056	0	0.0002	0.7942	0
Jazzman	MT	MT	0.1295	0	0.8686	0.0002	0.0016
Neptune	MT	MT	0.1843	0	0.8049	0.0007	0.0101
Caffey	MT	MT	0.2197	0.0001	0.6778	0.0018	0.1006
Templeton	MT	MT	0.1232	0.0001	0.8427	0.0002	0.0338
Taggert	S	S	0.0283	0	0.0015	0.9702	0
Jazzman2	S	HS[#]	0.9617	0	0.0050	0.0333	0
Jes	HS	HS	0.9005	0	0.0175	0.0819	0
CL162	MT	MT	0.0140	0.0075	0.9468	0	0.0317
CL181	HS	HS	0.9484	0	0.0237	0.0279	0
CL111	HS	HS	0.9203	0	0.0733	0.0063	0.0001
CL131	HS	HS	0.8603	0	0.1042	0.0333	0.0022
Cypress	MT	MT	0.0242	0	0.8615	0	0.1143
CL161	HS	HS	0.9659	0	0.0288	0.0053	0
LA0702085	S	S	0.0013	0	0	0.9987	0
CL261	S	S	0.1518	0	0	0.8482	0

[#] Rice genotypes misclassified

[$] HT, Highly tolerant; T, Tolerant; MT, Moderately tolerant; S, Susceptible; HS, Highly susceptible

B.4 Canonical discriminant analysis between salinity grouping and trait responses to salinity stress

Canonical Discriminant Function	Canonical Correlation	Squared Canonical Correlation	Proportion of variance explained	Test of Significance Pr > F
1	0.943	0.889	0.811	<.0001
2	0.742	0.551	0.124	<.0001
3	0.579	0.336	0.051	0.0035
4	0.349	0.122	0.014	0.1371

B.5 Total canonical structure of canonical discriminant function and class means of salinity group to canonical discriminant function

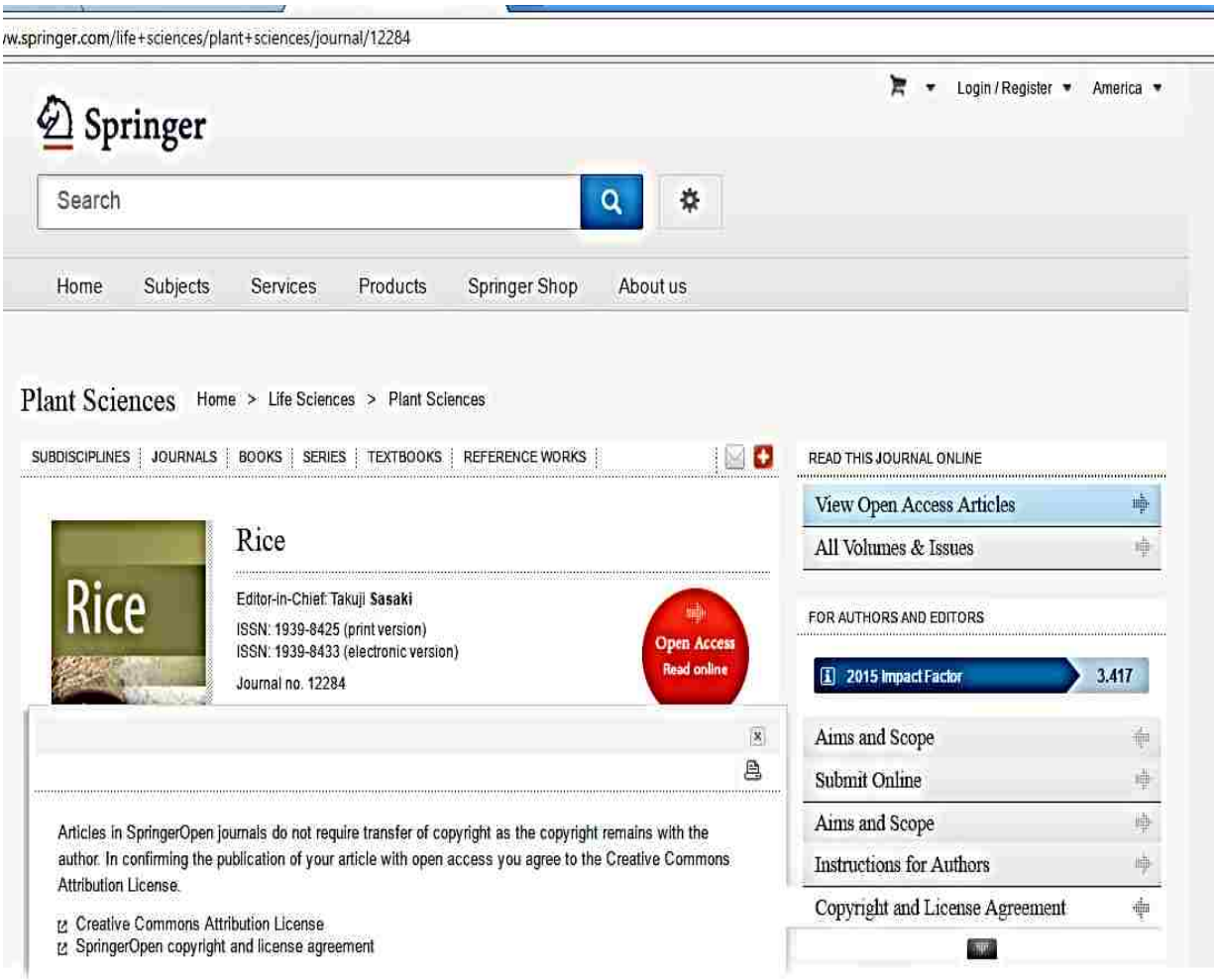
Variable[¥]	Can1	Can2
SIS	0.87	0.10
Ch_R	0.82	0.49
ShL_R	0.59	-0.49
Ion_leak	0.66	-0.40
Sh_K	-0.73	0.48
Sh_Na/K	0.77	-0.21
Salinity Group[$]		
HS	1.86	-0.23
HT	-3.96	1.17
MT	-0.34	-0.81
S	3.37	0.90
T	-1.55	-1.84

[¥] SIS, salt injury score; Chl_R, % reduction in chlorophyll; ShL_R, shoot length % reduction; Ion_leak, index of injury by ion leakage; Sht_K, shoot potassium content; Sh_Na/K, Na/K ratio in shoot.
[$] HT, Highly tolerant; T, Tolerant; MT, Moderately tolerant; S, Susceptible; HS, Highly susceptible

B.6 P-value for pairwise comparison of LS means between salinity groups (i/j)

i	j	SIS	Chl_R	ShL_R	Ion_leak	Sh_K	Sh_Na/K	Overall pair contrast
HT	T	0.868	0.436	0.012	<.0001	<.0001	0.072	<.0001
HT	MT	0.002	0.244	<.0001	0.016	0.000	0.000	<.0001
HT	S	<.0001	<.0001	0.000	<.0001	<.0001	<.0001	<.0001
HT	HS	<.0001	<.0001	<.0001	0.000	<.0001	<.0001	<.0001
T	MT	0.087	0.009	0.694	0.144	0.949	0.461	0.007
MT	S	0.002	<.0001	0.948	0.023	0.462	0.589	<.0001
S	T	<.0001	<.0001	0.961	0.989	0.931	0.026	<.0001
HS	S	1.000	0.015	0.919	0.339	0.999	0.992	0.001
HS	MT	0.002	0.061	1.000	0.649	0.319	0.829	0.001
HS	T	<.0001	<.0001	0.626	0.756	0.839	0.067	<.0001

APPENDIX C: PERMIT TO REPRINT CONTENT OF DE LEON, T.B., LINSCOMBE, S., AND SUBUDHI, P.K. (2016). MOLECULAR DISSECTION OF SEEDLING SALINITY TOLERANCE IN RICE (ORYZA SATIVA L.) USING A HIGH-DENSITY GBS-BASED SNP LINKAGE MAP. *RICE* 9:52. DOI: 10.1186/S12284-016-0125-2



APPENDIX D: SUPPORTING TABLE TO CHAPTER 3

D.1 Di-genic epistatic QTLs for traits related to salt tolerance at seedling stage in Bengal/Pokkali F6 RIL population identified by interval mapping.

Phenotype	QTL	Chr	Position (cM)	Left Marker	Right Marker	QTL	Chr	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add	Add	Add x Add
Na^+ conc.	*qNa4.25*	4	90	S4_25549517	S4_26622324	*qNa4.29*	4	110	S4_29966056	S4_29968457	3.08	11.02	57.92	-21.10	-102.91
	qNa3.26	3	135	S3_26536286	S3_26542118	*qNa5.008*	5	0	S5_87749	S5_96410	3.24	7.90	-13.88	-0.72	78.59
	qNa1.12	1	75	S1_12583448	S1_12685974	*qNa6.2*	6	10	S6_2266152	S6_2272501	3.34	9.09	43.52	-14.46	90.61
	qNa6.17	6	80	S6_17631626	S6_17780076	*qNa6.19*	6	85	S6_19446057	S6_19585327	3.10	15.64	106.90	-89.69	-208.67
	qNa6.4a	6	25	S6_4631489	S6_4771954	*qNa10.21*	10	85	S10_21364298	S10_21407693	3.83	13.99	86.22	46.66	88.20
	qNa6.4b	6	30	S6_4890290	S6_5269698	*qNa11.1*	11	5	S11_1086712	S11_1293020	3.26	13.91	58.04	39.35	80.06
	qNa3.2	3	15	S3_2171559	S3_2250307	*qNa11.23*	11	100	S11_23611942	S11_23708208	3.16	8.69	5.27	10.52	82.87
K^+ conc.	*qK1.7*	1	60	S1_7778029	S1_8656025	*qK2.3*	2	15	S2_3207423	S2_3207477	3.50	21.42	-44.62	-22.75	36.16
	qK2.25	2	115	S2_25166702	S2_25192275	*qK3.22*	3	115	S3_22976923	S3_23020366	3.04	8.02	5.08	-5.45	31.47
	qK1.40	1	190	S1_40584495	S1_40894634	*qK7.19*	7	70	S7_19334046	S7_19406235	3.14	9.07	-12.92	1.97	-32.60
	qK1.7	1	50	S1_7520182	S1_7569628	*qK12.17*	12	55	S12_17065005	S12_17195754	3.23	9.03	-13.81	8.04	-32.71
	qK11.19	11	70	S11_19222100	S11_19245359	*qK12.18*	12	60	S12_18687038	S12_18741493	3.63	10.31	16.55	-3.15	-34.00
NaK ratio	*qNaK1.42*	1	195	S1_42138516	S1_42310908	*qNaK3.21*	3	110	S3_21445493	S3_21628785	3.08	8.71	0.10	0.02	-0.24
	qNaK6.30	6	145	S6_30296317	S6_30370989	*qNaK8.2*	8	20	S8_2341829	S8_2949528	3.09	8.68	0.07	-0.13	0.26
	qNaK6.4a	6	25	S6_4631489	S6_4771954	*qNaK10.213*	10	85	S10_21364298	S10_21407693	3.58	17.65	0.34	0.12	0.26
	qNaK7.22	7	90	S7_22936622	S7_22936634	*qNaK10.217*	10	90	S10_21749293	S10_21786307	3.47	8.73	-0.03	0.03	-0.26
	qNaK5.16	5	65	S5_16290294	S5_16307102	*qNaK11.2*	11	15	S11_2838776	S11_3716306	3.55	10.80	-0.05	0.09	-0.26
	qNaK3.2	3	20	S3_2776106	S3_2780171	*qNaK11.24*	11	105	S11_24319577	S11_24335733	3.46	9.89	0.00	-0.12	0.26
	qNaK1.5	1	35	S1_5501756	S1_5792183	*qNaK12.19*	12	65	S12_19926993	S12_20016304	3.01	8.66	0.06	-0.08	0.24
Salt injury score	*qSIS6.2a*	6	15	S6_2927160	S6_2962502	*qSIS6.30*	6	145	S6_30296317	S6_30370989	3.82	15.04	0.04	0.04	0.07
	qSIS5.18	5	80	S5_18942631	S5_18997491	*qSIS9.9*	9	15	S9_9351804	S9_9857266	3.16	12.06	0.05	0.05	0.06
	qSIS3.10	3	65	S3_10992290	S3_11053944	*qSIS10.2*	10	5	S10_2799960	S10_2837737	4.11	11.59	0.01	0.04	0.07
	qSIS2.20	2	85	S2_20153436	S2_20182321	*qSIS10.11*	10	25	S10_11045261	S10_11244588	3.07	14.67	-0.07	0.02	-0.06
	qSIS3.11	3	70	S3_11848358	S3_11865689	*qSIS12.2*	12	15	S12_2315570	S12_2397199	3.33	7.96	0.00	0.00	0.06
Chlorophyll content	*qCHL1.20*	1	90	S1_20242882	S1_21276489	*qCHL1.21*	1	95	S1_21276489	S1_21352851	7.27	29.81	-4.09	4.17	-5.07
	qCHL3.17	3	105	S3_17083355	S3_17143997	*qCHL3.21*	3	110	S3_21445493	S3_21628785	4.59	28.05	4.16	-4.42	-4.73
	qCHL3.21	3	110	S3_21445493	S3_21628785	*qCHL7.7*	7	50	S7_7781645	S7_7839200	3.31	8.45	-0.29	-0.20	-1.23
	qCHL8.23	8	90	S8_23657286	S8_24738259	*qCHL8.24*	8	95	S8_24763939	S8_25110888	5.38	35.72	-4.73	5.06	-3.76

(D.1 continued)

Phenotype	QTL	Chr	Position (cM)	Left Marker	Right Marker	QTL	Chr	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add	Add	Add x Add
	qCHL9.12	9	25	S9_12217170	S9_12366675	*qCHL9.12*	9	30	S9_12915373	S9_14359383	3.89	34.51	-4.77	4.51	-4.09
	qCHL10.18	10	65	S10_18819950	S10_19941928	*qCHL10.18*	10	70	S10_18819950	S10_19941928	5.68	34.16	-4.44	4.32	-4.33
	qCHL7.27	7	110	S7_27772814	S7_27803479	*qCHL10.21*	10	90	S10_21749293	S10_21786307	3.52	13.31	1.14	0.91	1.28
	qCHL11.4	11	35	S11_4854309	S11_4863888	*qCHL11.6*	11	40	S11_6970703	S11_7012013	3.97	11.76	-1.18	1.25	-2.15
	qCHL3.4	3	25	S3_4116916	S3_4311471	*qCHL11.24*	11	105	S11_24319577	S11_24335733	3.68	11.82	0.28	-0.53	-1.30
Shoot length	*qSHL2.1*	2	10	S2_1653448	S2_2064517	*qSHL2.5*	2	40	S2_5800279	S2_5848583	3.84	9.77	-0.12	-0.48	-2.02
	qSHL4.25	4	95	S4_25549517	S4_26622324	*qSHL5.008*	5	0	S5_87749	S5_96410	4.32	10.89	-0.16	-0.22	-2.07
	qSHL4.27	4	100	S4_27678052	S4_27715999	*qSHL8.1*	8	15	S8_1995144	S8_2005542	3.81	10.15	-0.23	0.09	1.97
	qSHL2.32	2	155	S2_32339457	S2_32429009	*qSHL9.12*	9	25	S9_12217170	S9_12366675	3.67	11.31	0.82	-1.54	2.23
	qSHL1.28	1	130	S1_28157998	S1_28247178	*qSHL9.19*	9	65	S9_19628929	S9_19696641	3.09	11.19	-0.72	-0.54	1.75
	qSHL2.34	2	165	S2_34519074	S2_34545438	*qSHL10.20*	10	80	S10_20682624	S10_20733813	3.34	9.36	-0.15	0.46	1.83
	qSHL4.32	4	130	S4_32867449	S4_33074444	*qSHL10.21*	10	90	S10_21749293	S10_21786307	3.47	10.80	-1.40	-0.05	-1.86
Root length	*qRTL1.32*	1	145	S1_32327040	S1_32418346	*qRTL3.10*	3	65	S3_10992290	S3_11053944	4.80	17.47	-0.03	0.30	0.41
	qRTL4.16	4	35	S4_16669714	S4_16706375	*qRTL6.25*	6	115	S6_25296416	S6_25363541	3.79	12.10	0.19	-0.12	0.37
	qRTL3.28	3	145	S3_28513488	S3_29240341	*qRTL8.23*	8	90	S8_23657286	S8_24738259	3.26	9.34	-0.11	-0.05	-0.38
	qRTL6.15	6	75	S6_15734275	S6_15881397	*qRTL9.16*	9	50	S9_16775205	S9_16882286	3.02	11.22	0.15	-0.29	0.36
	qRTL4.33	4	135	S4_33557881	S4_33861248	*qRTL10.19*	10	75	S10_19941928	S10_20082337	4.15	10.32	-0.01	0.02	-0.41
Dry weight	*qDWT3.17*	3	105	S3_17083355	S3_17143997	*qDWT6.7*	6	50	S6_7662391	S6_7749349	3.20	10.64	0.00	0.00	-0.01
	qDWT6.4	6	30	S6_4890290	S6_5269698	*qDWT6.30*	6	145	S6_30296317	S6_30370989	3.06	12.61	0.00	-0.01	-0.01
	qDWT7.1	7	5	S7_1021298	S7_1051320	*qDWT7.27*	7	110	S7_27772814	S7_27803479	3.43	10.08	0.00	0.00	0.01
	qDWT5.2	5	20	S5_2483311	S5_2495045	*qDWT10.16*	10	50	S10_16848745	S10_16898283	3.30	16.39	-0.01	0.00	-0.01
	qDWT4.16	4	35	S4_16669714	S4_16706375	*qDWT10.19*	10	75	S10_19941928	S10_20082337	3.34	9.61	0.00	0.00	0.01
	qDWT3.5	3	35	S3_5859095	S3_5904925	*qDWT12.09*	12	10	S12_977852	S12_1386213	3.80	12.95	0.00	0.00	-0.01
	qSRR2.1	2	5	S2_1103758	S2_1653448	*qSRR4.27*	4	100	S4_27678052	S4_27715999	3.47	11.08	0.00	-0.19	0.35
Shoot-root ratio	*qSRR5.2*	5	15	S5_2116055	S5_2167880	*qSRR5.5*	5	40	S5_5798670	S5_5909747	3.85	9.93	0.12	-0.07	-0.39
	qSRR2.3	2	25	S2_3978527	S2_4234638	*qSRR9.14*	9	40	S9_14976723	S9_15092089	3.20	10.65	-0.14	0.24	-0.39
	qSRR1.20	1	90	S1_20242882	S1_21276489	*qSRR9.21*	9	75	S9_21030508	S9_21083576	3.09	10.97	-0.11	-0.13	0.37
	qSRR4.18	4	45	S4_18779374	S4_18826971	*qSRR10.001*	10	0	S10_103050	S10_160013	3.18	9.97	-0.13	-0.16	0.34

D.2 Comparison of 38 SNP calls by GBS and resequencing data

Chr.	SNP	Reference Seq.	GBS_SNP call			re-sequenced SNP call	
	Coordinate (bp)	Nipponbare	Bengal	RIL108 (T)	RIL192 (S)	Bengal	Pokkali
1	2,803,270	C	A	C	A	A	C
1	2,815,901	G	A	G	A	A	G
1	2,818,023	A	C	A	C	C	A
1	2,818,449	A	C	A	C	C	A
1	20,242,882	A	G	A	G	G	A
1	23,551,887	T	T	C	T	T	C
1	41,318,979	-	G	A	G	G	A
1	8,656,025	G	G	T	G	G	T
1	8,901,503	A	A	G	A	A	G
1	11,529,325	G	G	G	T	G	T
1	11,581,799	T	T	C	N	T	C
1	38,611,845	A	A	A	G	A	G
1	38,636,497	T	T	T	C	T	C
1	38,768,787	G	G	A	A	G	A
1	38,794,029	C	C	T	T	C	T
1	39,047,133	C	C	T	N	C	T
4	12,276,210	G	A	G	A	A	G
4	14,183,398	A	T	A	T	T	A
4	18,201,322	G	T	A	T	T	A
4	20,625,405	C	T	T	N	T	G
4	33,074,444	A	A	A	A	A	T
5	1,195,956	C	G	A	G	G	A
5	1,454,837	T	T	C	T	T	C
5	4,565,557	T	T	A	A	T	A
5	4,699,921	A	A	G	G	A	G
6	5,269,698	T	G	T	G	G	T
6	5,533,752	T	T	T	T	T	G
6	21,253,244	T	T	C	T	T	C
6	21,256,132	C	C	T	C	C	T
9	8,608,506	G	G	G	G	G	A
9	9,070,610	G	G	G	G	G	A
10	5,164,804	G	A	G	A	G	hetero (A/G)
10	5,645,587	C	T	C	N	T	C
10	7,443,745	T	C	T	C	C	T
10	15,875,631	C	T	A	A	T	A
10	22,433,572	A	A	G	G	A	G
11	2,304,268	C	C	G	G	C	G
11	19,945,249	A	A	A	C	A	C

T, salt tolerant RIL; S, sensitive RIL.

APPENDIX E: SUPPORTING TABLES TO CHAPTER 4

E.1 List of SSR markers used in IL mapping

Marker	Chromosome	Position (Mb)
RM84	1	1.0
RM220	1	4.4
RM23	1	8.0
RM580	1	9.6
RM493	1	12.3
RM466	1	17.3
RM1297	1	28.6
RM11706	1	32.1
RM5389	1	35.7
RM8278	1	36.6
RM3810	1	39.5
RM5362	1	41.1
RM3362	1	43.0
RM154	2	1.1
RM211	2	3.0
RM145	2	7.7
RM341	2	20.7
RM3762	2	22.4
RM263	2	25.9
RM250A	2	32.8
RM166	2	34.3
RM266	2	37.6
RM138	2	39
RM3203	3	0.8
RM5819	3	4.3
RM5480	3	5.3
RM7	3	8.0
RM6080	3	13.9
RM3180	3	18.3
RM8208	3	22.4
RM5626	3	24.9
RM15575	3	25.1
RM15721	3	27.7
RM15859	3	29.9
RM130	3	33.4
RM7389	3	36.2
RM8213	4	4.4
RM16502	4	6.7
RM5953	4	9.4
RM5633	4	13.1
RM16739	4	16.5
RM252	4	23.8
RM6089	4	29.4
RM348	4	32.6
RM17749	5	0.4

(E.1 continued)

Marker	Chromosome	Position (Mb)
RM5579	5	1.9
RM3419	5	5.3
RM7449	5	14.2
RM6054	5	22.8
RM6545	5	24.9
RM26	5	27.3
RM190	6	1.8
RM225	6	3.4
RM253	6	5.4
RM3431	6	8.7
RM8225	6	9.3
RM4924	6	18.8
RM7193	6	20.2
RM20416	6	25.2
RM340	6	28.6
RM1150	6	30.4
RM3831	7	1.2
RM1353	7	3.3
RM7121	7	5.6
RM214	7	12.8
RM5793	7	17.5
RM11	7	19.3
RM3555	7	27.9
RM248	7	29.3
RM3710	8	0.38
RM3819	8	3.0
RM330B	8	6.0
RM8264	8	19.8
RM556	8	22.3
RM23444	8	25.6
RM3496	8	27.8
RM316	9	0.3
RM1328	9	9.2
RM3769	9	11.7
RM257	9	17.7
RM107	9	20.1
RM24718	9	21.2
RM205	9	22.7
RM244	10	3.0
RM8207	10	9.8
RM8201	10	13.8
RM271	10	15.0
RM269	10	17.0
RM3773	10	19.9
RM20B	11	0.0
RM25956	11	0.18
RM26062	11	2.2

(E.1 continued)

Marker	Chromosome	Position (Mb)
RM1124	11	3.8
RM295B	11	9.0
RM206	11	22.0
RM187	11	23.0
RM6440	11	24.9
RM224	11	26.8
RM3483	12	1.6
RM5927	12	2.2
RM101	12	8.8
RM1337	12	11.9
RM1261	12	17.5
RM277	12	20.0
RM3331	12	23.5
RM6953	12	26.1

E.2 Phenotypic mean performance of ILs in nine traits under salinity stress 12dSm-1

Line	SIS	Na^+ ($mmolkg^{-1}$)	K^+ ($mmolkg^{-1}$)	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL51	5.40	1201.03	518.34	2.34	17.38	40.27	9.10	4.43	0.09
IL52	5.40	1427.06	478.16	3.63	19.57	32.40	8.80	3.67	0.09
IL53	6.10	1174.74	430.26	2.69	18.58	35.43	8.57	4.27	0.08
IL54	5.60	935.57	475.84	1.99	21.17	36.28	8.91	4.13	0.08
IL55	6.53	1387.64	455.75	3.14	20.29	33.77	8.03	4.25	0.08
IL56	5.27	1482.26	469.66	3.71	20.18	36.13	8.63	4.27	0.09
IL57	5.17	1111.67	442.62	2.58	18.51	30.90	7.14	4.34	0.08
IL58	5.77	1256.22	485.89	2.71	19.75	34.52	8.18	4.29	0.09
IL59	5.33	1158.98	616.08	1.97	20.36	33.98	8.27	4.12	0.08
IL60	6.89	1187.89	488.2	2.48	18.79	32.37	7.63	4.29	0.07
IL61	5.07	1022.3	453.44	2.30	20.60	31.83	8.67	3.68	0.08
IL62	6.47	1080.12	382.35	2.79	19.94	28.80	8.48	3.43	0.07
IL63	5.53	1250.97	389.69	3.22	20.98	36.78	9.37	3.94	0.09
IL64	6.60	1442.83	470.05	3.08	18.05	33.53	8.57	3.93	0.07
IL65	5.13	1198.4	474.68	2.74	19.51	37.00	9.33	4.01	0.10
IL66	6.33	1027.56	420.6	2.52	21.35	35.73	9.17	3.91	0.09
IL67	5.00	1148.46	497.09	2.37	21.49	34.96	9.01	3.94	0.10
IL68	4.47	1166.86	444.55	2.67	20.26	36.00	8.27	4.38	0.10
IL69	6.73	1198.4	439.91	2.73	21.07	32.33	8.60	3.79	0.08
IL70	7.50	1319.3	538.81	2.61	19.13	29.60	6.77	4.46	0.06
IL71	5.93	1190.51	377.33	3.15	21.87	36.80	9.43	3.90	0.09
IL72	7.13	1090.64	473.91	2.36	16.61	32.65	7.83	4.21	0.07
IL73	6.27	1143.2	453.82	2.57	20.22	35.07	8.93	3.96	0.08
IL74	5.40	1224.68	438.75	2.84	19.09	32.87	9.03	3.68	0.08
IL75	6.33	1321.93	494	2.75	18.65	34.67	8.73	4.00	0.08
IL76	6.47	1135.32	616.85	1.87	18.70	55.80	8.93	6.80	0.08
IL77	7.80	1064.35	505.98	2.08	15.85	32.27	9.90	3.26	0.07

(E.2 continued)

Line	SIS	Na^+ ($mmolkg^{-1}$)	K^+ ($mmolkg^{-1}$)	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL78	4.77	1103.78	504.04	2.23	19.27	34.48	8.60	4.03	0.07
IL79	7.33	1074.87	366.13	2.88	17.28	33.61	7.03	4.33	0.06
IL80	6.57	1553.23	449.57	3.50	18.90	33.70	8.46	4.02	0.08
IL81	6.73	1198.4	502.89	2.45	21.11	34.07	9.37	3.66	0.08
IL82	5.90	1277.25	429.1	2.98	21.15	35.00	9.40	3.77	0.09
IL83	5.53	1398.15	436.44	3.35	19.77	35.10	10.21	3.45	0.10
IL84	3.22	943.45	599.46	1.68	21.75	50.33	9.83	5.14	0.13
IL85	7.13	1185.26	390.85	3.11	20.59	34.25	8.73	3.94	0.08
IL86	5.50	1261.48	401.28	3.24	22.60	35.83	9.18	3.96	0.10
IL87	7.13	1201.03	415.19	2.87	21.21	30.07	6.83	4.47	0.06
IL88	6.43	1298.28	416.35	3.17	40.13	35.05	8.53	4.11	0.08
IL89	5.53	1253.59	558.51	2.32	19.83	38.67	8.07	4.83	0.09
IL90	5.27	1182.63	576.28	2.08	20.61	35.47	9.70	3.68	0.10
IL91	4.33	1240.45	411.71	3.03	21.82	34.52	7.87	4.42	0.10
IL92	6.13	1250.96	444.16	2.84	19.13	35.97	8.33	4.33	0.09
IL93	4.53	1253.59	476.61	2.63	21.17	37.83	9.87	3.89	0.10
IL94	5.80	1258.85	595.21	2.19	18.03	32.07	8.50	3.78	0.08
IL95	7.37	1211.54	447.25	2.74	19.90	33.50	9.04	3.78	0.07
IL96	6.33	1095.89	410.17	2.73	40.70	35.73	8.60	4.17	0.08
IL97	7.27	1463.86	473.52	3.18	39.38	29.40	7.80	3.78	0.07
IL98	5.53	1369.24	483.95	2.83	44.77	31.07	8.88	3.50	0.08
IL99	4.37	1061.73	549.24	1.93	19.67	33.80	9.25	3.75	0.08
IL100	5.82	1316.67	501.73	2.75	17.87	34.69	8.02	4.42	0.09
IL101	6.20	1153.72	463.87	2.70	23.42	34.80	8.67	4.01	0.09
IL102	6.64	1077.5	392.01	2.75	19.96	35.68	8.37	4.26	0.13
IL103	7.00	1271.99	518.72	2.51	17.22	28.13	7.78	3.65	0.06
IL104	5.93	1266.73	458.46	2.84	19.66	34.40	8.80	3.94	0.08
IL105	6.87	1316.67	526.45	2.70	19.12	53.28	9.16	5.83	0.07
IL106	5.27	1032.81	559.28	1.92	19.16	36.47	9.73	3.74	0.09
IL107	6.90	1308.79	499.41	2.72	19.46	53.75	9.40	5.63	0.08
IL108	6.33	1093.27	475.07	2.28	18.39	35.28	8.27	4.34	0.07
IL109	6.87	1403.41	528.38	2.66	18.44	34.80	8.93	3.93	0.08
IL110	6.33	1369.24	499.79	2.73	20.56	37.32	8.70	4.32	0.09
IL111	6.13	1379.75	466.18	2.97	20.78	37.17	8.92	4.17	0.10
IL112	7.00	1279.88	435.28	2.93	16.45	32.42	8.13	3.99	0.07
IL113	6.61	1172.11	440.69	2.81	17.46	33.93	9.05	3.80	0.08
IL114	6.07	1174.75	507.91	2.36	17.61	29.47	8.73	3.39	0.08
IL115	6.47	1232.57	444.94	2.78	16.85	32.13	8.03	4.00	0.07
IL116	4.40	1185.26	442.62	2.71	21.17	33.93	9.30	3.67	0.09
IL117	7.31	1369.24	473.14	2.93	19.17	32.36	8.76	3.69	0.09
IL118	6.00	1158.97	471.21	2.56	18.03	32.47	7.97	4.10	0.07
IL119	4.33	1151.09	425.62	2.79	22.61	30.73	9.00	3.46	0.07
IL120	6.97	1293.02	499.02	2.70	41.59	34.17	8.66	3.95	0.07
IL121	5.67	1193.14	454.59	2.72	19.30	35.73	8.37	4.37	0.08
IL122	6.33	1116.92	424.07	2.62	17.01	32.80	9.23	3.57	0.08

(E.2 continued)

Line	SIS	Na^+ ($mmolkg^{-1}$)	K^+ ($mmolkg^{-1}$)	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL123	6.83	1053.84	488.98	2.16	15.87	34.58	8.40	4.16	0.07
IL124	6.60	1261.48	417.12	3.11	18.15	33.93	9.27	3.70	0.08
IL125	6.87	1187.89	394.33	3.00	16.19	30.93	8.70	3.56	0.07
IL126	6.33	1048.58	440.68	2.38	19.14	31.28	8.07	3.86	0.07
IL127	8.00	1261.48	320.93	3.92	19.63	26.61	7.86	3.38	0.06
IL128	6.87	1222.06	481.64	2.51	17.25	31.47	8.17	3.90	0.06
IL129	4.73	1308.79	468.12	2.77	16.96	35.08	8.27	4.28	0.08
IL130	5.17	1298.28	456.14	2.97	19.77	32.93	8.62	3.84	0.07
IL131	5.40	1182.63	502.11	2.53	18.24	35.13	8.50	4.17	0.08
IL132	6.07	940.82	389.3	2.44	16.60	31.68	7.37	4.32	0.08
IL133	6.87	1340.33	499.79	2.74	20.30	32.83	9.08	3.65	0.07
IL134	5.53	1232.57	454.59	2.75	18.83	36.20	8.57	4.23	0.08
IL135	7.27	980.25	480.09	2.13	18.53	33.20	8.40	3.97	0.07
IL136	6.78	1345.59	471.59	2.96	17.77	34.91	8.28	4.23	0.08
IL137	5.80	1140.58	444.93	2.60	23.10	35.73	9.80	3.66	0.09
IL138	5.31	1442.83	427.55	3.38	22.76	36.62	8.67	4.29	0.10
IL139	5.82	1177.37	440.69	2.79	20.96	35.53	9.08	4.07	0.08
IL140	5.80	1377.13	544.22	2.53	42.25	38.20	8.37	4.63	0.10
IL141	6.87	1319.3	455.37	2.97	17.53	33.40	8.17	4.13	0.07
IL142	8.07	1258.85	472.36	2.65	18.35	30.73	7.10	4.34	0.06
IL144	7.53	1064.35	533.02	1.98	17.75	32.58	8.14	4.03	0.07
IL145	7.23	1156.35	440.69	2.59	16.72	31.78	8.29	3.88	0.07
IL146	7.13	1040.7	505.97	2.16	17.73	36.00	9.30	3.87	0.07
IL147	5.93	1208.91	442.62	2.76	19.47	36.47	8.77	4.16	0.08
IL148	5.29	1164.23	546.92	2.15	20.12	33.85	8.47	4.03	0.08
IL149	6.07	1214.17	581.69	2.09	19.69	35.33	8.90	4.03	0.09
IL151	5.50	1329.81	619.16	2.14	24.92	37.17	9.23	4.02	0.10
IL152	6.73	1424.43	759.4	1.88	21.83	36.07	9.87	3.67	0.11
IL153	5.20	1337.7	764.42	1.79	25.82	35.33	8.87	4.00	0.09
IL154	6.73	1319.3	670.93	1.97	22.88	34.15	9.00	3.88	0.08
IL155	6.47	1279.88	674.02	1.90	24.07	29.00	8.83	3.35	0.07
IL156	6.33	1458.6	753.22	1.94	21.83	32.87	9.10	3.63	0.08
IL157	7.67	1637.33	677.88	2.41	22.66	29.62	8.30	3.63	0.07
IL158	5.53	1442.84	746.65	1.95	22.38	32.13	8.27	3.93	0.08
IL159	6.73	1400.78	725.02	1.96	22.89	31.47	8.57	3.70	0.07
IL160	5.67	1327.19	706.09	1.88	23.39	31.07	8.37	3.75	0.07
IL161	7.40	1705.67	836.66	2.01	24.53	35.00	8.23	4.26	0.07
IL162	7.67	1390.27	561.22	2.51	23.67	27.60	9.00	3.08	0.06
IL163	6.73	1290.39	619.94	2.08	23.57	33.80	9.07	3.73	0.07
IL164	7.53	1429.69	685.61	2.08	24.54	33.27	9.57	3.50	0.07
IL165	7.40	1366.61	652	2.12	21.52	33.40	8.40	3.97	0.07
IL166	7.80	1245.71	562.76	2.28	23.95	32.87	8.70	3.80	0.07
IL167	6.63	969.73	495.54	1.97	22.45	34.78	9.23	3.80	0.08
IL168	7.13	1369.24	668.61	2.07	23.26	37.27	9.77	3.81	0.07
IL169	7.27	1232.57	635.78	2.01	24.45	34.40	9.53	3.61	0.07

(E.2 continued)

Line	SIS	Na^+ ($mmolkg^{-1}$)	K^+ ($mmolkg^{-1}$)	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL170	7.80	1335.07	596.37	2.19	23.34	30.87	9.23	3.35	0.06
IL171	6.47	1579.51	661.66	2.40	19.75	30.33	9.70	3.13	0.07
IL172	8.07	1153.72	620.33	1.89	18.57	26.23	7.83	3.35	0.05
IL173	7.13	1193.14	609.51	2.03	18.95	28.40	7.78	3.66	0.06
IL174	7.27	1632.07	703	2.34	20.85	31.18	7.83	3.97	0.07
IL175	6.20	1421.81	662.05	2.18	20.16	28.93	7.70	3.77	0.06
IL176	7.00	1090.64	580.53	1.91	24.37	33.27	9.23	3.63	0.07
IL177	6.70	1274.62	585.17	2.26	28.73	32.53	9.60	3.40	0.08
IL178	5.40	1348.21	753.99	1.86	20.69	39.27	8.23	4.74	0.08
IL179	5.27	1198.4	738.15	1.68	23.89	34.87	8.37	4.19	0.10
IL180	6.60	1098.52	584.4	1.92	24.73	32.47	9.13	3.55	0.07
IL181	6.03	922.42	507.91	1.99	25.37	29.70	8.35	3.56	0.07
IL182	5.27	925.05	538.43	1.77	23.35	31.18	8.77	3.57	0.07
IL183	6.87	864.6	479.7	1.75	21.69	31.33	8.43	3.74	0.07
IL184	6.60	1148.46	614.53	2.04	22.04	29.08	7.54	3.83	0.07
IL185	7.00	1072.24	642.35	1.68	23.44	31.27	9.47	3.32	0.07
IL186	5.77	938.2	507.13	1.88	21.56	30.93	7.03	4.38	0.08
IL187	5.93	1377.12	676.73	2.03	17.49	33.80	9.03	3.74	0.08
IL188	5.73	1250.97	660.12	1.95	23.44	35.53	7.63	4.70	0.10
IL189	6.07	959.22	565.47	1.69	19.85	32.73	7.77	4.21	0.07
IL190	5.67	1266.73	580.92	2.18	22.67	33.97	8.57	3.95	0.08
IL191	7.13	1379.75	683.68	2.07	23.30	31.22	8.81	3.54	0.06
IL192	6.47	1424.43	701.84	2.05	21.29	32.00	9.37	3.51	0.07
IL193	7.40	1547.97	754.38	2.10	21.55	32.00	8.97	3.56	0.07
IL194	7.00	1545.34	667.84	2.37	21.18	29.57	7.90	3.76	0.07
IL195	6.87	1148.46	456.53	2.53	20.30	31.60	9.20	3.46	0.07
IL196	7.17	1206.28	474.68	2.55	21.92	33.18	8.42	3.95	0.07
IL197	5.53	1017.05	515.63	1.99	22.14	33.67	8.73	3.87	0.08
IL198	7.00	1245.71	629.21	2.01	21.31	30.47	8.53	3.56	0.06
IL199	7.20	1306.16	631.92	2.23	21.43	34.03	7.83	4.35	0.07
IL200	6.07	1245.71	536.49	2.40	23.25	34.40	9.67	3.57	0.07
IL201	6.47	1337.7	678.28	1.99	24.73	28.73	8.50	3.39	0.07
IL202	7.13	1306.16	638.1	2.11	23.28	29.80	7.70	3.87	0.07
IL203	7.07	1208.91	614.92	1.98	24.22	31.47	10.37	3.04	0.07
IL204	6.73	859.34	446.1	1.98	23.02	29.73	8.73	3.40	0.07
IL205	6.40	1374.5	596.76	2.34	23.27	30.67	8.43	3.63	0.07
IL206	5.33	1051.21	523.75	2.20	24.29	33.20	9.33	3.56	0.08
IL207	7.40	1345.59	598.69	2.29	19.01	31.87	9.00	3.55	0.07
IL208	7.27	1366.61	597.53	2.44	21.49	31.73	9.17	3.48	0.07
IL209	7.27	1195.77	563.92	2.20	20.44	31.73	10.27	3.12	0.07
IL210	6.87	1513.8	698.75	2.21	20.67	30.87	8.67	3.58	0.07
IL211	7.53	1290.39	687.16	1.90	23.39	31.67	9.30	3.39	0.07
IL212	7.27	1285.13	609.51	2.15	22.71	29.80	8.60	3.45	0.06
IL213	7.67	1256.22	588.65	2.13	25.80	31.73	9.30	3.41	0.07
IL214	7.67	1571.62	663.98	2.36	23.43	29.20	9.10	3.21	0.06

(E.2 continued)

Line	SIS	Na^+ ($mmolkg^{-1}$)	K^+ ($mmolkg^{-1}$)	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL215	7.40	1568.99	760.95	2.07	22.12	31.40	9.57	3.30	0.07
IL216	6.87	1295.65	621.1	2.09	22.61	30.75	9.26	3.32	0.07
IL217	6.07	1348.21	718.06	1.91	21.96	32.13	9.67	3.33	0.07
IL218	6.20	1474.37	667.46	2.20	24.35	34.07	9.60	3.55	0.09
IL219	6.20	1550.6	763.26	2.12	22.79	30.80	8.73	3.53	0.07
IL220	6.33	1500.66	637.32	2.35	22.58	31.33	8.17	3.84	0.08
IL221	6.90	1172.11	488.2	2.42	24.28	32.63	8.58	3.83	0.08
IL222	7.23	1500.66	592.51	2.61	22.09	30.85	7.87	4.01	0.07
IL223	7.40	1547.97	668.61	2.37	22.83	31.47	8.87	3.55	0.07
IL224	6.53	1198.4	650.07	1.82	22.47	33.53	9.70	3.46	0.08
IL225	6.07	1248.34	681.75	1.85	22.99	34.42	10.08	3.44	0.08
IL226	6.87	1448.09	650.46	2.30	23.01	26.53	9.13	2.91	0.06
IL227	7.40	1374.5	663.6	2.07	21.29	31.13	8.50	3.68	0.06
IL228	8.20	1169.49	577.83	2.08	21.97	29.00	8.73	3.33	0.07
IL229	7.53	1329.82	633.84	2.09	23.46	33.27	9.70	3.50	0.08
IL230	4.10	1264.11	601.01	2.10	25.27	37.68	9.72	3.92	0.11
IL231	7.07	1466.49	701.84	2.15	22.00	28.48	8.30	3.44	0.08
IL232	7.00	1771.38	723.86	2.46	22.04	35.33	8.83	4.04	0.08
IL233	5.27	1287.76	746.65	1.75	20.81	35.67	9.73	3.68	0.09
IL234	6.87	1064.36	503.66	2.18	21.36	33.80	9.90	3.41	0.09
IL235	7.40	1492.77	664.75	2.24	21.10	33.80	9.00	3.75	0.08
IL236	6.87	1253.59	713.81	1.77	24.05	30.87	8.70	3.57	0.08
IL237	6.87	1219.43	558.9	2.22	23.71	33.18	8.88	3.73	0.08
IL238	7.67	1356.1	661.27	2.01	22.27	33.20	7.97	4.18	0.06
IL239	7.13	1224.68	643.5	1.92	23.55	32.80	9.07	3.63	0.06
IL240	7.53	1174.74	617.62	1.85	21.51	30.47	8.93	3.42	0.06
IL241	6.38	1206.28	582.47	2.09	25.18	31.56	8.43	3.81	0.08
IL242	7.53	1306.16	653.93	2.06	21.38	32.40	8.63	3.76	0.08
IL243	7.13	1332.44	579.76	2.30	22.29	31.80	8.60	3.72	0.09
IL244	7.27	1455.98	749.74	1.93	23.40	33.67	9.57	3.52	0.08
IL245	8.17	1503.29	658.18	2.25	20.10	27.83	8.08	3.45	0.07
IL246	7.87	1166.86	627.28	1.87	22.32	24.40	4.05	6.19	0.05
IL247	6.87	1332.45	687.16	1.94	23.27	28.80	8.43	3.45	0.07
IL248	7.13	1327.19	639.64	2.07	22.55	32.53	8.83	3.76	0.08
IL249	6.90	1350.84	618.39	2.17	23.57	34.20	9.63	3.58	0.08
IL250	8.17	1408.67	660.89	2.15	25.55	30.53	8.17	3.78	0.08
IL251	6.87	1371.87	624.96	2.28	24.26	31.20	8.33	3.74	0.07
IL252	7.13	1403.41	666.69	2.10	25.57	34.40	10.00	3.44	0.07
IL253	6.20	1279.88	581.31	2.19	25.62	34.40	10.07	3.42	0.08
IL254	6.33	1821.31	816.96	2.23	26.75	35.20	9.53	3.69	0.09
IL255	6.60	1471.75	686.77	2.14	25.18	34.40	9.17	3.74	0.08
IL256	7.80	1211.54	460	2.67	22.23	30.93	9.67	3.22	0.07
IL257	8.07	1156.35	611.83	1.91	22.56	30.40	9.63	3.17	0.06
IL258	7.13	1114.29	577.44	1.99	20.62	32.53	8.77	3.72	0.07
IL259	6.80	1153.72	592.51	1.96	22.59	30.87	9.07	3.40	0.07

(E.2 continued)

Line	SIS	Na^+ ($mmolkg^{-1}$)	K^+ ($mmolkg^{-1}$)	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL260	6.33	1363.99	610.28	2.30	22.13	33.20	8.70	3.82	0.07
IL261	7.67	1427.06	615.69	2.31	23.69	32.40	9.27	3.49	0.07
IL262	7.57	1080.12	537.27	2.02	21.55	24.67	8.14	3.04	0.05
IL263	5.93	1032.81	530.7	2.04	24.13	31.07	8.60	3.65	0.08
IL264	7.43	1222.05	537.65	2.15	22.73	30.73	8.90	3.55	0.07
IL265	6.73	1264.11	634.23	2.02	23.75	33.93	9.10	3.74	0.07
IL266	6.07	1106.41	626.89	1.80	23.79	30.40	8.17	3.72	0.08
IL267	6.73	1332.45	513.7	2.63	21.55	30.47	9.62	3.17	0.07
IL268	6.47	1484.89	665.14	2.22	21.90	30.47	8.50	3.59	0.07
IL269	6.47	1295.65	764.03	1.72	22.14	32.87	9.17	3.62	0.08
IL270	7.83	1540.08	618.78	2.53	20.18	30.64	9.17	3.34	0.05
IL271	6.90	1206.28	507.52	2.37	20.71	34.47	8.70	3.98	0.08
IL272	5.67	1119.55	695.66	1.62	24.51	38.02	8.12	4.70	0.08
IL301	6.47	1332.45	689.09	1.96	24.00	41.10	9.97	4.17	0.08
IL302	5.87	1232.57	654.32	1.90	25.35	34.40	9.60	3.58	0.08
IL303	6.57	1555.85	768.29	2.10	25.08	42.05	8.60	4.89	0.08
IL304	9.00	1505.91	594.44	2.47	22.27	27.80	6.90	4.03	0.05
IL305	6.73	1524.31	631.92	2.40	22.33	33.67	8.87	3.80	0.07
IL306	7.27	1293.02	646.21	1.98	23.15	35.27	8.70	4.04	0.07
IL307	8.47	1077.5	481.64	2.30	25.05	29.33	8.23	3.57	0.06
IL308	7.27	1371.87	666.68	2.04	25.66	32.73	8.32	4.01	0.07
IL309	7.13	1172.12	516.79	2.30	27.21	34.27	8.97	3.81	0.08
IL311	8.47	1340.33	512.93	2.67	22.07	29.93	8.20	3.64	0.06
IL312	7.20	1190.52	561.6	2.20	23.58	30.93	8.90	3.46	0.07
IL313	5.13	1424.43	639.25	2.23	22.63	35.73	9.43	3.79	0.09
IL314	7.13	1235.19	557.35	2.27	23.15	33.00	8.87	3.72	0.07
IL315	6.47	1066.98	528.77	2.13	25.48	32.60	9.23	3.52	0.07
IL316	7.53	1421.81	587.1	2.43	21.65	31.57	8.97	3.51	0.07
IL317	7.90	1264.11	640.41	1.99	23.59	34.30	9.20	3.73	0.09
IL318	6.60	1224.68	604.1	2.05	20.37	32.73	9.13	3.58	0.08
IL319	6.93	1237.82	591.73	2.13	21.97	33.70	9.27	3.64	0.07
IL320	6.60	1158.97	657.8	1.80	23.04	30.60	8.50	3.63	0.06
IL321	6.73	1424.44	749.36	1.97	23.30	35.00	9.17	3.83	0.08
IL322	7.53	1697.78	755.15	2.26	24.81	32.53	9.03	3.59	0.07
IL323	5.65	1703.04	748.58	2.36	23.08	44.93	9.57	4.72	0.09
IL324	4.20	1463.86	677.5	2.25	23.38	22.40	6.73	3.32	0.06
IL325	6.47	1180	506.75	2.32	23.23	33.13	9.50	3.50	0.08
IL326	7.00	1198.4	568.56	2.16	19.99	41.12	8.72	4.71	0.07
IL328	7.27	1222.05	504.81	2.45	21.97	32.47	9.47	3.42	0.07
IL329	7.40	1411.3	566.24	2.60	22.10	32.20	8.80	3.67	0.07
IL330	7.80	1098.52	481.64	2.32	22.44	26.80	8.80	3.05	0.07
IL331	7.13	1198.4	624.96	1.99	24.14	29.93	8.61	3.54	0.07
IL332	8.47	1245.71	655.87	1.91	22.91	30.43	8.67	3.50	0.07
IL333	5.93	1308.79	725.4	1.84	23.59	32.33	8.07	4.09	0.07
IL334	6.60	1017.04	565.85	1.81	21.41	30.50	7.53	4.04	0.07

(E.2 continued)

Line	SIS	Na^+ (mmolkg^{-1})	K^+ (mmolkg^{-1})	NaK (ratio)	CHL (SPAD unit)	SHL (cm)	RTL (cm)	SRR (ratio)	DWT (g)
IL335	6.93	1082.75	561.22	1.95	22.27	32.30	9.85	3.28	0.07
IL336	7.40	1306.16	635.39	2.04	21.63	31.00	9.47	3.30	0.07
IL337	8.20	1416.55	702.61	2.00	22.31	26.93	8.10	3.33	0.06
IL338	6.57	1653.1	776.4	2.13	23.43	32.20	8.88	3.64	0.07
IL339	6.73	1495.4	736.61	2.01	22.23	32.00	8.33	3.84	0.07
IL340	6.12	1524.31	766.35	2.00	23.26	32.65	8.47	3.87	0.06
IL341	5.67	1668.87	813.49	2.10	21.33	32.93	9.90	3.34	0.07
IL342	6.20	1576.88	830.87	1.89	22.48	32.67	9.00	3.63	0.07
IL343	6.60	1390.27	684.45	2.07	21.73	32.20	9.60	3.38	0.07
IL344	5.93	1534.83	774.08	1.97	22.17	35.20	9.63	3.68	0.08
IL345	4.33	1482.26	761.33	1.99	21.11	36.07	9.67	3.73	0.10
IL346	7.13	1253.59	557.74	2.30	21.97	31.45	8.40	3.76	0.07
IL347	7.27	1269.36	559.67	2.30	22.59	30.73	10.07	3.06	0.07
IL348	6.17	1466.49	736.61	1.98	22.37	36.88	8.51	4.35	0.07
IL349	7.93	1348.21	556.58	2.45	21.71	30.47	8.40	3.62	0.06
IL350	7.00	1416.55	653.55	2.24	45.32	29.93	9.43	3.15	0.07
IL351	7.13	1088.01	510.22	2.16	22.75	31.93	9.13	3.54	0.07
IL352	6.87	1316.67	573.58	2.32	23.33	34.87	9.13	3.88	0.09
IL353	5.93	1011.79	462.71	2.25	23.32	32.15	8.43	3.83	0.07
IL354	5.67	1095.89	568.56	2.00	24.15	28.47	8.47	3.38	0.06
IL355	6.87	930.31	474.29	2.04	23.49	32.67	8.83	3.71	0.06
IL356	7.13	1371.87	621.87	2.30	20.93	34.67	8.87	3.90	0.07
IL357	6.73	1335.07	597.15	2.26	21.93	32.07	8.60	3.73	0.08
IL358	7.40	1045.96	451.12	2.35	23.34	31.87	8.83	3.60	0.07
IL359	6.87	1271.99	542.29	2.44	22.53	33.40	9.13	3.67	0.08
IL360	7.13	1406.04	670.16	2.12	22.63	31.20	8.60	3.63	0.07
IL361	7.20	1350.84	651.62	2.07	24.44	34.20	9.17	3.78	0.07
IL362	7.40	1416.55	712.27	1.99	21.54	33.53	8.43	3.99	0.06
IL363	7.00	1148.46	564.69	2.05	24.50	31.80	8.53	3.75	0.07
IL364	6.60	1253.59	579.38	2.25	23.45	32.00	8.37	3.82	0.07
IL365	7.40	1219.43	527.61	2.38	21.39	31.60	8.83	3.57	0.06
IL366	7.40	1327.19	605.26	2.23	22.49	32.40	9.17	3.55	0.07
IL367	6.47	1137.95	628.05	1.81	22.59	32.20	8.17	3.95	0.07
IL368	6.33	1095.89	560.44	1.93	25.05	32.00	9.53	3.36	0.08
IL369	7.60	1324.56	632.69	2.09	24.41	31.43	8.20	3.85	0.06
IL370	7.00	1571.62	680.98	2.31	21.18	33.13	8.37	3.97	0.06
IL371	5.67	1287.76	691.02	2.00	24.01	37.67	9.63	3.94	0.08
IL372	7.27	1311.42	658.95	2.24	23.61	31.27	9.13	3.44	0.07
IL310_1	8.03	1547.97	615.3	2.45	22.39	29.09	8.90	3.28	0.07
IL310_2	7.70	1243.08	525.29	2.40	21.36	34.35	9.00	3.82	0.07
BENGAL	7.80	1232.57	548.08	2.29	18.99	31.67	8.68	3.66	0.07
POKKALI	3.00	940.82	590.19	1.59	16.05	47.20	9.97	4.75	0.14

E.3 Genome statistics of 72 ILs covering the rice genome by SSR markers

Line	% recurrent genome	% donor genome	# of donor segments	# of chr. w/ segments	Chromosomes bearing segments
IL53	98.77	1.23	1	1	2
IL58	94.47	5.53	3	3	1, 2, 9
IL59	88.32	11.68	6	5	1, 2, 2, 5, 8, 9
IL61	94.49	4.55	3	3	3, 5, 7
IL62	96.69	3.31	3	3	3, 7, 11
IL65	95.37	4.63	4	4	1, 7, 8, 11
IL67	98.73	1.00	2	2	1, 5
IL69	93.48	6.52	3	3	2, 3, 8
IL70	95.61	4.39	3	3	2, 3, 8
IL75	96.38	2.06	3	3	2, 7, 11
IL77	94.47	5.53	3	2	3, 3, 9
IL84	86.19	13.81	8	7	1, 2, 3, 4, 6, 7, 9, 9
IL86	97.54	1.01	1	1	1
IL87	91.06	8.29	5	4	2, 2, 8, 9, 11
IL93	97.26	2.74	4	4	2, 4, 7, 8
IL95	97.19	2.81	2	2	10, 11
IL96	99.16	0.84	1	1	8
IL101	97.84	2.16	2	2	2, 5
IL102	91.90	8.10	6	4	2, 2, 4, 6, 10, 10
IL105	94.58	5.42	5	4	2, 4, 5, 12, 12
IL110	97.06	2.94	2	2	5, 6
IL111	95.71	4.29	4	4	2, 5, 11, 12
IL114	90.71	9.29	7	6	2, 3, 4, 6, 11, 12, 12
IL119	95.62	4.38	2	2	7, 9
IL126	96.87	2.42	3	3	3, 7, 9
IL130	91.84	8.16	6	4	1, 3, 3, 7, 8, 8
IL133	98.39	1.20	1	1	1
IL134	95.70	4.30	3	2	1, 9, 9
IL136	95.13	4.87	3	3	1, 10, 12
IL137	92.89	7.11	4	4	1, 2, 3, 4
IL138	96.03	2.73	3	3	4, 7, 8
IL144	99.00	1.00	1	1	5
IL152	98.71	1.29	1	1	9
IL153	93.16	6.84	2	2	7, 9
IL154	98.13	1.87	1	1	4
IL157	92.05	7.95	6	6	2, 4, 5, 8, 10, 11
IL162	96.46	2.78	2	2	3, 5
IL173	93.02	6.98	6	5	1, 2, 3, 3, 5, 10
IL175	91.02	8.98	4	4	2, 3, 4, 10
IL178	98.38	1.62	2	2	1, 4
IL184	91.90	6.98	4	3	1, 1, 9, 10
IL185	92.64	7.36	5	4	3, 5, 6, 10, 10
IL188	92.15	7.85	4	4	2, 4, 5, 7
IL194	95.41	4.59	2	2	3, 7
IL198	95.90	4.10	2	2	7, 9

(E.3 continued)

Line	% recurrent genome	% donor genome	# of donor segments	# of chr. w/ segments	Chromosomes bearing segments
IL201	95.81	2.64	1	1	11
IL205	93.05	6.20	3	3	2, 6, 9
IL206	94.83	3.96	2	2	1, 10
IL220	95.54	4.46	2	2	2, 5
IL222	99.06	0.94	1	1	7
IL224	96.65	3.14	4	4	1, 2, 3, 4
IL230	85.92	14.08	7	7	2, 4, 5, 6, 7, 9, 12
IL233	91.73	8.27	5	5	2, 4, 5, 6, 10
IL237	97.17	2.83	2	2	2, 4
IL240	97.44	2.56	1	1	2
IL247	98.68	1.32	1	1	3
IL260	98.22	1.78	1	1	3
IL264	99.19	0.81	1	1	8
IL271	97.40	2.60	2	2	8, 12
IL307	95.58	4.42	4	2	2, 2, 2, 11
IL312	99.25	0.75	1	1	6
IL313	92.92	7.08	3	3	5, 9, 10
IL331	93.77	6.23	2	2	4, 11
IL336	96.19	3.81	4	3	5, 6, 12, 12
IL339	96.18	3.48	3	3	2, 3, 9
IL340	91.25	8.48	5	5	1, 3, 5, 9, 12
IL342	87.89	10.13	7	7	1, 5, 6, 7, 8, 9, 12
IL347	94.38	5.62	3	3	1, 8, 10
IL348	96.87	3.13	4	4	1, 3, 7, 10
IL354	98.71	1.29	2	2	7, 11
IL362	94.45	5.55	2	2	1, 12
IL364	96.04	3.96	3	3	2, 4, 9
average:	95.34	4.43	2.8	2.7	
Max	99.25	14.08	7.00	7.00	
Min.	85.92	0.75	1.00	1.00	

E.4 Genome statistics of 88 ILs by SNP markers

Line	% recurrent genome	% donor genome	# of donor segments[1]	# of chr. w/ segments	Chromosomes bearing segments
IL51	92.29	7.13	60	5	1, 1, 7, 8, 10
IL52	94.71	5.29	50	11	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12
IL53	98.21	1.79	21	7	2, 4, 5, 6, 8, 9, 12
IL55	97.45	2.55	45	7	2, 3, 4, 5, 6, 8, 11
IL57	95.63	4.37	36	5	1, 3, 4, 8, 11
IL59	93.31	6.69	49	6	1, 2, 4, 5, 7, 8,
IL61	97.05	2.69	20	6	1, 2, 3, 5, 7, 8
IL63	95.75	4.25	28	6	1, 5, 8, 9, 10, 11

(E.4 continued)

Line	% recurrent genome	% donor genome	# of donor segments[1]	# of chr. w/ segments	Chromosomes bearing segments
IL64	94.73	4.94	31	8	1, 4, 6, 7, 8, 9, 10, 11
IL65	91.82	8.18	56	11	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
IL67	97.63	1.5	21	8	1, 2, 3, 4, 5, 7, 8, 11
IL68	98.55	1.45	21	7	1, 2, 3, 4, 5, 8, 10
IL70	94.91	5.09	36	8	1, 2, 3, 4, 5, 7, 8, 11
IL74	94.98	5.02	47	7	1, 4, 6, 8, 9, 10, 11
IL76	96.76	2.96	38	9	1, 3, 4, 5, 7, 8, 9, 10, 11
IL77	97.22	2.78	39	9	1, 2, 3, 5, 7, 8, 9, 10, 12
IL78	99.41	0.59	19	8	1, 2, 3, 4, 6, 8, 9, 11
IL83	93.32	6.68	36	7	1, 2, 3, 4, 5, 8, 9
IL84	90.98	9.02	70	11	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12
IL86	97.68	2.32	19	6	1, 3, 5, 8, 9, 11
IL89	95.23	4.77	46	7	1, 3, 5, 8, 9, 10, 11
IL90	98.06	1.81	19	10	2, 3, 4, 6, 7, 8, 10, 11, 12
IL91	94.12	5.88	42	9	1, 2, 3, 4, 5, 6, 8, 10, 11
IL92	93.19	6.48	42	8	1, 2, 3, 4, 7, 8, 9, 12
IL93	97.26	2.74	55	6	1, 2, 4, 5, 7, 8,
IL94	98.85	1.15	29	9	1, 2, 3, 4, 7, 8, 9, 10, 11
IL98	96.69	3.31	28	6	1, 3, 4, 7, 8, 9
IL99	95.95	3.72	30	9	1, 2, 4, 5, 6, 7, 8, 9, 10
IL106	97.07	2.93	143	1	8
IL107	97.26	2.74	29	9	1, 3, 4, 5, 6, 7, 8, 10, 11
IL111	95.54	4.46	24	9	2, 3, 4, 5, 6, 7, 8, 11, 12
IL116	97.51	2.49	27	4	1, 3, 9, 11
IL119	95.54	4.46	19	5	1, 4, 7, 8, 9
IL127	97	3	32	10	1, 2, 3, 4, 5, 6, 8, 9, 10, 12
IL129	97.45	2.28	43	11	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12
IL130	97.97	2.03	48	10	1, 2, 3, 4, 5, 7, 8, 9, 10, 11
IL131	98.45	1.55	24	6	1, 2 ,3, 4, 8, 9
IL134	95.83	4.17	41	9	1, 3, 4, 5, 6, 8, 9, 11, 12
IL137	96.47	3.28	48	8	1, 2, 3, 4, 6, 8, 9, 10
IL138	98.01	1.99	40	9	1, 3, 4, 5, 6, 7, 8, 9, 11
IL140	98.77	0.9	29	7	1, 2, 4, 6, 7, 8, 12
IL153	94.38	5.62	37	6	1, 2, 4, 6, 7, 8, 9,
IL158	97.49	2.51	25	8	1, 2, 4, 5, 6, 7, 8, 9
IL160	96.88	3.12	41	11	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
IL162	97.46	2	26	7	1, 3, 4, 5, 6, 8, 12
IL166	93.69	6.17	30	9	1, 3, 4, 6, 7, 8, 9, 11, 12
IL172	94.21	5.79	31	8	1, 3, 5, 6, 8, 10, 11, 12
IL174	97.95	2.05	25	6	1, 3, 4, 6, 8, 10
IL178	95.97	3.25	31	10	1, 2, 4, 5, 6, 7, 8, 9, 11, 12
IL185	93.25	6.37	28	9	1, 2, 4, 5, 6, 7, 8, 10, 11
IL186	90.79	9.21	39	9	1, 2, 3, 5, 6, 7, 8, 9, 11
IL188	92.01	7.99	58	7	1, 2, 4, 5, 7, 8, 9,
IL190	95.75	4.25	20	6	5, 6, 7, 8, 11, 12

(E.4 continued)

Line	% recurrent genome	% donor genome	# of donor segments[1]	# of chr. w/ segments	Chromosomes bearing segments
IL194	98.73	1.27	11	6	1, 3, 4, 5, 9, 11
IL197	98.7	1.3	29	5	3, 6, 7, 8, 9
IL198	96.63	3.37	27	7	1, 2, 3, 4, 6, 7, 9
IL199	93.79	6.21	72	9	1, 2, 3, 4, 6, 7, 8, 10, 11
IL206	95.23	4.77	32	7	1, 2, 4, 5, 7, 8, 10,
IL219	92.57	6.53	47	10	1, 2, 3, 4, 5, 7, 8, 9, 11, 12
IL230	87.4	12.46	56	10	1, 2, 4, 5, 6, 7, 8, 9, 10, 12
IL232	91.92	7.54	35	5	1, 4, 7, 8, 10
IL233	94.14	5.59	58	9	1, 2, 4, 5, 6, 8, 9, 10, 12
IL235	98.2	1.34	15	9	1, 2, 4, 5, 8, 9, 10, 11, 12
IL238	95.86	4.14	41	4	1, 2, 3, 4,
IL248	98.66	1.34	10	6	5, 6, 7, 8, 10, 11
IL253	96.94	3.06	35	6	1, 3, 4, 5, 8, 12
IL256	96.4	3.35	16	5	1, 7, 8, 10, 11
IL262	94.81	4.94	45	5	1, 3, 4, 8, 11
IL263	95.9	4.1	30	5	3, 4, 6, 8, 11
IL265	96.2	3.55	36	6	1, 2, 4, 5, 8, 11
IL266	90.94	9.06	63	9	1, 2, 4, 5, 6, 8, 9, 10, 12
IL267	91.65	8.08	55	10	1, 2, 3, 4, 5, 7, 8, 10, 11, 12
IL271	96.39	3.61	33	5	1, 3, 5, 8,12
IL303	91.07	8.93	46	10	1, 3, 4, 5, 6, 7, 8, 9, 11, 12
IL307	96.88	2.84	20	10	1, 2, 3, 4, 6, 7, 8, 9, 10, 11
IL311	98.07	1.93	26	5	1, 3, 4, 8, 11
IL313	95.7	4.3	32	5	1, 5, 8, 9, 10
IL323	98.96	1.04	16	4	1, 5, 8, 9
IL332	98.23	1.77	22	7	1, 3, 5, 7, 8, 10, 11
IL336	96.62	3.38	39	8	1, 2, 3, 5, 6, 8, 9, 12
IL340	92.47	7.53	44	6	1, 3, 5, 8, 9, 12
IL346	97	3	34	3	1, 3, 8,
IL348	93.47	6.26	51	8	1, 2, 3, 5, 7, 8, 9, 10
IL350	98.38	1.62	18	4	1, 4, 8, 11
IL352	96.89	3.11	45	6	1, 2, 4, 7, 8, 9
IL353	92.06	7.67	57	10	1, 2, 3, 4, 5, 6, 7, 8, 10, 11,
IL354	95.52	4.48	25	6	3, 4, 7, 8, 11, 12
IL366	99.82	0.18	6	5	1, 2, 5, 7, 8
average	95.25	4.62	34.63	7	
Min	87.4	0.18	6	3	
Max	99.82	12.46	72	10	

[1]number of SNPs having Pokkali allele out of 6797SNP markers.

E.5 Phenotypic attributes and genome composition of tolerant ILs.

	Mean phenotypic value under salt stress EC12 dSm^{-1}									Line statistics based on 107 SSR markers					Line statistics based on 6797 SNP markers				
BP IL	SIS	Na^+ (mmol kg^{-1})	K^+ (mmol kg^{-1})	NaK (ratio)	CHL (spad unit)	SHL (cm)	RTL (cm)	SRR	DWT (g)	# of donor segments	# of chr. with segments	% recurrent genome	% donor genome	Chromosomes bearing segments	# of donor segments	# of chr. w/ segments	% recurrent genome	% donor genome	Chromosomes bearing segments
84	3.2	943	599	1.7	21.7	50.3	9.8	5.1	0.133	8	7	86.19	13.81	1, 2, 3, 4, 6, 7, 9	70	11	90.98	9.02	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12
230	4.1	1264	601	2.1	25.3	37.7	9.7	3.9	0.111	7	7	85.92	14.08	2, 4, 5, 6, 7, 9, 12	56	10	87.40	12.46	1, 2, 4, 5, 6, 7, 8, 9, 10, 12
119	4.3	1151	426	2.8	22.6	30.7	9.0	3.5	0.075	2	2	95.62	4.38	7, 9	19	5	95.54	4.46	1, 4, 7, 8, 9
91	4.3	1240	412	3.0	21.8	34.5	7.9	4.4	0.097	6	5	92.48	7.52	3, 5, 6, 8, 11,	42	9	94.12	5.88	1, 2, 3, 4, 5, 6, 8, 10, 11,
99	4.4	1062	549	1.9	19.7	33.8	9.3	3.8	0.082	2	2	96.19	3.81	5, 9	30	9	95.95	3.72	1, 2, 4, 5, 6, 7, 8, 9, 10
116	4.4	1185	443	2.7	21.2	33.9	9.3	3.7	0.091	1	1	95.90	4.10	9	27	4	97.51	2.49	1, 3, 9, 11
68	4.5	1167	445	2.7	20.3	36.0	8.3	4.4	0.096	4	4	97.39	2.33	1, 3, 5, 10	21	7	98.55	1.45	1, 2, 3, 4, 5, 8, 10
93	4.5	1254	477	2.6	21.2	37.8	9.9	3.9	0.097	4	4	97.26	2.74	2, 4, 7, 8	55	6	97.26	2.74	1, 2, 4, 5, 7, 8, 9, 11, 12
129	4.7	1309	468	2.8	17.0	35.1	8.3	4.3	0.079	5	4	96.38	3.62	1, 4, 7, 11	43	11	97.45	2.28	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12
78	4.8	1104	504	2.2	19.3	34.5	8.6	4.0	0.073	3	3	94.32	5.68	3, 8, 9	19	8	99.41	0.59	1, 2, 3, 4, 6, 8, 9, 11
67	5.0	1148	497	2.4	21.5	35.0	9.0	3.9	0.102	2	2	98.73	1.00	1, 5	21	8	97.63	1.50	1, 2, 3, 4, 5, 7, 8, 11
61	5.1	1022	453	2.3	20.6	31.8	8.7	3.7	0.084	3	3	94.49	4.55	3, 5, 7	20	6	97.05	2.69	1, 2, 3, 5, 7, 8
313	5.1	1424	639	2.2	22.6	35.7	9.4	3.8	0.091	3	3	92.92	7.08	5, 9, 10	32	5	95.70	4.30	1, 5, 8, 9, 10,
65	5.1	1198	475	2.7	19.5	37.0	9.3	4.0	0.102	4	4	95.37	4.63	1, 7, 8, 11	56	11	91.82	8.18	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,
130	5.2	1298	456	3.0	19.8	32.9	8.6	3.8	0.073	6	4	91.84	8.16	1, 3, 7, 8	48	10	97.97	2.03	1, 2, 3, 4, 5, 7, 8, 9, 10, 11
57	5.2	1112	443	2.6	18.5	30.9	7.1	4.3	0.083	1	1	97.36	2.64	11	36	5	95.63	4.37	1, 3, 4, 8, 11
Bengal	7.8	1233	548	2.3	19.0	31.7	8.7	3.7	0.071										
Pokkali	3	941	590	1.6	16.1	47.2	10.0	4.8	0.141										

VITA

Teresa Bermejo De Leon was born in the Philippines. She attended the University of the Philippines Los Baños, where she completed her Bachelor of Science in Biology within three and half years. Upon graduation, she was hired immediately to work on generation of transgenic banana and papaya for virus resistance. Later on, she received a scholarship at the International Rice Research Institute (IRRI) and worked on microarray and candidate gene expression studies for tungro resistance in rice. She received her Master of Science in Molecular Biology and Biotechnology at the same university in June 2007. She also worked shortly on phosphorus deficiency tolerance in rice. From February of 2008 to July of 2011, she worked as a Research Associate under the supervision of Dr. Prasanta K. Subudhi at the LSU AgCenter to work on QTL mapping of rice seed dormancy, shattering, and other agronomic traits. In January 2012, she was admitted into the doctoral degree program in the School of Plant, Environmental and Soil Sciences at Louisiana State University Agricultural and Mechanical College. She has since then worked on molecular genetics of salinity tolerance in rice under the guidance of Dr. Subudhi for the improvement of Louisiana rice varieties.