IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR SALINITY TOLERANCE IN RICE (*Oryza sativa* L.) USING IR29/HASAWI MAPPING POPULATION

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ABSTRACT

Thirty days old rice seedlings of 300 $F_{5:6}$ recombinant inbred lines (RILs) derived from a cross between a salt sensitive, IR29 (*indica*), and a salt tolerant, Hasawi (*indica*), were evaluated for salinity tolerance and thereafter QTLs linked to salinity tolerance were identified. Large variation in salinity tolerance among the RILs was detected. Final salinity injury scores ranged from highly tolerant to highly sensitive with a transgressive segregation towards sensitive parent. However, for the other studied parameters, RILs showed transgressive segregation on both parental sides. One hundred ninety four polymorphic SNP markers were used to construct a genetic linkage map involving 142 sorted RILs based on final salt injury scores. The SNPs covered 1441.96 cM genome with an average distance of 7.88 cM between loci. Twenty QTLs (LOD>3)were identified on chromosome 1, 2, 4, 6, 8, 9, and 12 using composite interval mapping for eight studied traits in salinized (EC 12 dSm^{-1}) Yoshida nutrient culture solution following IRRI standard protocol. Two QTLs were located on chromosome 1; three QTLs on chromosome 2; three QTLs on chromosome 4; three QTLs on chromosome 6; one QTL on chromosome 8, two on chromosome 9, and six QTLs on chromosome 12. The QTLs identified on chromosome 1 (*qSL1.1*) were located on long arm of the chromosome 1, which is a very different position from *Saltol* locus. This was the major QTL explaining 20.6% of the total phenotypic variation for shoot length. Five out of six QTLs identified on chromosome 12 were located at 6.9 cM position. Fine mapping of these novel QTLs in a different genetic background is suggested. Novel QTLs could be useful to enhance the level of tolerance through MAS for the pyramiding of different QTLs in one background.

DECLARATION

I, Jean Berchmans Bizimana, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any institution.

_____ _____

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The above declaration is confirmed by

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To my lovely wife, Virginie Nahishakiye, to my gorgeous sons, Johny Stephy Gateka and Aubin Becky Himbaza, This Master’s dissertation is dedicated.

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LIST OF ABBREVIATIONSAND SYMBOLS

%	Percentage
% Red RDW	Percentage reduction of root dry weight
% Red RFW	Percentage reduction of root fresh weight
% Red RL	Percentage reduction of root length
% Red SDW	Percentage reduction of shoot dry weight
% Red SFW	Percentage reduction of shoot fresh weight
% Red SHL	Percentage reduction of shoot length
⊗	Self-pollination
$(NH_4)Mo_7O_{24}.4H_2O$	Ammonium molybdate, 4-hydrate
< -	Less than
> -	Greater than
°C	degree Celsius
A	Adenine
ACPFG	Australian Centre for Plant Functional Genomics
AFLPs	Amplified Fragment Length Polymorphisms
ANOVA	Analysis of variance
BC	Backcross
BC_1	First backcross generation
BC_1F_2	Second backcross generation after selfing
BecA	Biosciences eastern and Central Africa
bp	Base pair
C	Cytosine
$C_{10}H_{12}FeN_2NaO_8Na_2.H_2O$	Ethylenediaminetetraacetic acid iron (III)
$CaCl_2.2H_2O$	Calcium chloride dihydrate
CAPS	Cleaved Amplified Polymorphic Sequences

Chr.	Chromosome number
Chr1	Chromosome 1
Chr12:	Chromosome 12.
CIM	Composite interval mapping
Cl^-	Chloride anion
cm	centimetre
cM	centiMorgan
CTAB	Cetyl Trimethyl Ammonium Bromide
$CuSO_4.5H_2O$	Cupric sulphate,5-hydrate
CV	Coefficient of variation
DArTs	Diversity Arrays Technology
DH	Double haploids
DNA	Deoxyribonucleic acid
DPE	Direction of Phenotypic Effect
dSm^{-1}	deciSiemens per meter
e.g.	for example
EC	Electrical conductivity
ECe	Electrical conductivityof saturated extract
EDTA	Ethylenediaminetetraacetate
E-QTLs	Epistatic QTLs
ESP	Exchangeable Sodium Percentage
ESTs	Expressed Sequence Tags
EtOH	Ethanol
F_1	First filial generation
F_{18-19}	Eighteenth filial generation advanced to nineteenth generation

F_2	Second filial generation
F_3	Third filial generation
F_4	Fourth filial generation
F_5	Fifth filial generation
$F_{5:6}$	Fifth filial generation advanced to sixth filial generation
F_6	Sixth filial generation
F_8	Eighth filial generation
FAO	Food and Agriculture Organization
g	Gram
G	Guanine
H	High
H_3BO	Boric acid
ha	Hectare
HCl	Hydrogen Chloride
i.e.	That is
ILRI	International Livestock Research Institute
IRRI	International Rice Research Institute
ISSR	Inter-simple sequence repeat
ISSRs	Inter-Simple Sequence Repeats
K^+	Potassium cation
K_2HPO	Potassium dihydrogen phosphate dibase
K_2SO_4	Potassium sulphate
kb	kilo base
kg ha^{-1}	kilogram per hectare
KH_2PO_4	Potassium dihydrogen orthophosphate
L	Low

LOD	Logarithm of the odds (LOD=LRS/4.6)
LRS	Likelihood ratio statistic
$LSD_{0.05}$	Least significant difference at 5% level of probability
M	Medium
MABC	Marker-assisted backcrossing
MAS	Marker Assisted Selection
Max	Maximum
Mb	Mega base
mg	milligram
mg kg^{-1}	milligram per kilogram
$MgSO_4.7H_2O$	Magnesium sulphate,7-hydrate
Min	Minimum
min	minute
ml	milliliter
mm	millimetre
mM	millimolar
MMAL	Molecular Marker Applications Laboratory
$MnCl_2.4H_2O$	Manganous chloride, 4-hydrate
MPa	Megapascal
M-QTLs	Main-effect QTLs
n-	nano-(10^{-9})
N	No
n.s.	no significant difference
Na^+	Sodium cation
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide

ng µl-1	nanogram per microliter
NH_4NO_3	Ammonium nitrate
^oC	degree Celcius
OD	Optical density
OPA	Oligo Pool All
P_1	Parent 1
P_2	Parent 2
PCR	Polymerase Chain Reaction
pH	Hydrogen ion concentration
Pr.	Probability
PVE	Phenotypic variation explained
QTLs	Quantitative Traits Loci
r	Correlation coefficient
r.p.m.	Revolutions per minute
R^2 -	Coefficient of determination
RAPDs	Randomly Amplified Polymorphic DNAs
RCBD	Randomized Complete Block Design
RDW	Root dry weight
RFLPs	Restriction Fragment Length Polymorphisms
RFW	Root fresh weight
RH	Relative humidity
RILs	Recombinant inbred lines
RL	Root length
RNA	Ribonucleic acid
RNase	Ribonuclease
SAR	Sodium adsorption ratio

SCAR	Sequence characterized amplified region
SDW	Shoot dry weight
SESF	Final salinity injury score
SESI	Fnitial salinity injury score
SFW	Shoot fresh weight
SL	Shoot length
SNP	Single Nucleotide Polymorphisms
SPS	Single plant selection
SPSS	Statistical Package for the Social Sciences
SSR	Simple Sequence Repeat (microsatellite)
STMS	Sequence tagged microsatellite
STR	Short-tandem repeats
SUA	Sokoine University of Agriculture
T	Thymine
T°	Temperature
TBE	Tris-borate-EDTA
TE	Tris-EDTA
Tris	Tris(hydroxymethyl)amino-methane
V	volts
VNTR	Variation in the number of tandem-repeat unit
Y	Yes
$ZnSO_4.7H_2O$	Zinc sulphate, 7-hydrate
µ	micro-(x10-6)
µg	microgram(s) = 10-6 gram
µl	microlitre(s) = 10-6 litre

CHAPTER ONE

1.0 INTRODUCTION

1. 1 Background Information

Rice (*Oryza sativa* L.) is one of the agronomically and nutritionally important cereal crops. It is a major source of food and energy for more than 2.7 billion people on a daily basis and is planted on about one-tenth of the earth's arable land. It is the single largest source of food energy to half of humanity; it is the staple food for more than half of the world's population, most of them in the developing countries. By the year 2025, 21% increase in rice production will be needed over that of year 2000 (Bhowmik*et al*., 2009; Ammar *et al*., 2007). Rice accounts for about 20% of the world's total grain production, second only to wheat (Acquaah, 2007). It is grown on 160.7 million hectares with a total production of 697.9 million tons giving an average productivity of 4.3 tons per hectare (FAO, 2010). Besides its economic significance, rice is rich in genetic diversity in the form of thousands of landraces and progenitor species (Ammar *et al*., 2007).

Despite its importance, a series of biotic and abiotic stresses limits its productivity worldwide, among which abiotic stress alone contributes to 50% of the total yield losses. Soil salinity is considered one of the major and widespread abiotic stresses limiting rice production in many rice-growing areas. Salt-affected areas can be found worldwide under almost all climatic conditions (Abrol *et al*., 1988; Munns and Tester, 2008), not only in arid and semi-arid regions, but also in sub-humid and humid regions. Salinity is particularly a major problem in coastal regions in the tropics where rice-based farming systems predominate. This is because of the intrusion of brackish water during the dry season and at the start of the wet season. Salt stress is also a worsening problem in inland areas because of the buildup of salinity as a consequence of excessive use of irrigation

water with improper drainage coupled with the use of poor quality irrigation water (Thomson *et al.,* 2010).

1. 2 Problem Statement and Justification

Salinity is one of the most serious biophysical constraints of rice production in many rice-producing areas of the world (Munns and Tester, 2008). At the present, salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increased rice production worldwide (Mohammadi-Nejad *et al*., 2010). Over 800 million hectares of land throughout the world are salt-affected, either by salinity (397 million ha) or the associated condition of sodicity (434 million ha). This is over 6% of the world's total land area. In Africa, out of 1899million hectares, 39 and 34 million hectares are saline and sodic soils respectively (Ahmed *et al*., 2010; Yadav *et al*., 2011).

Worldwide, the research to overcome problems related to the salt is based on three approaches; (a) either change the growing environment (make it normal) suitable for the normal growth of plants i.e "better soils for the crops we have"; (b) select the crop and/or change genetic architecture of the plant so that it could be grown in such areas i.e "better crops for the soil we have";(c) or hybrid approach. The first approach requires major engineering structures and provision of fresh water irrigation, though, it is very expensive and difficult to implement. The second approach i.e. breeding for salt tolerance offers more promising, energy saving, cost effective, and socially acceptable approach (Sharma and Gupta, 1986; Ray and Islam, 2008). The third one is the combination of environment modification and plant based approach.

Rice yield is a very complex character comprising of yield many components. These yield components are related to final grain yield which are also severely affected by salinity. Plant height, total number of tillers, panicle length, grain weight per panicle,

1000seedsweight, quality and quantity of grains decrease progressively with increase in salinity levels (Islam *et al.*, 2007).Tolerance to salinity is genetically and physiologically complicated and inherited quantitatively since it is known that the salt tolerance in rice is controlled by many genes known as quantitative traits (also “polygenic”, “multifactorial” or “complex” traits). These genes have additive and dominant effects (Baby *et al*., 2010). The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs) (Collard *et al*., 2005). Salt tolerant related traits are complex and to facilitate the development of new varieties with a high level of salinity tolerance, it is required to clearly understand the genetic control mechanisms for salt tolerance.

Breeding for salt tolerance requires the appropriate identification of QTLs which is not possible by using conventional phenotypic evaluation; but only the molecular markers could be used to tag quantitative trait loci and to evaluate their contributions to the phenotype (salt tolerance) by selecting for favorable alleles at these loci (Sabouri and Sabouri, 2008; Thomson, 2009).

A major quantitative trait locus (QTL) for salt tolerance named *Saltol* was mapped on short arm of chromosome 1between 14.7-18.6cM(Gregorio, 1997, Thomson, 2009; Singh *et al*.,2007)using F_8 recombinant inbred lines (RILs) of Pokkali/IR29 cross, which is responsible for low Na^+, high K^+ uptake and maintaining Na^+/K^+ homeostasis in the rice shoots. As many as thirty three polymorphic SSR markers located on short arm of chromosome 1 were also used to determine the impact of QTLs associated with salt tolerance in rice (Mohammadi-Nejad *et al*., 2008).The *Saltol*, a major QTL on chromosome1 reported to confer salinity tolerance at seedling stage in rice but not providing enough tolerance.

1. 3 Objectives

1. 3. 1 Overall objective

The aim of this research was to identify the novel big-effect QTLs from the novel source of salt-tolerance that could be further used for pyramiding of different QTLs to enhance the level of salt tolerance and for the value addition in selection of the adapted rice varieties in the region through marker assisted backcrossing (MABC) selection.

1. 3. 2 Specific objectives

The specific objectives of the study were:

i. To survey polymorphic single nucleotide polymorphic (SNP)markers between IR29 and Hasawi across the rice genome;
ii. To screen the $F_{5:6}$ RILs population derived from cross IR29/Hasawi for salinity tolerance at seedling stage;
iii. To genotype the F_5 plants using genome wide polymorphic SNP markers;
iv. To construct the linkage map and identify the big effect putative QTLs responsible for salinity tolerance.

CHAPTER TWO

2.0 LITERATURE REVIEW

2. 1 Description of Rice

Rice (*Oryza sativa* L.) is an "autogamous" crop in grass family, distributed throughout the tropics and subtropics but, grown more easily in the tropics (Acquaah, 2007). To-date, six basic genome sets, AA, BB, CC, EE, FF and GG, and four genomic combinations, BBCC, CCDD, HHJJ and HHKK, have been identified and designated in diploid and tetraploid species of *Oryza*, respectively.There is ample polymorphism in rice DNA compared to other plants. One centimorgan (cM) of rice equals approximately 250 kb, compared to more than 500 kb in tomato, 750 kb in potato, 3200 Mb in maize and 17 200 Mb in wheat (Bennet and Smith, 1976; Tanksley *et al.*, 1989; Sasaki *et al*., 2001).The rice species are either diploid with 2n=24 chromosomes or tetraploids with 2n=2x=48 chromosomes with basic chromosome number n=12 and has a total length of 382.17Mb corresponding to about 1500cM with predicted 40000 to 50 000 genes (Kurata *et al*., 2002; Zhou *et al*., 2007).

The genus consists of 22 wild and weedy species and two cultivated species (24 species in total), *viz*., the Asian *O. sativa* L. and the African *O. glaberrima* Steud. The *O. sativa*, domesticated in Asia has now spread to all the rice growing areas of the world, while *O. glaberrima*, first domesticated in the River Niger Bassin is confined to western tropical Africa zone alone (Li *et al*., 2001; Kshirod, 2010). Of the 22 wild and weedy species, six are in the primary gene pool of *O. sativa* complex and these wild species are easily crossable with the major cultivated species. These have the same AA genome as *O. sativa* and *O. glaberrima*. However, there are ten wild species under *O. officinalis* complex having BB, CC, BBCC, CCDD, EE and FF genomes. The wild species of this complex are in the secondary gene pool and are cross incompatible with *O. sativa*. There are six most

distantly related wild species with either diploids or tetraploids of GG, HHJJ and HHKK genomes and are highly cross incompatible with *O. sativa* and constitute the tertiary gene pool.

All the 22 wild species of *Oryza* are a vast reservoir of valuable genes of resistance for biotic and abiotic stresses (Randhawa *et al.*, 2006; Kshirod, 2010). Cultivars of common rice (*O. sativa*) are divided into three eco-geographical races: indica, javanica, and sinica (or japonica) (Matsuo *et al*., 1997).

2. 2 Salt-affected Soils and Classification

In general, the term salinity includes all the problems due to salts present in the soil however in strict sense, salinity is because of the presence of excessive amounts of soluble salt that hinders or affects the normal function needs for plant growth. It is measured in terms of electric conductivity (EC). Another kind of salt stress is called sodicity or alkalinity which is due to excessive carbonates and bicarbonates of sodium ion present on the colloidal clay complex of soil. Sodicity is measured in terms of the exchangeable sodium percentage (ESP) or sodium absorption ratio (SAR) and pH of saturated soil paste extract. Sodium is a dominant cation of the salt-affected soils presentin both major soil categories i.e. sodic (or alkali) and saline. A third type of salt-stress is also found in some affected areas and called saline-sodic soils. The major differences between these two types of salinity are the nature of anions and the pH of the soil (Flowers and Flowers, 2005; IRRI, 2006; Munns and Tester, 2008; Baby *et al*., 2010).

2. 2. 1 Saline soils

Saline soils occur in arid regions, estuaries, and coastal fringes. Saline soils are again dominated by sodium cations with electrical conductivity of saturated extract (ECe) >4 dSm^{-1} which is equivalent to approximately 40mM NaCl and generates an osmotic pressure of approximately 0.2 MPa; but the dominant ions are usually soluble chloride and

sulphate. Exchangeable sodium percentage (ESP) < 15 and pH values of these soils are much lower than in sodic soils (pH<8.5)(Flowers and Flowers, 2005; Fairhurst*et al*., 2007; IRRI, 2006; Munns and Tester, 2008; Baby *et al*., 2010).Based on ECe, soils could be classified as non-saline (0–1.5 dSm^{-1}), slightly saline(1.5 to 2 dSm^{-1}), moderately saline (2 to 6 dSm^{-1}), strongly saline (6 to 15dSm^{-1}), and very strongly saline (>15dSm^{-1}) (Yadav *et al*., 2011).

2. 2. 2 Sodic soils or alkaline soils

Sodic or alkaline soils are widely distributed in arid and semi-arid regions. They have high concentrations of free carbonate and bicarbonate and excess of sodium on the exchangeable site of clay particles. They show the deficiency of nitrogen, phosphorus and zinc due to high pH. In such soils, ECe is less than 4, the pH is greater than 8.5 and sometimes up to 10.7; the ESP is greater than 15% with poor soil structure. Clay fraction and organic matter are dispersed, thus soils are sticky when wet and hard when dry. There is high impedance to root growth due to very poor hydraulic conductivity (IRRI, 2006; Fairhurst *et al*., 2007).

Saline-sodic soils are common in arid and semi-arid regions and are intermediate type of salt-affected soils with an ECe greater than 4 dSm^{-1}, ESP greater than 15% and pH more than 8.5 (Flowers and Flowers, 2005; Fairhurst *et al*., 2007; Munns and Tester, 2008; Baby *et al*., 2010).

2. 2. 3 Origin of salts

The main source of all salts in the soil is the primary minerals in the exposed layer of the earth's crust (Abrol *et al*., 1988).Most of this salt-affected land has arisen from natural causes, from the accumulation of salts over long periods of time in arid and semi-arid zones (Yadav *et al*., 2011). During the process of chemical weathering ofparental rocks which involves hydrolysis, hydration, solution, oxidation, carbonation and other

processes, the salt constituents are gradually released and made soluble. Various types of soluble salts released such as chlorides of sodium (mainly), calcium, magnesium, and to a lesser extent, sulphates and carbonates (Abrol *et al*., 1988; Flowers and Flowers, 2005; Fairhurst *et al*., 2007; Munns and Tester, 2008).The other cause of accumulation is the deposition of oceanic salts carried in wind and rain. Rainwater contains6 to 50 $mgkg^{-1}$ of sodium chloride; the concentration decreases with distance from the coast. Rain containing 10 mg $kg^{-1}$of sodium chloride would deposit 10 kg ha^{-1} of salt for each100 mm of rainfall per year (Fairhurst *et al*., 2007; Munns and Tester, 2008).

Apart from natural salinity, a significant proportion of recently cultivated agricultural land has become saline owing to land clearing and/or irrigation, both of which cause water tables to rise and concentrate the salts in the root zone (Munns and Tester, 2008).Indeed, if a significant amounts of water are provided by irrigation, with no adequate provision of drainage for the leaching and removal of salts, resulting in the soils becoming salty and unproductive. They also concentrate ions toxic to plants and may degrade the soil structure (FAO, 2005; Fairhurst *et al*., 2007).

2. 3 Salt Stresses on Rice Crop

Extreme high salt stress conditions kills the plant but the moderate to low salt stress affect the plant growth rate and thereby manifest symptoms which could be associated with morphological, physiological or biochemical alterations (IRRI,2006).

2. 3. 1 Morphological effects

Salinity affects the growth of rice plant at all stages of its life cycle. But, several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2 to 3 leaf stage), gains tolerance during vegetative growth stage, becomes

sensitive during reproductive stage (panicle initiation, anthesis, and fertilization), and then becomes increasingly more tolerant at maturity.

Morphological symptoms are physical indications of the injurious salt of salt stress. The extent of which can be known by making critical comparison with plants growing under comparable conditions (normal versus salt stressed). Salt stressed plants appear to be stunted. Salinity may directly or indirectly inhibit cell division and may cause enlargement at the plant's growing point. Reduced shoot growth, which starts from the growing tissues, is the reason why leaves and stems of salt stressed plants appear stunted (Fairhurst *et al*., 2007; Singh *et al*., 2010; Singh and Flowers, 2010).

Most of the parameters like low tillering, spikelet sterility, less florets per panicle, low 1000 grain weight and quality, and leaf scorching, are affected uniformly under both sodicity and salinity; however it is not a rule of thumb. Major symptoms are:(a)white leaf tip followed by tip burning (salinity), (b)leaf browning and death (sodicity), (c)stunted plant growth, (d) low tillering, (e) spikelet sterility, (f) low harvest index, (g) less florets per panicle, (h) less 1000 grain weight, (i) low grain yield, (j)change in flowering duration, (k) leaf rolling, (l) white leaf blotches, (m) poor root growth, and (n) patchy growth in field (Gregorio *et al*., 1997;IRRI,2006; Fairhurst *et al*., 2007; Islam *et al*., 2007; Bhowmik *et al*., 2007).

2. 3. 2 Physiological and biochemical effects

High salt stress disrupts homeostasis in water potential and ion distribution; which lead to physiological disruption, growth arrest and even death of plants. The rate at which new leaves are produced depends largely on the water potential of the soil solution, in the same way as for drought stressed plants. Under salt stress, there is a change in the pattern of gene expression, and both qualitative and quantitative changes in protein synthesis.

Salinity changes the levels of plant hormones, such as abscisic acid and cytokinin (Moorby and Besford, 1983). It has been suggested that salt affects cellular and nuclear volume; induces endopolyploidy, and induces nucleic acid and protein synthesis. Several steps involved in protein synthesis are very sensitive to changes in ionic environment and may result in impairment of protein metabolism (IRRI, 2006).

2. 4 Salt Tolerance Mechanisms

Salt effects are the combined result of the complex interaction among different morphological, physiological and biochemical processes. Under low salinity, tolerance in plant can be due to controlled salt uptake.Plants have evolved three main mechanisms to enable them to tolerate salinity stress.

2. 4. 1 Osmotic tolerance

Osmotic tolerance involves the plant's ability to tolerate the drought aspect of salinity stress and to maintain leaf expansion and stomatal conductance (Rajendran *et al*., 2009), but the resulting increased leaf area would benefit only plants that have sufficient soil water (Munns and Tester, 2008).The osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure. Greater leaf area expansion would be productive when a supply of water is ensured such as in irrigated food production systems, but could be undesirable in water-limited systems, and cause the soil water to be used up before the grain is fully matured (Munns and Tester, 2008; Rajendran*et al*.,2009).

2. 4. 2 Sodium ion (Na^+) exclusion

Reducing Na^+ accumulation in the shoot by manipulating root Na^+ transport processes to minimize Na^+ delivery to the shoot. Na^+ exclusion by roots ensures that Na does not accumulate to toxic concentrations within leaves. A failure in Na^+ exclusion manifests its

toxic effect after days or weeks, depending on the species, and causes premature death of older leaves(Munns and Tester, 2008; Singh *et al*., 2010;ACPFG, 2010).

2. 4. 3 Sodium ion (Na^+) tissue tolerance

Tolerating Na^+ or in some species Cl^-that builds up in the leaf by compartmentalizing them into organelles within a cell (increased Na^+ sequestration into vacuoles). This avoids toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf. Toxicity occurs with time, after leaf Na^+ increases to high concentrations in the older leaves (Munns and Tester, 2008; ACPFG, 2010).

Therefore, a good breeding strategy for salinity tolerance in rice pass through the pyramiding of different salt tolerance mechanisms such as a limited daily Na^+ uptake, big capacity of tonoplasts to hold more toxic ions like Na^+ and high uptake of balancing ion K^+(Singh *et al*., 2010).

2. 5 Mapping Population

Segregating populations such as F_2, F_3 or backcross (BC) populations are frequently used as mapping populations. However, populations that can be maintained and produced permanently, such as recombinant inbred lines (RILs) and doubled haploids (DH), are preferable because they allow replicated and repeated experiments. These types of populations may not be applicable to outbreeding cereals where inbreeding depression can cause non-random changes in gene frequency and loss of vigour of the lines (Collardand Mackill, 2007).

The construction of a linkage map in plants requires a segregating population (i.e. a population derived from sexual reproduction). The parents selected for the mapping population will differ for one or more traits of interest. Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals, however

larger populations are required for high-resolution mapping. The scheme of how different mapping populations are produced is shown in Fig.1 (Collard *et al*., 2005). The RIL and DH populations are more advantageous since they produce homozygous or "true-breeding" lines that can be multiplied and reproduced without genetic change occurring. This allows for the conduct of replicated trials across different locations and years. Thus, both RIL and DH populations represent "eternal" resources for QTL mapping (Collard *et al.,* 2005). By emphasizing on RILs, they are developed by single plant selection (SPS) from individual plants of F_2 population either by selfing or sibling mating. Single plant selection is repeated for several generations. Importantly, each of the lines is fixed for many recombination events; thereby they contain the segregation adequately fixed to maximum homozygosity. Because RILs are essentially homozygous, only additive gene action can be measured (Vinod, 2006).

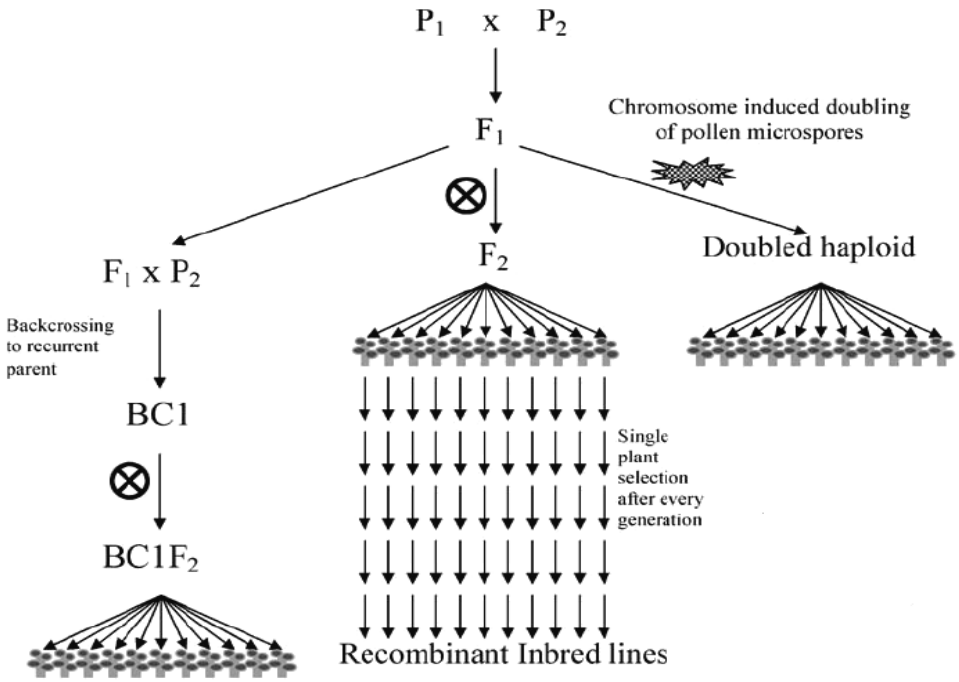

Figure 1: Diagram of main types of mapping populations for self-pollinating species

P_1: parent 1 used in crossing, P_2: parent 2 used in crossing, F_1: first filial generation, X: crossed with, F_2: second filial generation, BC_1: first backcross generation, BC_1F_2: second backcross generation after selfing, ⊗: self-pollination.

2. 6 Overview of Markers, QTL Mapping and Marker-Assisted Selection

A marker is a defined measurable characteristic, “signpost” that can be used to identify something or indicate the presence of something that is not directly measured. There are three major types of markers: (a) morphological markers, (b) biochemical markers, and (c) DNA markers.

2. 6. 1 Morphological markers

Morphological (also “classical” or “visible”) markers are phenotypic traits or characters. Morphological markers are usually visually characterized phenotypic characters such as flower colour, seed shape, growth habits or pigmentation (Collard *et al*., 2005). These are the traditional markers and, unfortunately, first these types of markers are highly dependent on environmental factors, secondly they have undesirable features such as dwarfism or albinism, and lastly performing breeding experiments with the morphological markers is time consuming, labour intensive and requires a large populations of plants and would need large plots of land and/or greenhouse space in which to be grown.

2. 6. 2 Biochemical markers

Biochemical markers, including allelic variants of enzymes called isozymes, are superior to morphological markers in that they are generally independent of environmental growth conditions. The only problem with isozymes in MAS is that most cultivars (commercial breeds of plants) are genetically very similar and isozymes do not produce a great amount of polymorphism and polymorphism in the protein primary structure may still cause an alteration in protein function or expression(Collard *et al*., 2005; Baby *et al*., 2010; Sabina *et al*., 2010).

2. 6. 3 DNA markers

Also known as molecular markers, reveal sites of variation in DNA. The DNA marker systems, which were introduced to genetic analysis in the 1980s, have many advantages over the traditional morphological and protein markers that are used in genetic and ecological analyses of plant populations. Firstly, an unlimited number of DNA markers can be generated; secondly, DNA marker profiles are not affected by the environment, and, thirdly DNA markers, unlike isozyme markers, are not constrained by tissue or developmental stage specificity (Park *et al*., 2009).

Different types of molecular markers have been developed and evolved, including, but not limited, to Restriction Fragment Length Polymorphism (RFLP), Amplified Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs) or microsatellites, single nucleotide polymorphisms (SNPs). The suitable molecular marker has following characteristics: (a) must be polymorphic, (b) has co-dominant inheritance, (c) randomly and frequently distributed throughout the genome, (d) easy and cheap to detect, and (e) reproducible (Baby *et al*., 2010; Sabina *et al*., 2010).

The DNA markers may be broadly divided into three classes based on the method of their detection: (a) hybridization-based; (b) polymerase chain reaction (PCR)-based and (c) DNA sequence-based (Semagn *et al*., 2006a). The DNA markers are particularly useful if they reveal differences between individuals of the same or different species. These markers are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers. Polymorphic markers could be codominant or dominant type. This is based on whether markers can discriminate between homozygotes and heterozygotes. Codominant markers indicate differences in size whereas dominant markers are either present or absent (Collard *et al*., 2005; Haq, 2009).

Today, in the context of plant improvement, single nucleotides polymorphisms (SNPs) are rapidly replacing simple sequence repeats (SSRs) as the DNA marker of choice for applications in plant breeding and genetics because they are more abundant, stable, amenable to automation, efficient, and increasingly cost-effective (Park *et al*., 2009). SNPs like ESTs belong to the third generation molecular markers. SNPs are the only new generation molecular markers for individual genotyping needed for molecular marker-assisted selection (Cooper *et al*., 1985; Gupta *et al*., 2001; Chen *et al*., 2011). They occur in both coding and non-coding regions of nuclear and plastid DNA. The abundance of these polymorphisms in plant genomes makes the SNP marker system an attractive tool for mapping, marker-assisted breeding and map-based cloning. As genetic markers, they represent sites in the genome where DNA sequence differs by a single base when two or more individuals are compared (Semagn*et al*. 2006b; McCouch*et al*. 2010). The key features of common molecular marker technologies are found in Appendix 1 (Farooq and Azam, 2002; Jeremy *et al*., 2007; Mondini *et al*., 2009).

2. 6. 4 QTLs mapping

A QTL is defined as a region of the genome that is associated with an effect on quantitative trait. Conceptually, a QTL can be a single gene, or it may be a cluster of linked genes that affect the trait (Vinod, 2006). Then, the objective of QTL mapping is to identify the loci that are responsible for variation in quantitative traits, such as salt tolerance. The QTL mapping is based on the principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Collard *et al*., 2005). Therefore, the identification of genomic regions that carry QTLs, allows breeders to use marker-assisted selection to precisely move beneficial QTLs into elite lines for crop improvement in breeding programmes (Ahmadi and Fotokian, 2011).A major breakthrough in the

characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s. One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis (Collard *et al*., 2005).

The detection of genes or QTLs controlling traits is possible due to genetic linkage analysis, which is based on the principle of genetic recombination during meiosis (Tanksley, 1993). This permits the construction of linkage maps composed of genetic markers for a specific population. The QTLs can be detected by single-marker analysis, simple interval mapping and composite interval mapping. Using statistical methods such as single-marker analysis or interval mapping to detect associations between DNA markers and phenotypic data, genes or QTLs can be detected in relation to a linkage map (Collard and Mackill, 2007).

2. 6. 5 Marker-assisted selection

The DNA markers that are tightly linked to agronomically important genes (called gene "tagging") may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (Collard *et al*., 2005). Selection based on genotype is not affected by environmental variation or by complexities of interaction affecting phenotypic selection. Marker-assisted selection offers a form of genotypic selection programme. It involves screening for a desired plant phenotype or phenotypic component based on the banding pattern of array of molecular markers linked to the gene(s) of interest. This involves assaying the DNA of an individual plant for the presence or absence of bands of expected molecular weight. The banding pattern of a molecular marker at a given locus is indicative

of the presence or absence of a specific chromosomal segment which is known to carry a desired gene or allele (Gregorio, 1997).

There are several advantages of MAS over conventional phenotypic selection: (a) selection can be carried out at the seedling stage. This may be useful for many traits, but especially for traits that are expressed at later developmental stages. Therefore, undesirable plant genotypes can be quickly eliminated; (b) single plants can be selected. Individual plants can be selected based on their genotype; (c)distinguish the homozygous from heterozygous; (d)selection of several traits; (e) target genotypes can be more effectively selected, which may enable certain traits to be 'fast-tracked', resulting in quicker line development and variety release;(f) reduction of the total number of lines that need to be tested over generations since many lines can be discarded after MAS early in a breeding scheme (Gregorio, 1997; Collard and Mackill, 2007).

2. 6. 6 Types of QTLs

To date, QTLs identified can be classified into two major types: main-effect QTLs (M-QTLs) and epistatic QTLs (E-QTLs), based largely on the presence or absence of epistasis. Distinction of the two types of QTLs is critical to the understanding of the genetic basis of quantitative trait variation (Li, 2001).

2. 6. 6. 1 M-QTLs

The M-QTLs are defined as single Mendelian factors at which effects (additive and/or dominance) on a given phenotype arise from allelic substitution and are detected by marker-trait associations using single-factor ANOVA or interval mapping models (Angaji, 2009). The M-QTLs include, major genes of very large effects on highly heritable traits, which are typically detected with very large LOD scores (>10.0), and each explains a large portion of the total trait variation in a mapping population (Li, 2001; Angaji, 2009).

For these types of QTLs, the trait values (phenotypes) are associated with specific alleles at single loci.

2. 6. 6. 2 E-QTLs

Loci at which trait values are determined by interactions between alleles at two or more loci and detected by associations between trait values and multilocus marker genotypes using epistatic models are known as E-QTLs (Li *et al*., 1997 and Wang *et al.*, 1999). Indeed, for such types of QTLs, the trait values (phenotypes) are associated with multilocus genotypes (Li, 2001; Angaji, 2009).

CHAPTER THREE

3. 0 MATERIALS AND METHODS

3. 1 Materials

A set of 300F_5 recombinant inbred lines (RILs) from International Rice Research Institute (IRRI) was used in this study (Appendix 2). Each RIL derived from seeds of a single F_4 plant from a cross between IR29 and Hasawi. IR29 is an elite rice variety developed by IRRI and highly salt sensitive (Bonilla *et al.,* 2002), thus it is being used as the sensitive check. It was used as female parent of the cross. Hasawi is an accession in gene bank of IRRI and while screening the germplasm for the salinity tolerance, it was rated as extremely tolerant to salinity stress (Singh, R. K. personal communication, 2011). So far Hasawi has neither been used as donor for salinity tolerance nor studied for tolerance mechanisms and QTL analysis; hence it is termed as novel source of salinity tolerance. While developing RILs population Hasawi was used as male parent.

3. 2 Methods

3. 2. 1 Screening for salinity at seedling Stage

The parents, Hasawi and IR29, and their $F_{5:6}$ Recombinant Inbred Lines (RILs) were evaluated for salinity tolerance in hydroponic system using IRRI standard protocol (Gregorio *et al*., 1997) at IRRI-Eastern and Southern Africa Regional Office, based in Dar-Es-Salaam, Tanzania. Seeds were heat-treated for 48 hours in a convection oven set at 40°C to break seed dormancy, and after that, the seeds were placed in petri dishes with two layers of paper towels, moistened with water during 48 hours for even germination. The germinated seeds were sown one seed per hole on a Styrofoam sheet with 96 holes, attached to a nylon net bottom, and the sheet was floated on modified Yoshida solution nutrient solution (Appendix 3) (Singh *et al.,* 2010) in a 60litres capacity plastic tray. The nutrient culture solution was prepared by adding 1.25 ml of stock solution for every liter

of deionized water (Yoshida *et al*., 1976). Only good-quality seedlings, with well-developed root and coleoptile were chosen. Parental checks [Hasawi, (tolerant) and IR29, (sensitive)] were included in every float. The germinated seeds were under nutrient solution culture from the first day.

Seedlings were salinized after 5 days using $6dSm^{-1}$ salt (NaCl) concentration (equivalent about to 50 mM NaCl). This concentration was increased to $12dSm^{-1}$ after two days of 6 dSm^{-1} treatment to reduce the immediate shock. The experiment was conducted in a plastic house with average minimum temperature of 24 and maximum of 37°C. The average minimum relative humidity was 51 and maximum 84%, and natural daylight of about 14 hours. Normal field temperature and relative humidity observation points during the experiment are given in Appendix 4.

The pH of the solution was adjusted and maintained to 5.0 to 5.1 every day during 30 days with acid (1N HCl) or base (1N NaOH) (Yoshida *et al*., 1976). The nutrient solution was renewed once every week to limit the effect of algae and replenish the nutrients. Initial scoring was recorded 12 days after the imposition of salinity stress and final scoring was done after 25 days. The IRRI modified standard evaluation system (SES) was used as shown in Appendix 5. The scores attributed to the different seedling are shown in Appendix 6.

Specifically, as plants were grown in floats in individual trays, these trays formed the fundamental unit of replication. Thus, an individual RIL typically had four plants per individual tray, and there were four trays (three salinized and one non-salinized) having the line. The specific assignment of RILs and their position within a tray was a randomized complete block design (RCBD) and a single salinity level was used. GenStat Discovery Edition 4 was used to allocate randomly the genotypes.

In addition to salt injury scores, the length of roots and shoots (RL, SL) from each 30 days old F_6 RILs were measured from three plants in cm and average length was calculated using Excel software 2010. Similarly, roots and shoot fresh weight (RFW, SFW) from each 30 days old F_6 RILs were weighed from three plant replicates using a top loading electronic balance and the average measurements (in g) was recorded. The roots and shoot dry weight (RDW, SDW) from each 30 days old F_6 RILs were dried for five days in an oven set at 75^{o}C. Upon drying, the root and shoot dry weights were weighed using a top loading electronic balance and the average measurements (in g) was recorded.

Relative percentage reduction of root and shoot length, root fresh and dry weight, shoot fresh and dry weight was calculated using average data of each genotype from three replications for all genotypes grown in saline conditions and those grown in non-saline conditions. The following formula was used:

$$\text{Relative Reduction of Trait (\%)} = \frac{\text{Trait in non saline conditions} - \text{Trait in saline conditions}}{\text{Trait in non saline conditions}} \times 100 \quad \ldots\ldots(1)$$

3. 2. 2 DNA extraction

Genomic DNA was isolated from young leaves of two plants of each parent lines while the leaves of individual plant were used for 300 F_5 RILs. The DNA was extracted using the CTAB (Cetyl Trimethyl Ammonium Bromide) mini-preparation method.The procedure of the method as well as the required equipment and chemicals used in this study are given in Appendix 7. A part of extraction was done in BecA Laboratory based at ILRI, in Nairobi Kenya and another part of DNA extraction and genotyping were done in the Molecular Marker Applications Laboratory (MMAL) based at IRRI-Philippines.

3. 2. 3 Quantification and quality control of DNA

The quality of the DNA was tested by staining DNA within SYBR® Safe DNA gel staining solution after electrophoresis in 1% agarose gel at 150V for 45 min in 0.5X TBE

buffer and the image was visualized with gel documentation system (Alpha imager). Two µl of DNA sample were mixed with 6 µl loading dye and then pipetted into the sample wells. Also 1 µl (50ng), 2 µl (100ng), and 4 µl (200ng) of ladder were loaded in first three wells for a qualitative comparison based on band thickness.

The DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260nm and 280nm through spectrophotometer (NanoDrop8000). One microliter of crude DNA was diluted to 2µl deionized water. One µl of each sample were placed on spectrophotometer. The concentration in $ng\mu l^{-1}$ was checked and the ratio OD260/OD280 was read to conclude the purity.

3. 2. 4 Scoring of SNPs and analysis of polymorphism

A chip comprised of three hundred eighty four (384) SNP markers scattered over the 12 chromosomes of rice genome was used for parental polymorphic survey between two parents (Hasawi and IR29).For each OPA (Oligo Pool All) reagent run, a plate of ninety six samples with 5µl of unamplified genomic DNA nomalized to 50 ng μl^{-1}concentration was genotyped using the "GoldenGate Genotyping Assay for VeraCode Manual Protocol" (Illumina Part # 11275211), following the manufacturer's instructions.

Scoring of SNP genotyping data was done using the BeadStudio genotyping computer software. The SNPs with the same genotype as Hasawi were scored as "1'', SNPs with the same genotype as IR29 were scored as "2'', heterozygous SNPs were scored as "3", and missing SNPs were scored "0".Since the SNP map obtained from the BeadStudio software gave marker position (in bp), the position was converted to genetic map (in cM) using the following equation (Tanksley *et al.,* 1989).

$1cM=2.5 \times 10^4$ bp ……..(2)

Where cM is centimorgan, a unit used to measure genetic map; bp is base pair, a unit used to measure physical map.

The calls for Hasawi and IR29 were checked carefully to find polymorphism. The markers with a missing call or showing same alleles for both parents were discarded. A total of 142 tolerant and sensitive plants were selected for molecular study. The selection was done by sorting out the extremes and intermediates based on combination of tolerance final salt injury scores SESF, shoot fresh and dry weight. Graphical genotype visualisation computer software, Flapjack (Milne *et al*., 2010) was used to visualize and present polymorphism.

3. 2. 5 Analysis of variances and correlations analysis

All the raw data obtained from salt stress and non-stress conditions were processed using Microsoft Excel 2010. Analysis of variances (ANOVA) and Pearson correlations (r) were performed for salt injury scores (SESI and SESF), root length (RL), shoot length (SL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight of (SFW),and shoot dry weight of (SDW) variables. The same analyses were performed for percentage reduction of RL, SL, RFW, RDW, SFW, and SDW. WindoStat 8.5 version computer software was used. The data were checked for frequency distribution of all traits using the software package SPSS 16.0 version for Windows. Charts for SES scores versus % reduction of RL, SL, RFW, RDW, SDW, and SDW were drawn using Microsoft Excel 2010. Based on SES scores and using ANOVA, phenotypic difference between the two parents under salinity stress was verified.

3. 2. 6 SNPs linkage map and QTL analysis

Data from all SNP markers that detected polymorphism between Hasawi and IR29 were used to construct the linkage map. Three hundred eighty four SNP were used for parental polymorphism survey. The polymorphic markers were selected for QTL analysis and construction of linkage map.

QGene software version 4.3.1 (Joehanes and Nelson, 2008) was used to construct the genetic linkage map by using functions suggested by Kosambi (1944) and linkage evaluation of P=0.001, based on genotypic and phenotypic data of $F_{5:6}$ RILs. The threshold of logarithm of odds (LOD) score for the test of independence of marker pairs was set at >3.0 (Collard *et al*., 2005) and the markers order with the highest LOD score were then selected. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (null hypothesis).

The QTL analysis was performed using QGene software version 4.3.1with LOD > 3 as threshold.Composite interval mapping (CIM) (Zeng, 1994) was performed to examine the association between phenotypic data and marker genotype. To increase the precision of putative QTLs, minimal logarithm of odd (LOD) value was analysed empirically from 1000 permutation tests (Churchill and Doerge, 1994). QGene software was again used to identify the effects and origins of alleles contributed by the Hasawi and IR29 parents. A positive-signed effect represented an increasing effect of allele from parent 1 (Hasawi); a negative-signed effect, an increasing effect of allele from parent 2 (IR29). The proportion of the total phenotypic variance explained by each QTL was calculated as R^2 value (R^2 = ratio of the sum of squares explained by the QTL to the total sum of squares).QTL positions were assigned to the point of maximum LOD score in the target regions. Windows QTL Cartographer version 2.5 (Wang *et al*., 2011) software was used to locate the identified QTLs involved in salinity tolerance on the particular chromosomes.

CHAPTER FOUR

4. 0 RESULTS

4. 1 Morphological Difference between Parents under Salinity Stress

The analysis of variance (Table 1) howed significant differences between the parents and among the F_6 RILs for all the eight traits (SESI, SESF, RL, SL, RFW, SFW, RDW and DW) evaluated during the study. The significant differences were also observed for the growth reduction for the RL, SL, RFW, SFW, RDW and DW (Table 2).

Initial and final salinity tolerance scores (SESI and SESF) discriminated Hasawi from IR29. Hasawi scored 1.1 and 1.9 as initial and final SES respectively while IR29 scored 7.7 and 9 (Table 3). The frequency distribution showed that the two parents were extremely diverse for salt injury scores (Fig. 3 and 4).

Similarly, all others traits (RL, SL, RFW, SFW, RDW and DW) discriminated the two parents after 25 days of plant growth under salinity stress (Table 3, Fig. 3 and 4). The same trend of discriminating the two parents was observed for the percentage of reduction of the above traits (Table 4); Hasawi (salinity tolerant) showed low percentage of growth than IR29 (salinity sensitive). The values of those seven traits showed more reduction in IR29 than in Hasawi when the plants were exposed to salinity stress, which was consistent with the fact that Hasawi is well-identified salt-tolerant genotype.

Table 1: Mean Squares for the SESI, SESF, RL, SL, RFW, SFW, RDW and SDW between two parents

Source of variation	df	Mean Squares			
		SESI	SESF	RL	SL
Replication	2	0.08^{ns}	0.08^{ns}	1.18^{ns}	2.667^{ns}
Genotype	1	64.68^{***}	75.62^{**}	150.0^{**}	2400.0^{***}
Error	2	0.06	0.08	0.500	2.000
CV (%)		5.62	5.19	4.51	3.79
Grand mean		4.42	5.45	16	37
$LSD_{0.05}$		0.87	0.99	2.48	4.97

and* indicate mean values significant at 1% and 0.1% level of probability, respectively, CV: Coefficient of variation, $LSD_{0.05}$: least significant difference at 5% level of probability, n.s.: no significant difference; SESI: initial salinity injury score, SESF: final salinity injury score, RL: root length, SL: shoot length.

Table 1: (Continued)

Source of variation	df	Mean Squares			
		RFW	SFW	RDW	SDW
Replication	2	0.007^{ns}	0.027^{ns}	0.0001^{ns}	0.0009^{ns}
Genotype	1	1.138^{**}	7.562^{**}	0.0128^{**}	0.213^{**}
Error	2	0.002	0.015	0.00003	0.0003
CV (%)		7.60	10.00	9.78	7.57
Grand mean		0.535	1.227	0.059	0.229
$LSD_{0.05}$		0.14	0.43	0.02	0.06

RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight.

Table 2: Mean Squares for the% reduction of RL, SL, RFW, SFW, RDW and SDW between two parents

Source of variation	df	Mean Squares		
		% Red RL	% Red SL	% Red RFW
Replication	2	31.19 ns	5.75 ns	139.11*
Genotype	1	922.81*	1473.92**	5574.79***
Error	2	10.36	2.81	4.99
CV (%)		15.90	3.39	5.27
Grand mean		20.25	49.47	42.36
$LSD_{0.05}$		11.31	5.89	7.85

*, ** and *** indicate mean values significant at 5%, 1% and 0.1% respectively; CV: Coefficient of variation; $LSD_{0.05}$: least significant difference at 5% level of probability, ns: no significant difference, % Red RL: Percentage reduction of root length, % Red SL: Percentage reduction of shoot length, % Red RFW: Percentage reduction of root fresh weight.

Table 2: (Continued)

Source of variation	df	Mean Squares		
		% Red SFW	% Red RDW	% Red SDW
Replication	2	13.056 ns	53.97 ns	29.43 ns
Genotype	1	1796.43**	3911.19**	2785.12***
Error	2	1.922	13.78	1.24
CV (%)		1.80	8.13	1.72
Grand mean		76.92	45.67	64.64
$LSD_{0.05}$		4.87	13.04	3.92

% Red SFW: Percentage reduction of shoot fresh weight, % Red RDW: Percentage reduction of shoot dry weight, % Red SDW: Percentage reduction of shoot dry weight.

Table 3: Average values for the SESI, SESF, RL, SL, RFW, SFW, RDW and SDW between two parents

GENO	SESI	SESF	RL (cm)	SL (cm)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
HASAWI	1.1	1.9	21	57	0.970	2.348	0.105	0.417
IR29	7.7	9.0	11	18	0.100	0.105	0.013	0.041

SESI: initial salinity injury score, SESF: final salinity injury score, RL: root length, SL: shoot length, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight; Geno: genotypes.

Table 4: Percentage reduction for the SESI, SESF, RL, SL, RFW, SFW, RDW and SDW between two parents

GENO	% Red RL	% Red SL	% Red RFW	% Red SFW	% Red RDW	% Red SDW
HASAWI	7.84	33.80	11.87	59.61	20.13	43.10
IR29	32.65	65.14	72.84	94.22	71.20	86.18

% Red RL: Percentage reduction of root length, % Red SL: Percentage reduction of shoot length, % Red RFW: Percentage reduction of root fresh weight, % Red RDW: Percentage reduction of root dry weight, % Red SFW: Percentage reduction of shoot fresh weight, % Red SDW: Percentage reduction of shoot dry weight**.**

4. 2 Genetic Polymorphism between Parents

Genetic survey was performed using SNP markers. One hundred ninety four SNPs out of 384 (50.52%) showed polymorphism between Hasawi and IR29. No heterozygote alleles were found through rice genome of both parents. Fig.2 shows, for each chromosome, different alleles across chromosomes contrasting the two parents. The physical position for the polymorphic markers is also given in base pair (bp).

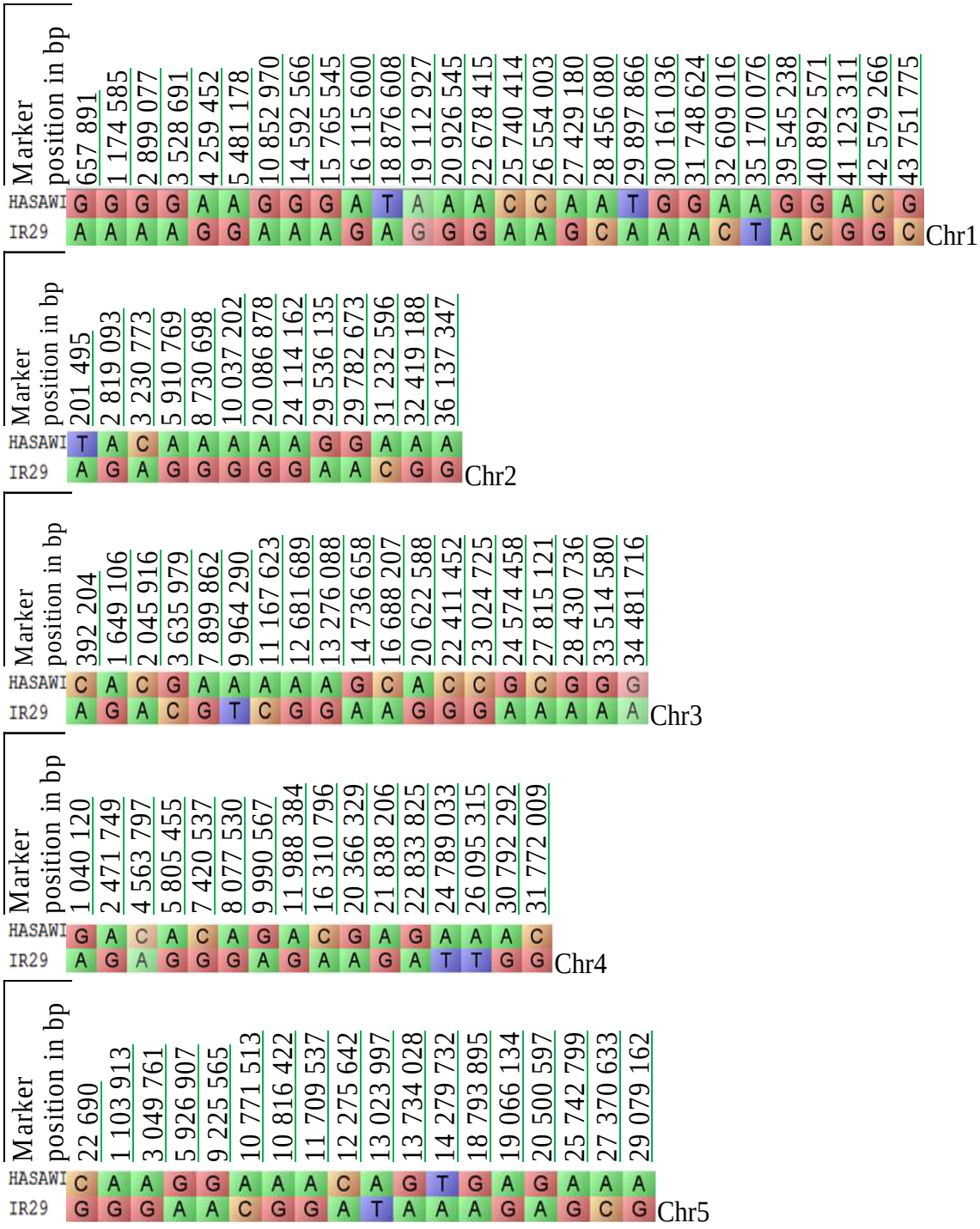

Figure 2: Alleles calls due to the presence of SNPs showing polymorphism between two parents used to develop recombinant inbred lines

The physical position of the polymorphic SNP is given in base pair (bp);A: Adenine, T: Thymine, G: Guanine, and C: Cytosine. Chr1 to Chr12: Chromosome 1 to Chromosome 12.

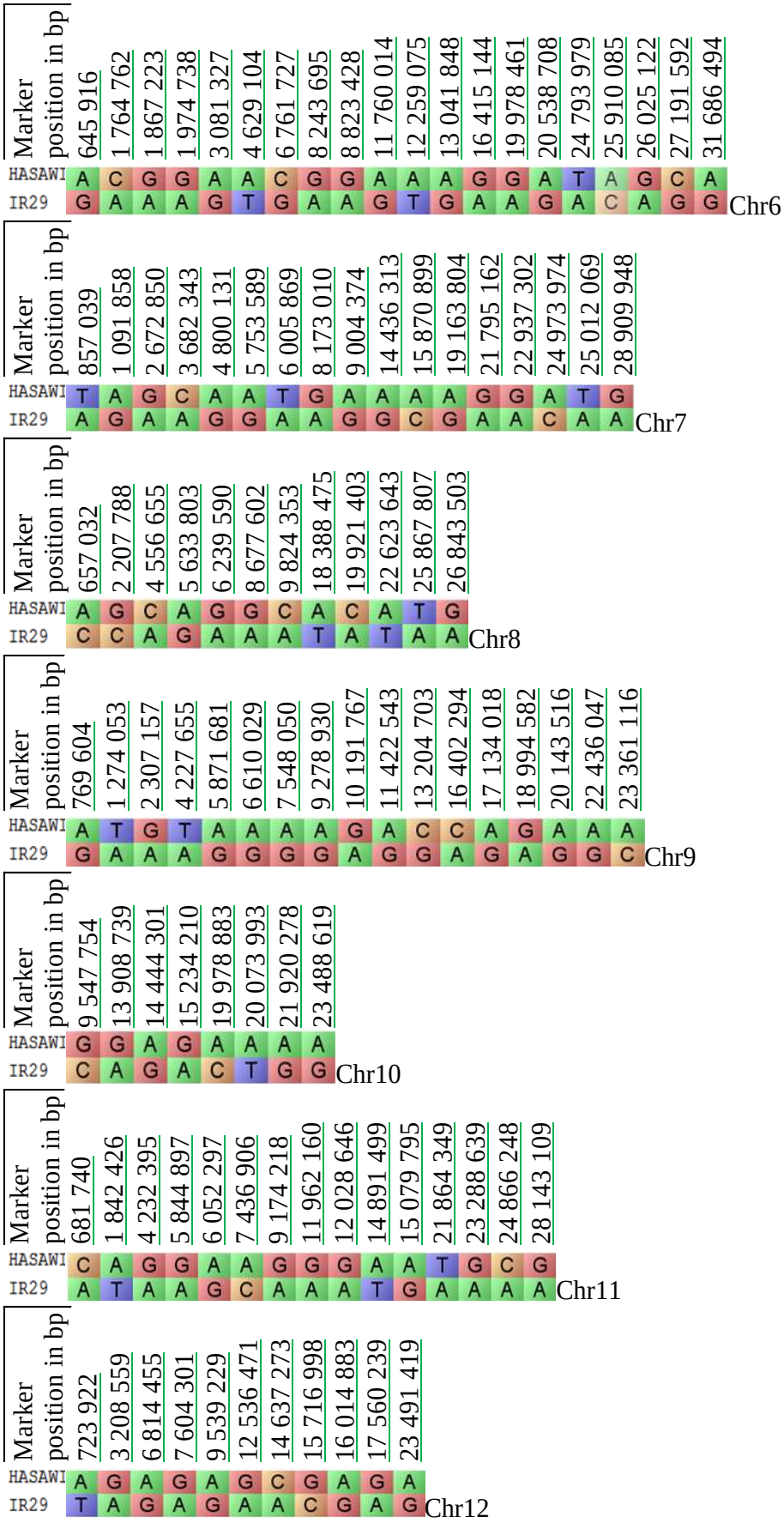

Figure 2: (Continued)

4. 3 Evaluation of Salt Tolerance Through Salinity Injury Scores

The salinity tolerance for the 300 RILs was evaluated at seedling stage in hydroponic system following the method described by Gregorio *et al.* (1997). The Appendix 6shows the leaf injuries due to the salinity stress. The Fig. 3 and 4 shows the frequency distribution of initial (SESI) and final (SESF) salinity tolerance scores for the 300 RILs after 12 and 25 days of the salt stress respectively. Analysis of variance for the final salinity tolerance score SESF is shown in Table 5. For initial salinity scores (SESI), the results showed normal distribution while were skewed towardsthesensitive parent for the final salinity scores SESF). The median and mean for this trait almost converged (mean = 4.59 and median = 4.67). But after 25 days under salt stress (SESF), the trend changed and the frequency distribution for the salinity tolerance scores among the genotypes showed skewed behaviour towards the sensitive parent. The mean and median diverged (mean=7.35 and median=8.33). At the same time the transgressive segregation was observed on Hasawi side.

Considering final salinity tolerance scores, among 300 RILs used in the study, 67 RILs were in the range of tolerant (highly tolerant, tolerant and moderate tolerant) with four RILs scored SESF=1, three scored SESF=1.7, one scored SESF=2.3, seven scored SESF=3, seven scored SESF=3.7, 10 scored SESF=4.3, fourteen scored SESF=5, and twenty one scored SESF=5.7. Seventy eight RILs were in the range of sensitive, and 155 RILS were in the range of highly sensitive.The meanings of different SES are presented in Appendix 5.

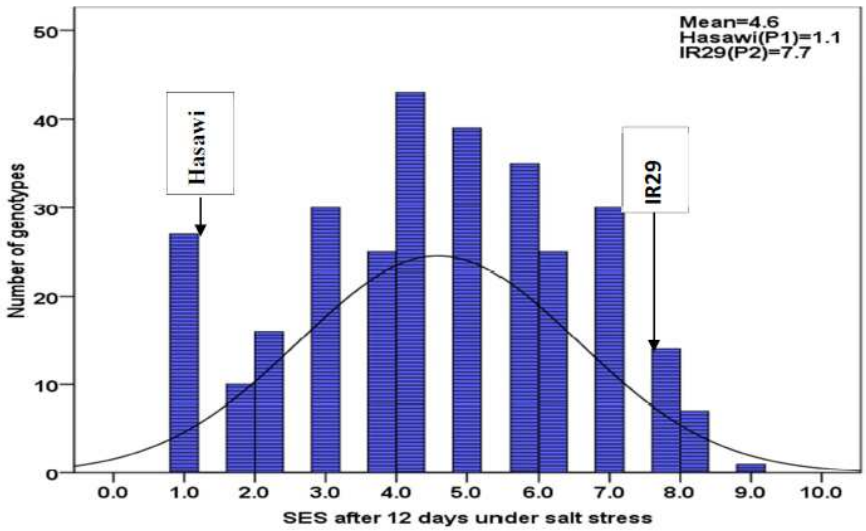

Figure 3: Salinity injury scores variation for 300 $F_{5:6}$ Recombinant Inbred Lines after 12 days under salt stress conditions

The grand mean and the mean of scores values of both parents (P1): Hasawi, (P2): IR29 are indicated.

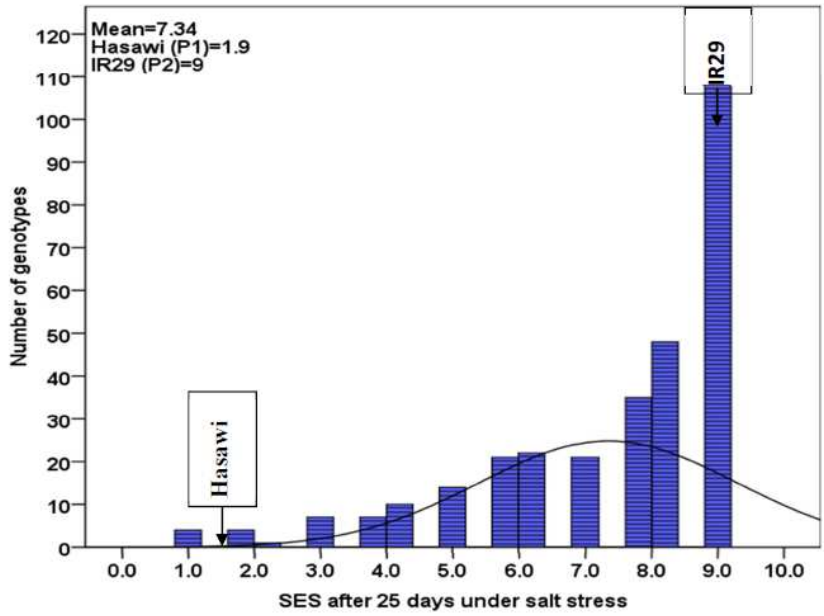

Figure 4: Salinity injury scores variation for 300 $F_{5:6}$ Recombinant Inbred Lines after 25 days under salt stress conditions

The grand mean and the mean of scores values of both parents (P1): Hasawi, (P2): IR29 are indicated.

Table 5: Analysis of variance showing Mean of Squares for the final salinity injury scores

Source of variation	df	Mean Squares	F-Value	Pr
Replications	2	13.224	4.66**	0.0098
Genotype	301	11.338	3.99***	<0.001
Residual	602	2.840		
Total	**905**	**5.690**		

CV (%)=22.93; LSD (P=0.05)=2.70; Grand mean=7.35.

and* indicate mean values significant at 1% and 0.1% level of probability, respectively, df: degree of freedom, CV: Coefficient of variation, Pr.: probability.

4. 4 Morphological Variation of $F_{5:6}$ Recombinant Inbred Lines under Stress

Together with their parents (Hasawi and IR29), the 300 $F_{5:6}$ RILs were subjected to salt stress of EC 12dSm^{-1}. The tolerant RILs were distinguished from the sensitive ones when the population was grown in salinized conditions (Fig. 5). Analysis of variance (ANOVA) showed highly significant difference between genotypes for all traits including their percentage reduction. Mean squares for each traits and significance at 0.1%, 1% and 5% level of probability are presented in Table 6 and 7 while descriptive statistics are shown in Table 8. Fig.6 to 11showfrequency distribution of RL, SL, RFW, RDW, SFW, and SDW. A transgressive segregation in both sides of parent was observed.

4. 4. 1 Root length and shoot length

The average root length for 300 RILs ranging from 8 to 28 cm was for IR29while for Hasawi was 11 and 21 cm. The results also showed that among the 300 RILs, 10 had same root length as Hasawi and 28 had greater root length than Hasawi. Thirteen RILs had same root length as IR29, twelve were shorter.

The average shoot length for RILs ranged from 16 to 60 while for IR29 and Hasawi was 18 and 57cm respectively. The results showed that among the 300 RILs, two had greater

shoot length than Hasawi. On other hand, one had the same shoot length as IR29 while three RILs were shorter. Normal distribution for RL and SL was observed (Fig. 6 and 7), the mean and the median were almost the same for both traits. The mean was16.69 and median was 16.55 for RL. The mean was 34.11 and median was 33.32 for shoot length (Table 8).

4. 4. 2 Root fresh and dry weight

Averages of root fresh weights ranged from 0.04 to 1.68 g and from 0.005 to 0.158g for dry weight. The average of root fresh weight for IR29 was 0.10g and 0.97g for Hasawi respectively. The results showed also that among the 300 RILs, four had higher root fresh weight than Hasawi. Five had same root fresh weight as IR29 while for 17 RILs, it was lower.

The average of root dry weight for IR29 was 0.013g and 0.150g for Hasawi. The results showed that among the 300 RILs, one had the same root dry weight as Hasawi while it was higher for 5 RILs. Seven had same root dry weight as IR29 while it was lower for 24 RILs. The frequency distribution of RFW and RDW didn't fit the normal distribution and was skewed toward the sensitive parent (Fig. 10 to 11); the mean and median for these traits were not same. The mean was 0.337 and median was 0.288 for RFW. The mean was 0.035and median was 0.029 for RDW (Table 8).

4. 4. 3 Shoot fresh and dry weight

Averages of shoot fresh weights ranged from 0.08 to 3.38 g and from 0.031 to 0.699g for dry weight. The average shoot fresh weight for IR29 was 0.10g and 2.25g for Hasawi. The results showed also that among the 300 RILs, three had higher root fresh weight than Hasawi and for the 297 RILs had lower. Four had same root fresh weight as IR29 and17 RILs had lower while 279 had higher.

The average of shoot dry weight for IR29 was 0.041g and 0.417g for Hasawi. The results showed that among the 300 RILs, ten had higher shoot dry weight than Hasawi while five had lower root dry weight than IR29. The frequency distribution of SFW and SDW didn't fit the normal distribution and was skewed toward the sensitive parent; the mean and median for these traits were not similar (Fig. 8 and 9). The mean was 0.635 and median was 0.494 for shoot fresh weight. The mean was 0.172 and median was 0.150 for shoot dry weight (Table 8).

Figure 5: Salinity tolerance screening at seedling stage using hydroponic system with EC 12 dSm^{-1}: (a) tolerant lines are shown with red arrow, (b) root density under salt conditions, (c) root density in normal conditions

Table 6: Mean Squares for various growth attributes of rice genotypes under salinity conditions

Source of variation	df	Mean Squares					
		RL	SL	RFW	SFW	RDW	SDW
Replications	2	60.041***	516.796***	0.189*	3.383**	0.003**	0.183***
Genotypes	301	39.872***	223.687***	0.162***	0.782***	0.002***	0.036***
Error	602	8.033	44.650	0.042	0.188	0.001	0.010
CV (%)		16.92	19.56	60.69	68.18	71.74	58.09
Grand mean		16.74	34.16	0.337	0.635	0.035	0.172
$LSD_{0.05}$		5.44	10.71	0.33	0.69	0.04	0.16

and* indicate mean values significant at 1% and 0.1% level of probability, respectively; CV: Coefficient of variation, $LSD_{0.05}$: least significant difference at 5% level of probability; RL: root length, SL: shoot length, RFW: root fresh weight, RDW: root dry weight, SFW: shoot fresh weight, SDW: shoot dry weight.

Table 7: Mean Squares for the % reduction of RL, SL, RFW, SFW, RDW and DW

Source of variation	df	Mean Squares					
		% Red RL	% Red SL	% Red RFW	% Red SFW	% Red RDW	% Red SDW
Replications	2	1490.074***	775.815***	2241.170*	2018.414***	3461.338**	5102.583***
Genotypes	301	1239.261***	369.325***	3114.343***	555.452***	1867.325***	1301.258***
Error	602	205.6563	71.7298	625.316	110.267	589.694	339.735
CV (%)		91.35	14.93	40.28	12.29	37.98	25.19
Grand mean		15.70	56.72	39.552	85.46	63.94	73.16
$LSD_{0.05}$		22.99	13.58	40.09	18.84	39.94	29.55

*, **and*** indicate mean values significant at 5%, 1% and 0.1% level of probability, respectively; CV: Coefficient of variation, $LSD_{0.05}$: least significant difference at 5% level of probability; % Red RL: percentage reduction of root length, % Red SL: percentage reduction of shoot length, % Red RFW: percentage reduction of root fresh weight, % Red RDW: percentage reduction of root dry weight, % Red SFW: percentage reduction of shoot fresh weight, % Red SDW: percentage reduction of shoot dry weight.

Table 8: Descriptive statistics for six morphological traits under salt stress

	SESI	SESF	RL	SL	RFW	SFW	RDW	SDW
Mean	4.59	7.35	16.69	34.11	0.337	0.635	0.035	0.172
Median	4.67	8.33	16.55	33.32	0.288	0.494	0.029	0.150
Skewness	-0.16	-1.29	0.32	0.46	2.184	1.653	1.954	1.704
Minimum	1	1	8.24	15.57	0.043	0.082	0.005	0.031
Maximum	9	9	27.53	60.29	1.680	3.382	0.150	0.699

SESI: initial salinity injury score, SESF: final Salinity injury score, RL: root length, SL: shoot length, RFW: root fresh weight, RDW: root dry weight, SFW: shoot fresh weight, SDW: shoot dry weight.

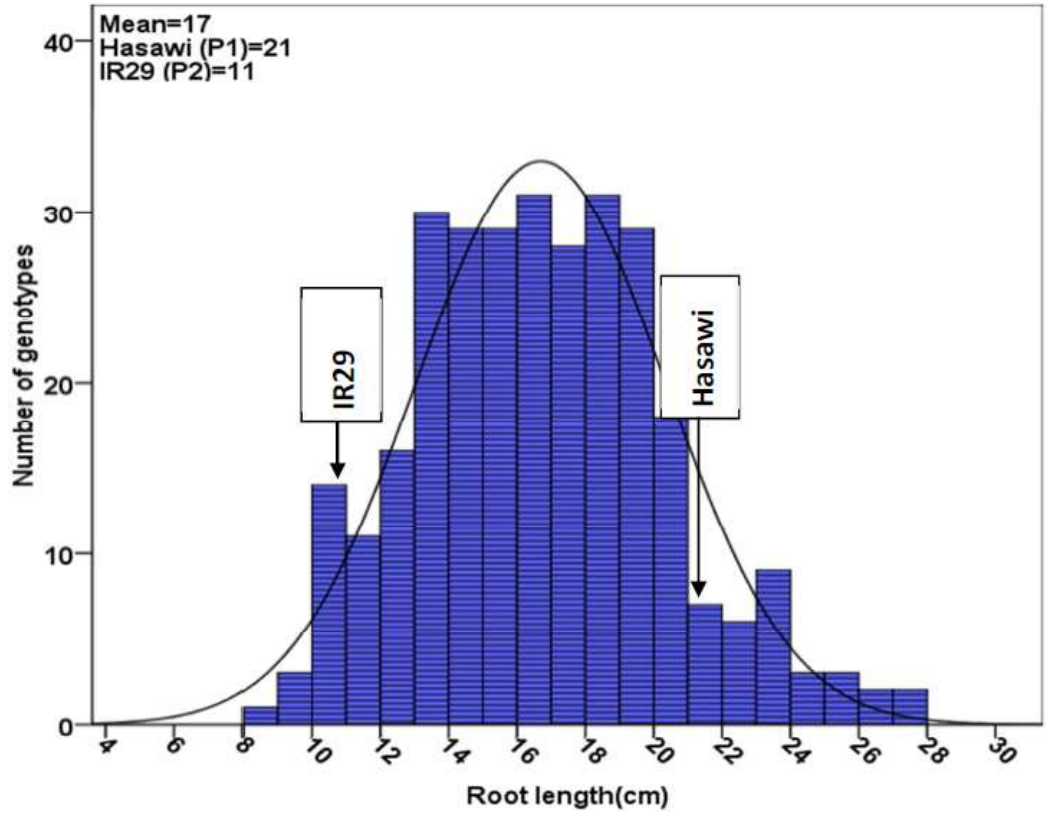

Figure 6: Frequency distribution of root length after 25 days of salt stress at EC 12 dSm^{-1} for the 300 recombinant inbred lines (F_6) derived from Hasawi and IR29 under salt stress

The grand mean and the mean of both parents; P1 = Hasawi, P2 = IR29 are indicated.

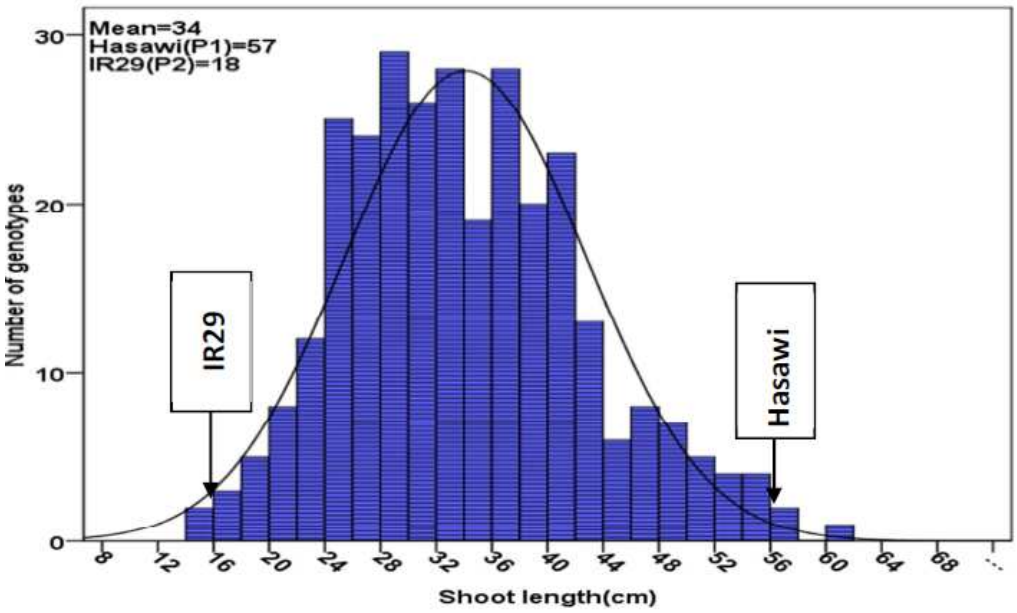

Figure 7: Frequency distribution of shoot length after 25 days of salt stress at EC 12 $dSm^{-1}$for the 300 recombinant inbred lines (F_6) derived from Hasawi and IR29 under salt stress

The grand mean and the mean of both parents; P1 = Hasawi, P2 = IR29 are indicated.

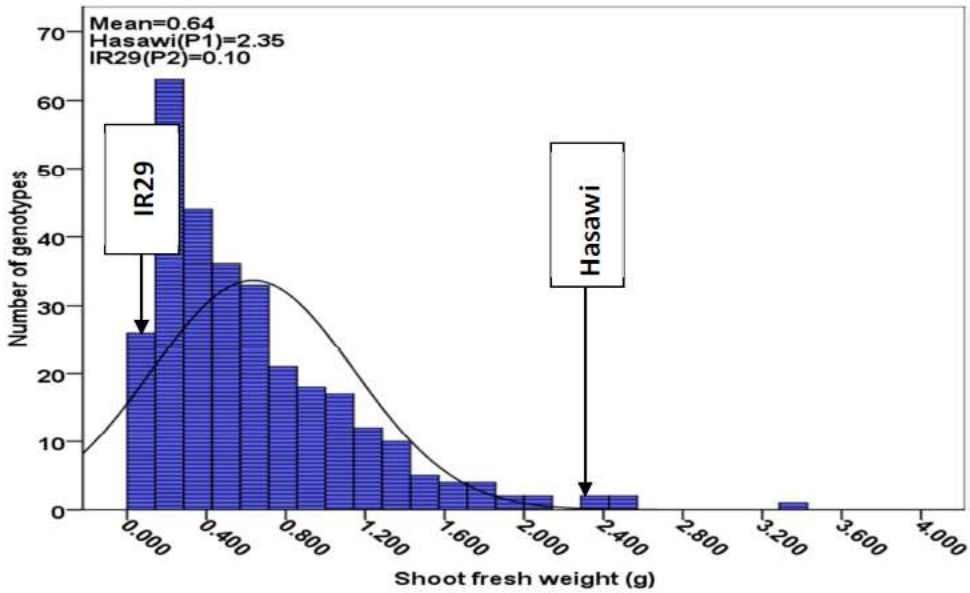

Figure 8: Frequency distribution of shoot fresh weight after 25 days of salt stress at EC 12 $dSm^{-1}$for the 300 recombinant inbred lines (F_6) derived from Hasawi and IR29 under salt stress

The grand mean and the mean of both parents; P1 = Hasawi, P2 = IR29 are indicated.

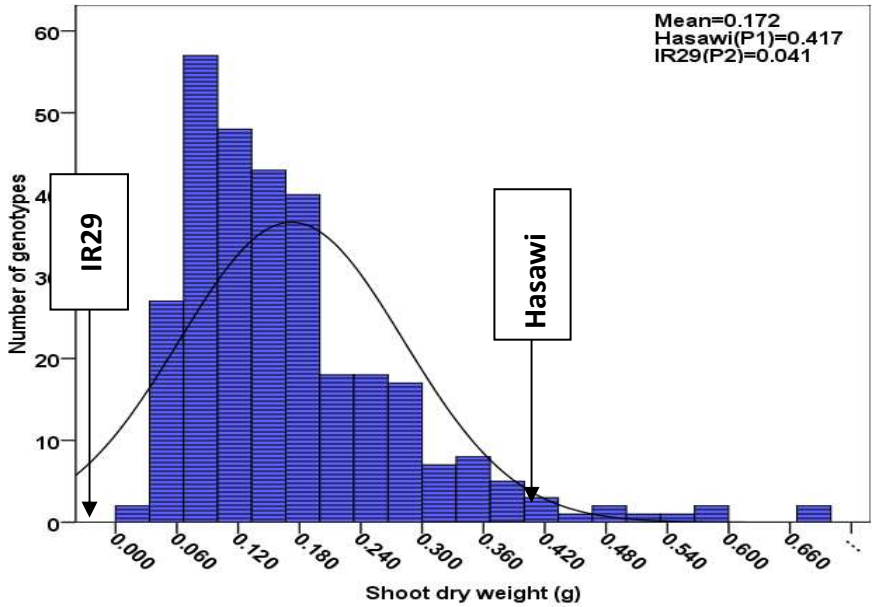

Figure 9: Frequency distribution of shoot dry weight after 25 days of salt stress at EC 12 dSm^{-1} for the 300 recombinant inbred lines (F_6) derived from Hasawi and IR29 under salt stress

The grand mean and the mean of both parents; P1 = Hasawi, P2 = IR29 are indicated.

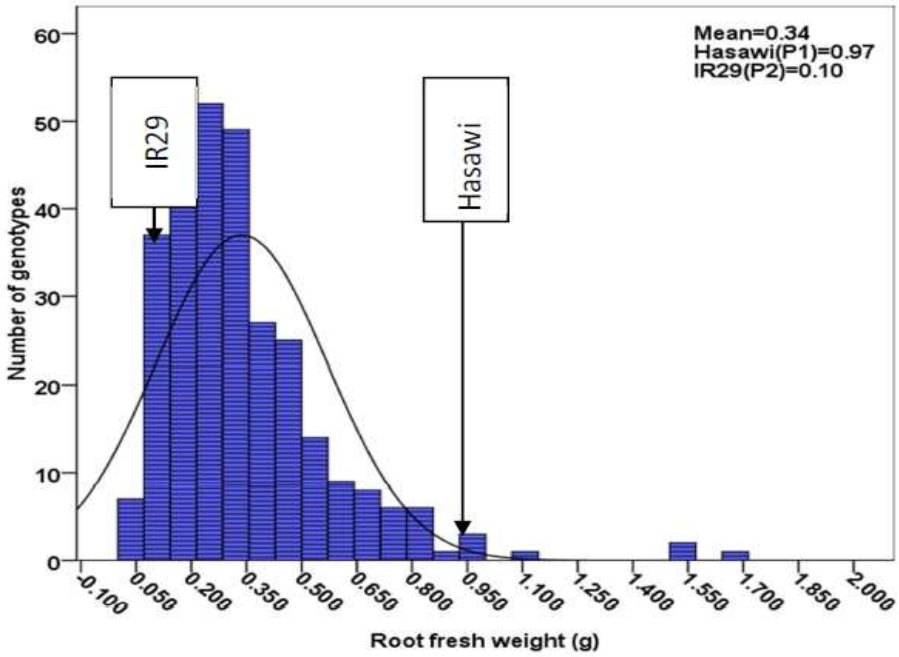

Figure 10: Frequency distribution of root dry weight after 25 days of salt stress at EC 12 dSm^{-1} for the 300 recombinant inbred lines (F_6) derived from Hasawi and IR29 under salt stress

The grand mean and the mean of both parents; P1 = Hasawi, P2 = IR29 are indicated.

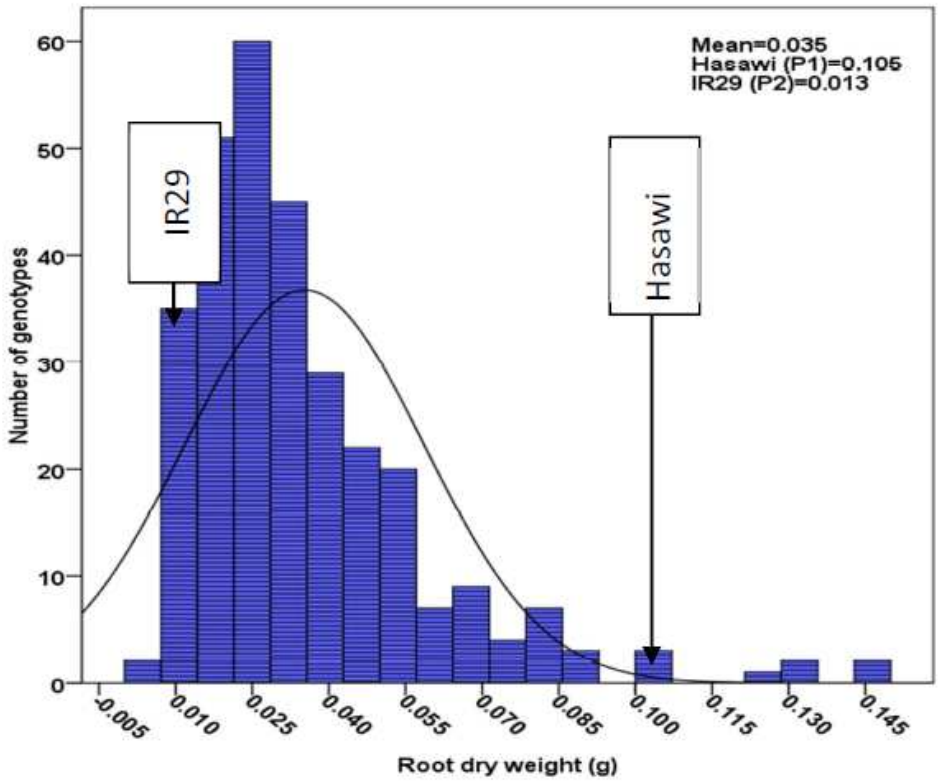

Figure 11: Frequency distribution of root fresh and dry after 25 days of salt stress at EC 12 $dSm^{-1}$for the 300 recombinant inbred lines (F_6) derived from Hasawi and IR29 under salt stress

The grand mean and the mean of both parents; P1= Hasawi, P2=IR29 are indicated.

4. 5 Correlation between Evaluated Traits and Growth Reduction

The correlation between traits was computed by regressing phenotypic values of one trait on those of other traits. The correlations among traits after 25 days of 12 dSm^{-1} salt stress in hydroponic system with deionized water are presented in Table 9.

Under salinized conditions, highly significant ($P \leq 0.001$) and positive correlations were found between the RL and SL, RL and RFW, RL and RDW, RL and RDW, RL and SDW and reciprocally. The same trend was observed between RFW and SFW, RFW and RDW, RFW and SDW. Between initial and final salt injury scores, a highly and significant correlation were also noticed but, both showed inverse and highly significant correlation

with other parameters. The salt injury score (SESI and SESF) showed inverse and highly correlation with others traits. For example, SESF showed inverse correlation with RL (r= -0.581**), SL (r= -0.537**), RFW (r= -0.708**), SFW (r= -0.782**), RDW (r= -0.601**), and with SDW (r= -0.703**) (Table 9). However, percentage of reduction of each trait is significant and positively correlated with SESI and SESF. Between the percentage reductions of all traits, a positive and highly significant were observed.

Generally, as the tolerance score (SESI and SESF) increases, the percentage reduction of root and shoot length, root and shoot fresh weight, root and shoot dry weight increases also for all RILs. Strangely, the results showed a different trend especially for root. For the genotypes with low tolerance score SESF, the RL, RFW, and RDW were better in saline than in normal conditions and this was confirmed with negative average value of percentage of reduction.

Table 9: Pearson correlation coefficients of different traits at seedling stage of rice under salinized conditions

	SESI	SESF	RL	SL	RFW	SFW	RDW	SDW
SESF	0.732**	1.000						
RL	-0.562**	-0.581**	1.000					
SL	-0.512**	-0.537**	0.612**	1.000				
RFW	-0.590**	-0.708**	0.661**	0.619**	1.000			
SFW	-0.614**	-0.782**	0.687**	0.686**	0.885**	1.000		
RDW	-0.497**	-0.601**	0.576**	0.524**	0.750**	0.741**	1.000	
SDW	-0.599**	-0.703**	0.651**	0.646**	0.809**	0.891**	0.684**	1.000
% Red RL	0.524**	0.562**	-0.839**	-0.571**	-0.598**	-0.668**	-0.523**	-0.636**
% Red SL	0.588**	0.632**	-0.616**	-0.780**	-0.670**	-0.730**	-0.571**	-0.660**
% Red RFW	0.381**	0.476**	-0.435**	-0.390**	-0.661**	-0.636**	-0.485**	-0.573**
% Red SFW	0.487**	0.626**	-0.554**	-0.500**	-0.749**	-0.850**	-0.607**	-0.758**
% Red RDW	0.434**	0.522**	-0.483**	-0.398**	-0.651**	-0.669**	-0.816**	-0.621**
% Red SDW	0.444**	0.526**	-0.470**	-0.411**	-0.619**	-0.706**	-0.516**	-0.821**

**: Highly significant at 0.1% level of probability, SESI: initial salt injury score, SESF: final salt injury score, RL: root length, SL: shoot length, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight, % Red RL: percentage reduction of root length, % Red SL: percentage reduction of shoot length, % Red RFW: percentage reduction of root fresh weight, % Red RDW: percentage reduction of root dry weight, % Red SFW: percentage reduction of shoot fresh weight, % Red SDW: Percentage reduction of shoot dry weight.

Table 9: (Continued)

	% Red RL	% Red SL	% Red RFW	% Red SFW	% Red RDW	% Red SDW
% Red RL	1.000					
% Red SL	0.623**	1.000				
% Red RFW	0.562**	0.610**	1.000			
% Red SFW	0.639**	0.703**	0.791**	1.000		
% Red RDW	0.558**	0.628**	0.717**	0.750**	1.000	
% Red SDW	0.575**	0.608**	0.713**	0.860**	0.709**	1.000

% Red RL: percentage reduction of root length, % Red SL: percentage reduction of shoot length, % Red RFW: percentage reduction of root fresh weight, % Red RDW: percentage reduction of root dry weight, % Red SFW: percentage reduction of shoot fresh weight, % Red SDW: Percentage reduction of shoot dry weight.

The growth reduction of the root showed strange trend. The genotypes which scored an average SESF ≤4.3, the average percentage reduction ranged -35.57 to -7.07% for SESF=1 and 4.3 respectively. For RFW, the genotypes which scored an average SESF≤1.7, the average percentage reduction was -3.31 and -1.85 % for SESF=1 and 1.7 respectively. The average percentage reduction was -1.77% for RDW at SESF=1. For other studied traits, generally, the growth reduction increases with the increment of SESF. The Fig.12 to 17 show average percentage reduction of RL, SL, RFW, SFW, RDW, and SDW for the 300 recombinant inbred lines derived from Hasawi and IR29 under salinity conditions due to salinity stress.

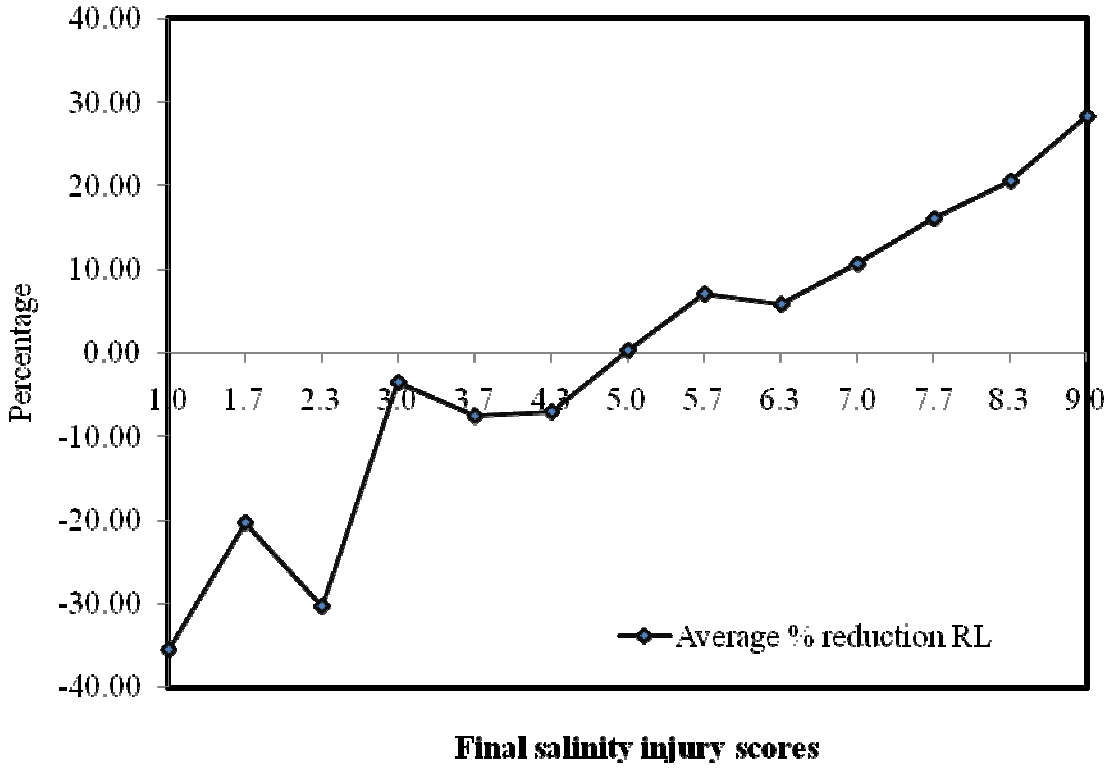

Figure 12: Final salinity injury scores versus average % reduction of root length (RL) for the 300 recombinant inbred lines ($F_{5:6}$) derived from Hasawi and IR29 under salinity conditions

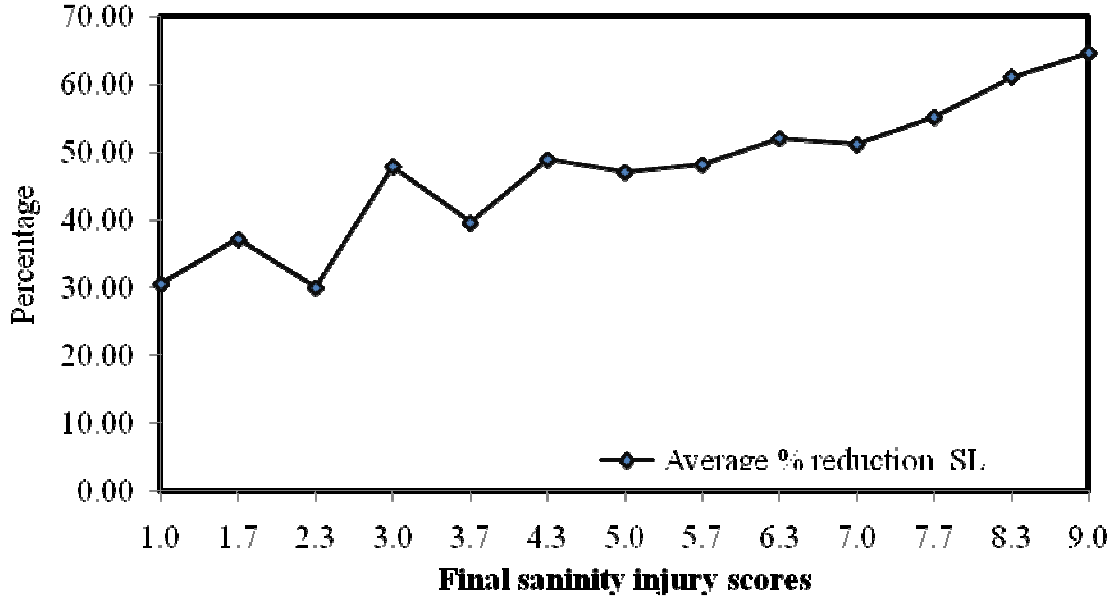

Figure 13: Final salinity injury scores versus average % reduction of shoot length (SL) for the 300 recombinant inbred lines ($F_{5:6}$) derived from Hasawi and IR29 under salinity conditions

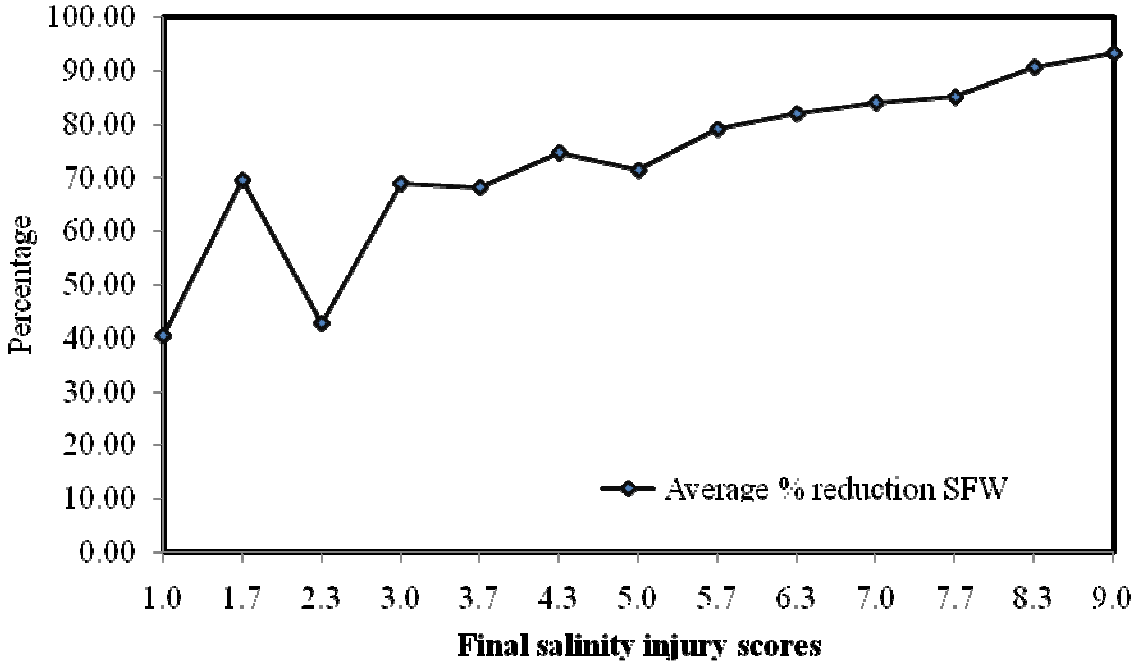

Figure 14: Final salinity scores versus average % reduction of shoot fresh weight (SFW) for the 300 recombinant inbred lines ($F_{5:6}$) derived from Hasawi and IR29 under salinity conditions

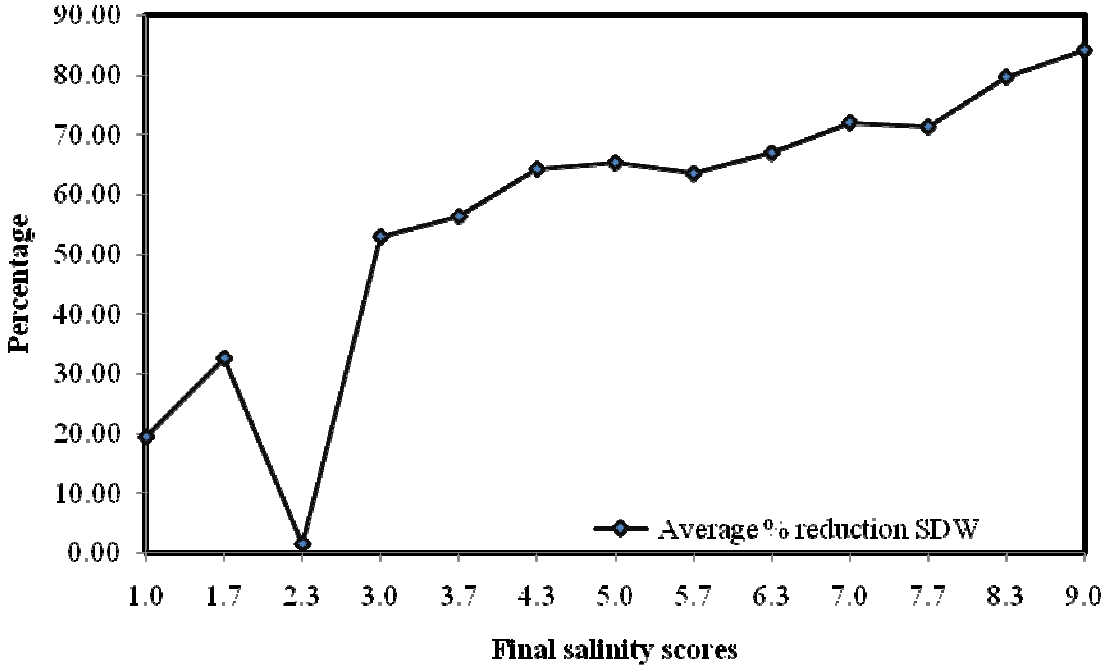

Figure 15: Final salinity scores versus average % reduction of shoot dry weight (SDW) for the 300 recombinant inbred lines ($F_{5:6}$) derived from Hasawi and IR29 under salinity conditions

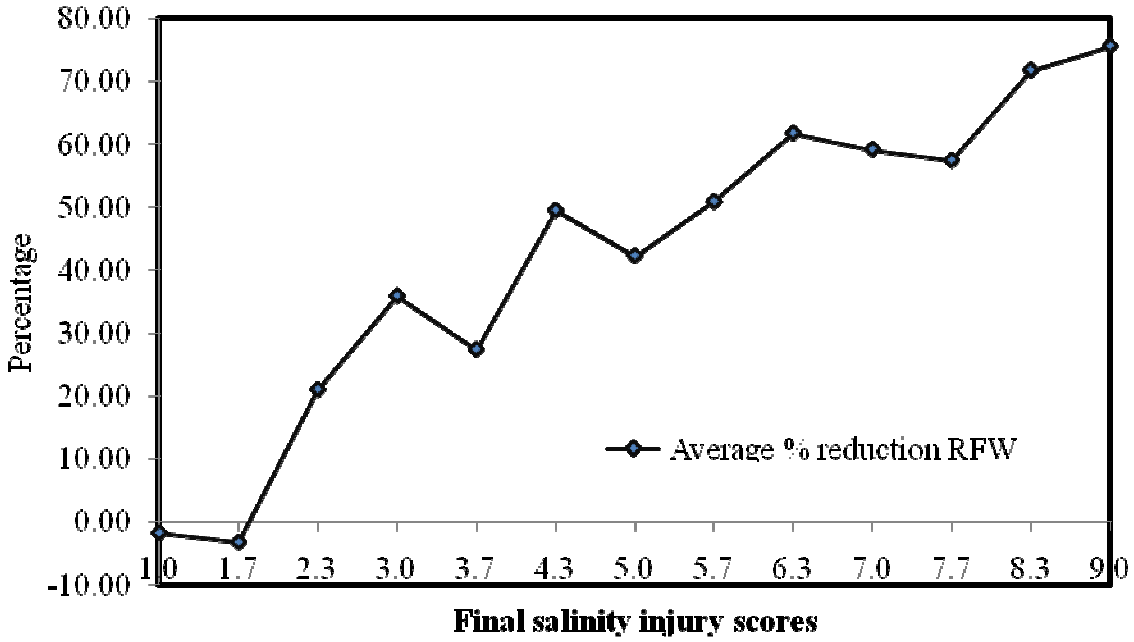

Figure 16: Final salinity injury scores versus average % reduction of root fresh weight (RFW) for the 300 recombinant inbred lines ($F_{5:6}$) derived from Hasawi and IR29 under salinity conditions

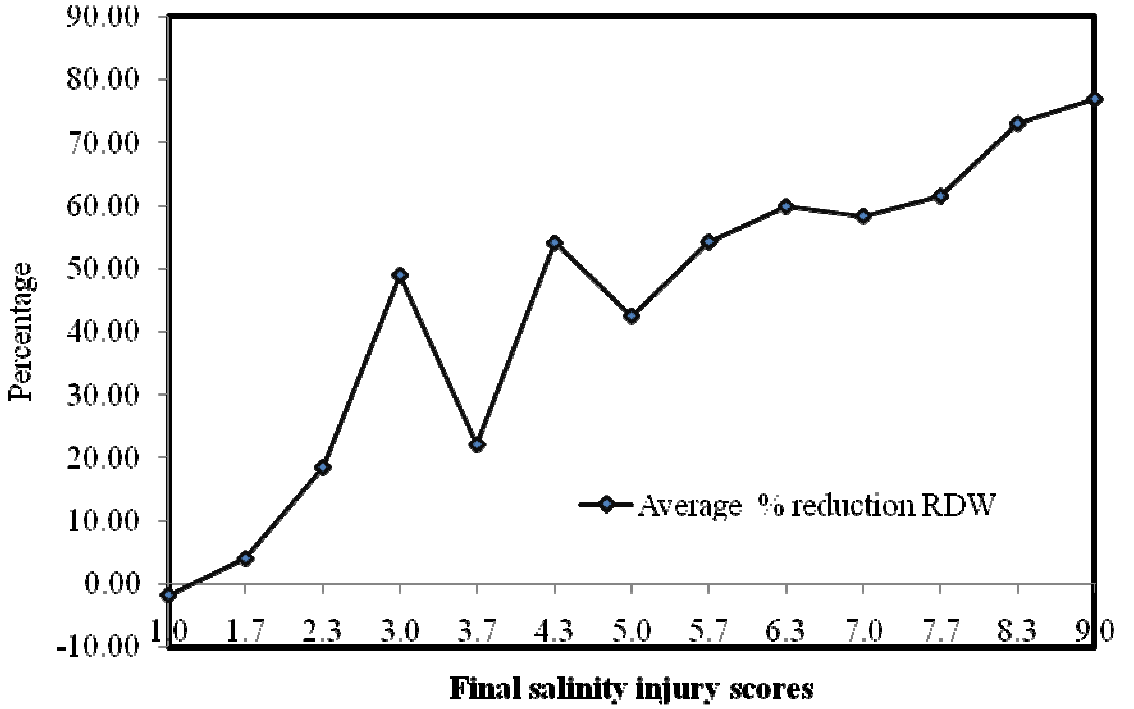

Figure 17: Final salinity injury scores versus average % reduction of root dry weight (RDW) for the 300 recombinant inbred lines ($F_{5:6}$) derived from Hasawi and IR29 under salinity conditions

4. 6 Distribution and Density of Polymorphic SNP Markers in Rice Genome

SNPs genotyping data was used to perform linkage analysis with 194 markers using QGene version 4.3.1. The distribution of the 194 markers throughout the rice genome is shown in Fig. 18, with a total length of 1441.96cM (Table 10). The average interval size between markers was 7.88cM. High markers distribution was found on chromosome 1 (28 markers over a total number of 194), with an average interval of 6.38cM and the largest average interval was in chromosome 10 (11.74 cM). The homozygous alleles were represented at 92.3% (Table 11). The nucleoside deoxyadenosine (A) represented highest percentage of 41.9 while deoxythymidine (T) was represented at 5.8%. The heterozygous alleles were represented at 6.9%. The missing alleles call was represented with 0.7%.

Table 10: Distribution and number of the polymorphic markers

Chromosome number	Number of polymorphic SNPs markers	Total interval size (cM)	Average interval size (cM)
1	28	172.38	6.38
2	13	144.55	11.12
3	19	137.93	7.26
4	16	127.09	7.94
5	18	116.32	6.46
6	20	126.75	6.34
7	17	115.64	6.80
8	12	107.37	8.95
9	17	93.44	5.50
10	8	93.95	11.74
11	15	112.57	7.50
12	11	93.97	8.54
Total	**194**	**1441.96**	**7.88**

Table 11: Allelesdistribution across each chromosome

Chromosome number	% of alleles								
	Missing	Homozygous				Heterozygous			
	-*	G	A	T	C	A/G	C/G	A/T	A/C
1	0.4	28.2	42.9	6.2	13.1	6.8	0.5	0.9	0.8
2	0.4	44.6	38.1	3.5	6.7	5.1	0	0.7	1.0
3	0.4	33.8	35.3	4.6	19.2	3.7	1.5	0.2	1.3
4	2.4	32.3	43.2	7.4	7.3	4.7	1.3	1.1	0.2
5	0.7	23.3	50.9	6.8	12.8	4.3	0.3	0.4	0.6
6	0.3	34	46.7	5.0	9.3	3.0	0.2	1.1	0.4
7	0.6	30.4	49.1	4.2	7.0	5.1	0.0	1.6	2.0
8	0.7	25.2	28.2	12	25.3	3.1	1.1	2.4	1.9
9	0.5	51.6	37.7	0.1	5.5	3.0	0.7	0.4	0.5
10	0.4	46.4	24.7	4.4	16.1	4.1	0.3	1.7	1.8
11	0.8	18.1	57.5	11.9	5.3	4.6	0.0	1.0	0.8
12	0.8	41.8	31.4	4.9	14.1	5.4	0.3	1.0	0.4
Total	**0.7**	**33.1**	**41.9**	**5.8**	**11.5**	**4.5**	**0.5**	**1.0**	**0.9**
	0.7		**92.3**				**6.9**		

*: Missingalleles; A:deoxyadenosine, G: deoxyguanosine, C: deoxycytosine, and T: deoxythymidine; A/G, C/G, A/T, and A/C are the heterozygous from the four different nucleosides of DNA.

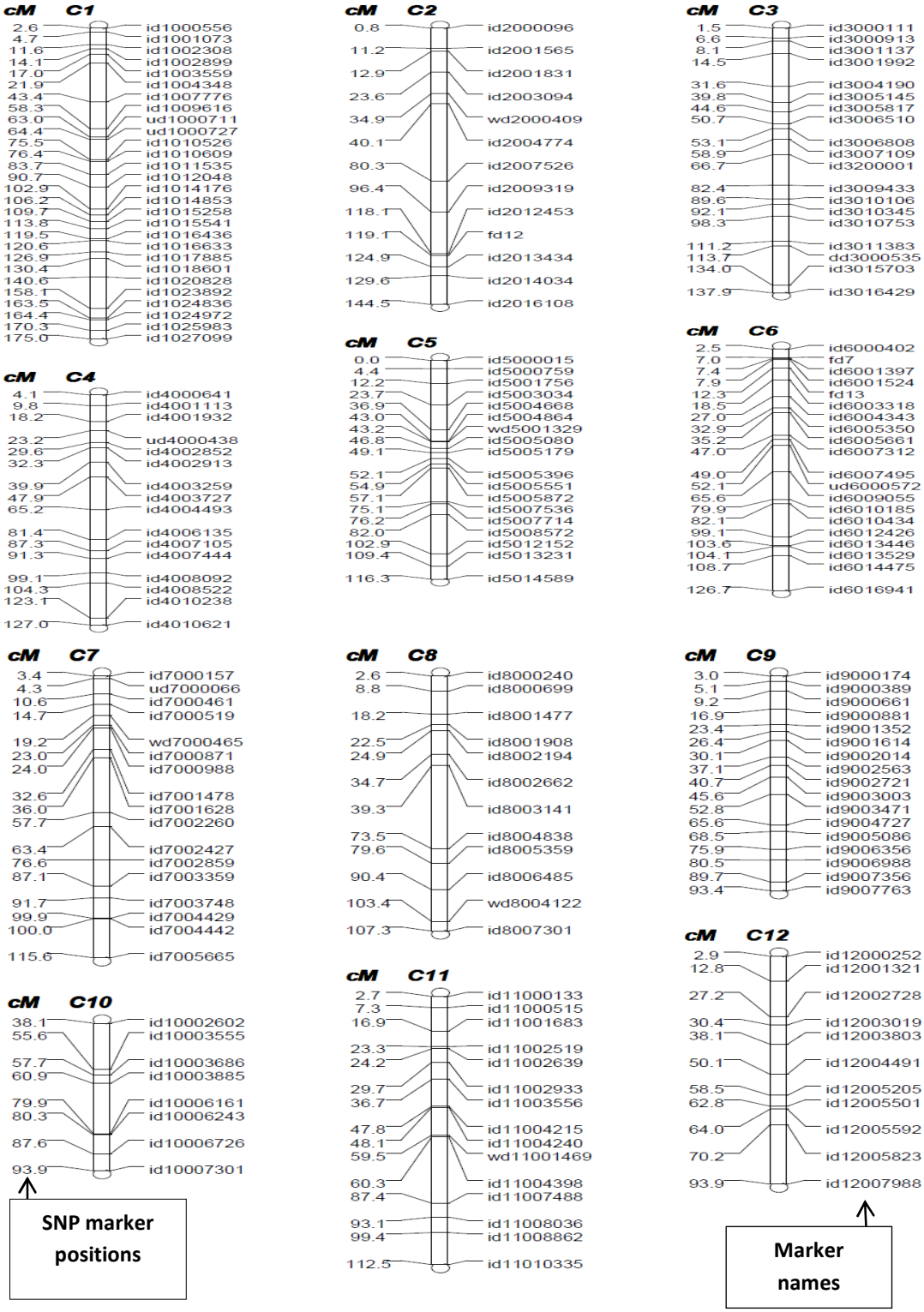

Figure 18: Linkage map showing density of the polymorphic SNPs markers used in this study

The labels on the right of the chromosomes reveal marker names while the numbers on the left indicate SNP maker positions in cM, C1 to C12: chromosome number.

4. 7 Quantitative Traits Loci Identified

The QTLs identified from 142 RILs mapping population derived from IR29/Hasawi cross are given Table 12 and Fig.19. Twenty putative and significant QTLs (represented by red bars) were identified for the visual initial and final tolerance score (SESI, SESF), seedling root length (RL), shoot length (SL), root fresh and dry weight (RFW, RDW), and shoot fresh and weight (SFW, SDW). Those QTLs were located on chromosomes number 1, 2, 4, 6, 8, 9, and 12.

Table 12: QTLs identified from the IR29/Hasawi population of 142 RILs under stress conditions

Chr.	Traits	QTL name	Peak Marker	Position (cM)	Flanking markers	Flanking markers position (cM)	Additive Effect (DPE)	Peak LOD CIM	R^2 (%)
1	Shoot length (SL)	*qSL1.1*	id1024836 *	162.6	id1023892 - id1024836	158.1 – 163.5	5.262(H)	7.21	20.6
		qSL1.2	id1025983 *	168.6	id1024972- id1025983	164.5-170.3	4.049(H)	3.93	11.8
2	Initial SES score (SESI)	*qSESI2.1*	id2004774 *	60.8	id2004774 - id2007526	40.1 – 88.3	-2.130(I)	3.50	10.6
	Shoot dry weight (SDW)	*qSDW2.1*	id2004774 *	62.8	id2004774 - id2007526	40.1 – 88.3	0.132(H)	3.84	11.6
	Final SES score (SESF)	*qSESF2.1*	id2004774 *	64.8	id2004774 - id2007526	40.1 – 88.3	-2.145(I)	3.67	11.1
4	Shoot fresh weight (SFW)	*qSFW4.1*	id4001932 *	18.1	id4001113 - id4001932	9.8 – 18.2	0.571(H)	4.28	12.8
	Root dry weight(RDW)	*qRDW4.1*	id4001932 *	18.1	id4001113 - id4001932	9.8 – 18.2	0.022(H)	3.29	10.0
	Root fresh weight (RFW)	*qRFW4.1*	id4008092 *	98.1	id4007444 - id4008092	91.4 - 99.16	0.100(H)	3.38	10.3

Chr. : Chromosome number; cM: Centimorgan; SESI: initial salinity injury score, SESF: final salinity injury score, RL: root length, SL: shoot length, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW, *: No peak marker at a particular postion but named is the nearest marker; Peak LOD CIM: Logarithm of Odds using composite interval mapping; R^2 : Percentage of total phenotypic variance explained bya particular QTL; Additive effect: The positive or negative value indicates that allele fromHasawi or IR29 increases the trait respectively; DPE: direction of phenotypic effect; H: Hasawi; I: IR29.

Table 12: (Continued)

Chr.	Traits	QTL name	Peak marker	Position (cM)	Flanking markers	Flanking markers position (cM)	Additive Effect (DPE)	Peak LOD CIM	R^2 (%)
6	Root length (RL)	*qRL6.1*	id6003318	18.5	fd13 - id6004343	12.3 – 27.0	1.496(H)	4.04	12.1
	Shoot length (SL)	*qSL6.1*	id6003318	18.5	fd13 - id6004343	12.3 – 27.0	3.631(H)	4.04	12.1
	Initial SES score (SESI)	*qSESI6.1*	ud6000572 *	52.5	ud6000572 - id6009055	52.1 – 65.6	1.121(H)	3.98	12.0
8	Shoot dry weight (SDW)	*qSDW8.1*	wd8004122 *	98.6	id8006485 - wd8004122	90.4 – 103.4	0.072(H)	3.07	9.4
9	Root fresh weight (RFW)	*qRFW9.1*	id9001614 *	27	id9001614 - id9002014	26.4 – 30.1	0.281(H)	3.34	10.1
	Shoot fresh weight (SFW)	*qSFW9.1*	id9001614 *	27	id9001614 - id9002014	26.4 – 30.1	0.684(H)	4.20	12.6
12	Final SES score (SESF)	*qSESF12.1*	id12000252 *	6.9	id12000252 - id12001321	2.9 – 12.8	-1.332(I)	3.52	10.6
	Root length (RL)	*qRL12.1*	id12000252 *	6.9	id12000252 - id12001321	2.9 – 12.8	2.378(H)	3.81	11.5
	Shoot length (SL)	*qSL12.1*	id12000252 *	6.9	id12000252 - id12001321	2.9 – 12.8	5.34(H)	3.24	9.8
	Shoot fresh weight (SFW)	*qSFW12.1*	id12000252 *	6.9	id12000252 - id12001321	2.9 – 12.8	0.343(H)	3.11	9.5
	Shoot dry weight (SDW)	*qSDW12.1*	id12000252 *	6.9	id12000252 - id12001321	2.9 – 12.8	0.071(H)	3.06	9.3
	Initial SES score (SESI)	*qSESI12.1*	id12000252 *	84.9	id12005823 - d12007988	70.2 – 93.9	-1.443(I)	3.54	10.7

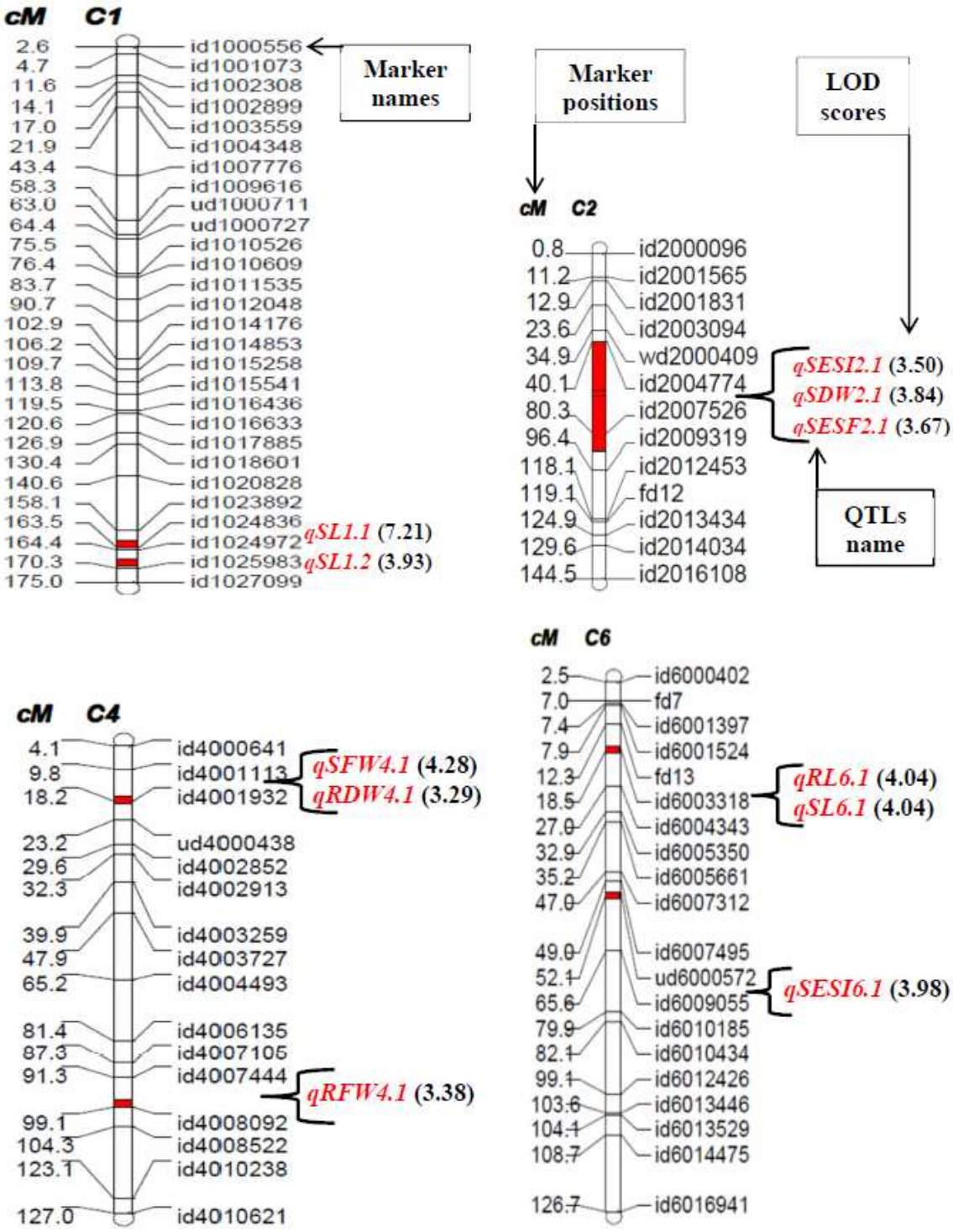

Figure 19: Linkage map with positions of QTLs for salinity tolerance using 142 RILs the cross IR29/Hasawi

The red colour indicates the QTLs location. The LOD scores are indicated in parenthesis and the labels on the right of the chromosomes reveal marker names while the numbers on the left indicate SNP maker positions in cM.

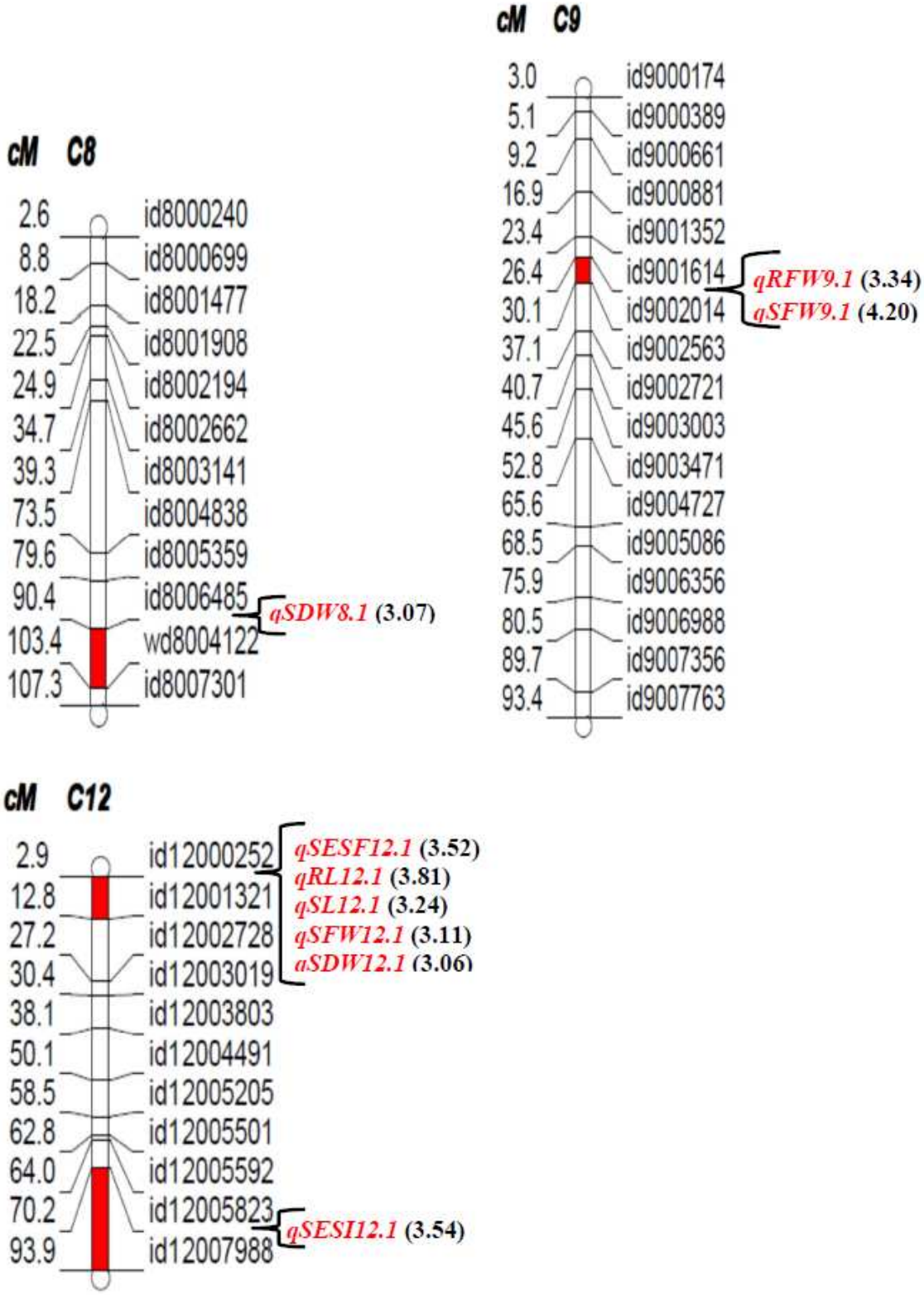

Figure 17: (Continued)

4. 7. 1 Initial salinity tolerance scores

Three putative QTLs, *qSESI2.1* (on chromosome 2); *qSESI6.1* (on chromosome 6); and *qSESI12.1* (on chromosome 12), conferring salt tolerance after 12 days of salt stress at the young seedling stage were mapped. *qSESI2.1* were flanking to id2004774 and id2007526; *qSESI6.1* to ud6000572 and id6009055; and *qSESI12.1* to id12005823 and id12007988 but was more close to id12005823. The interval between the flanking markers to *qSESI12.1* is 23.7cM; this interval is the highest for all QTLs identified. The phenotypic variation explained (PVE) by *qSESI2*.1 was10.6%. For *qSESI6.1* and *qSESI12.1*,PVE were 12.0 and 10.7% respectively. The parental additive effects of the three QTLs were -2.130, -1.121, and -1.443 respectively. The IR29 allele increased the salinity injury scores at all three loci. In other words, *qSESI2*.1 contributed an additional value of 2.13 SES to the initial salinity injury scores; *qSESI6.1* contributed 1.121 SES; and *qSESI12.1* contributed 1.443SES (Table 12 and Fig.19). The Fig. 20 to 25 shows the position of these QTLs.

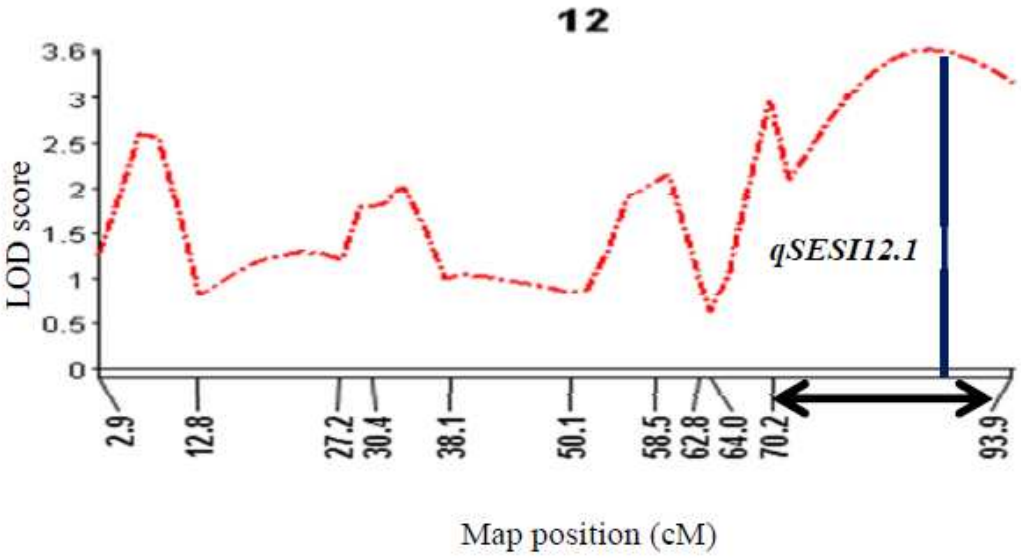

Figure 20: LOD plots showing position of putative QTLs for SESI, SESF, RL, SL SFW, and SDW chromosome 12

The gap at distal portion is shown by double horizontal arrow**.**

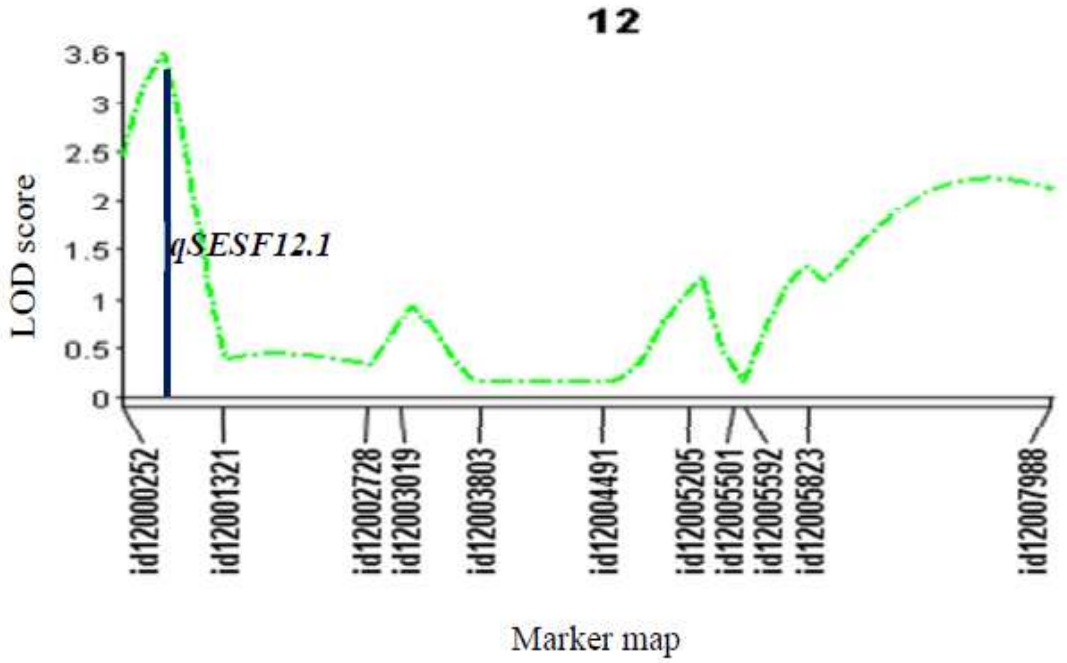

Figure 21: LOD plots showing position of putative QTL for final salinity injury scores (SESF) on chromosome 12

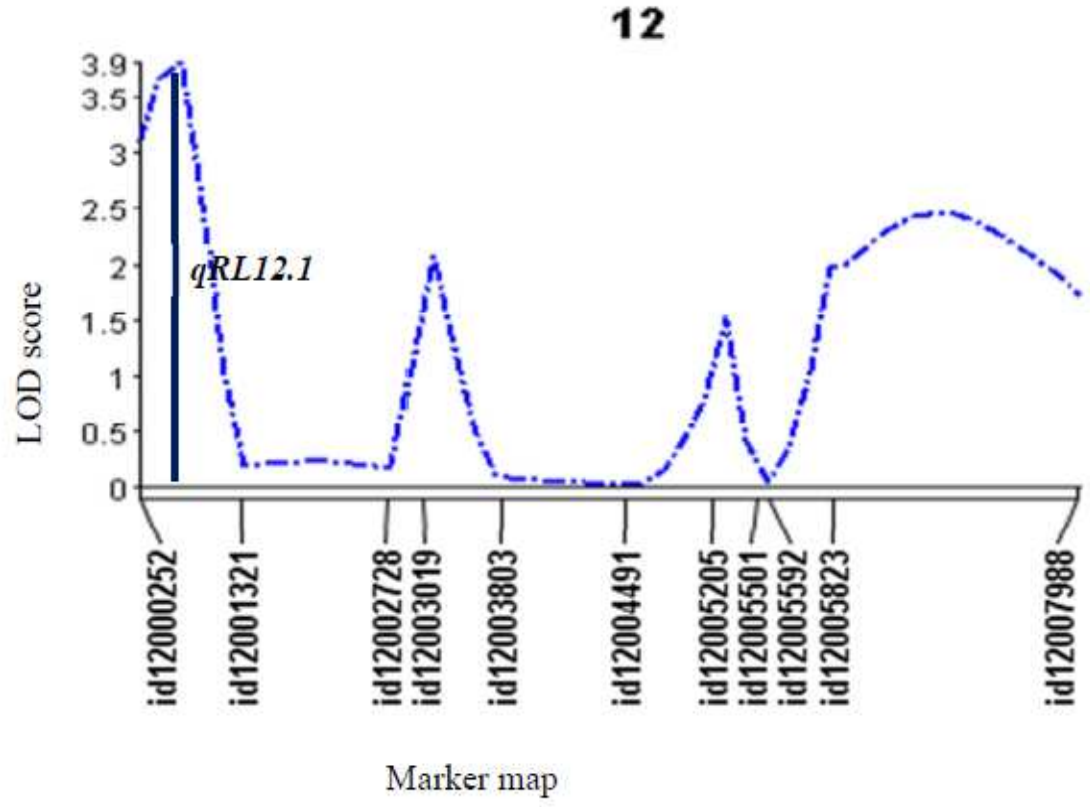

Figure 22: LOD plots showing position of putative QTL for root length (RL) on chromosome 12

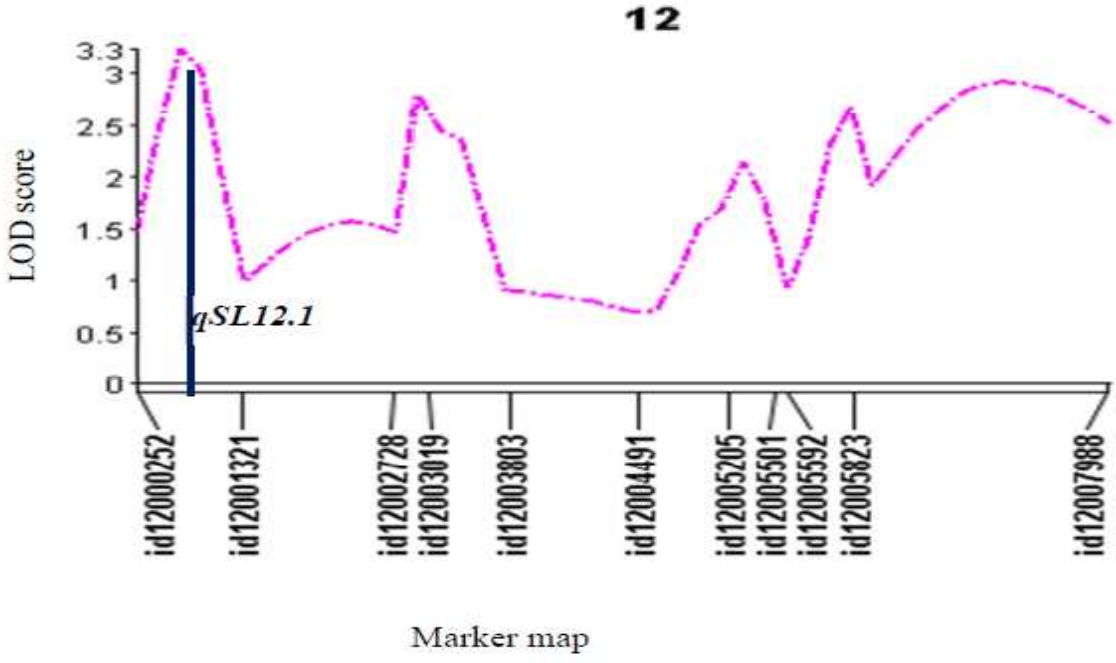

Figure 23: LOD plots showing position of putative QTL for shoot length (SL) on chromosome 12

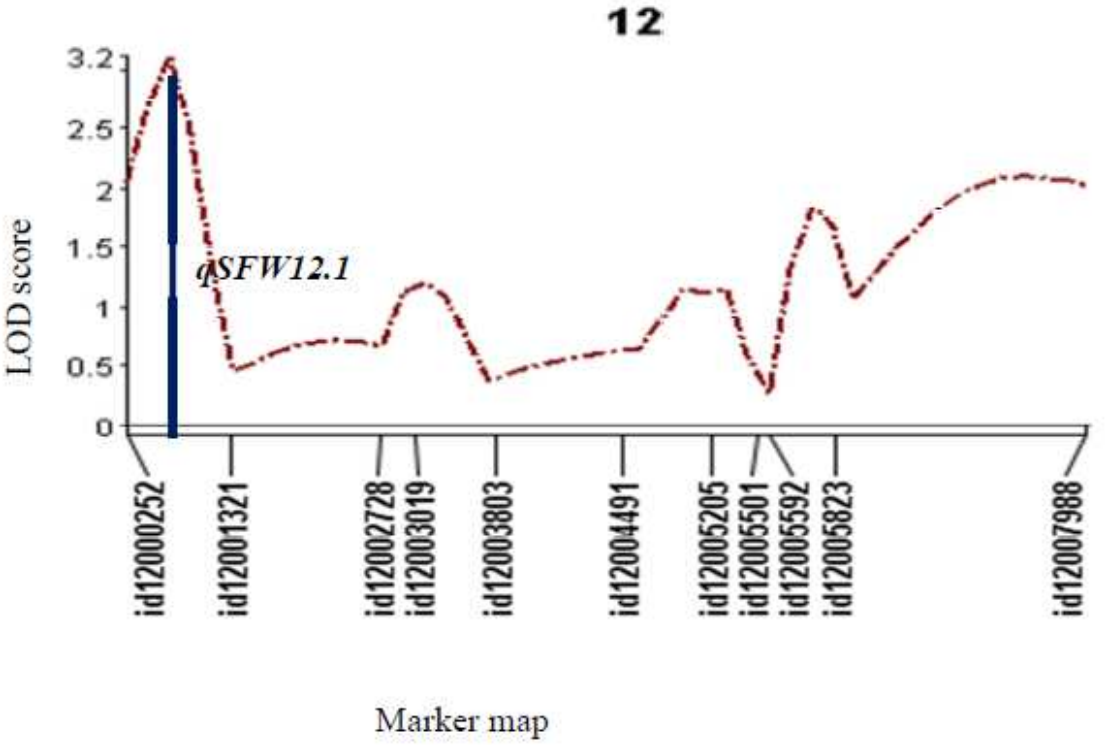

Figure 24: LOD plots showing position of putative QTL for shoot fresh weight (SFW) on chromosome 12

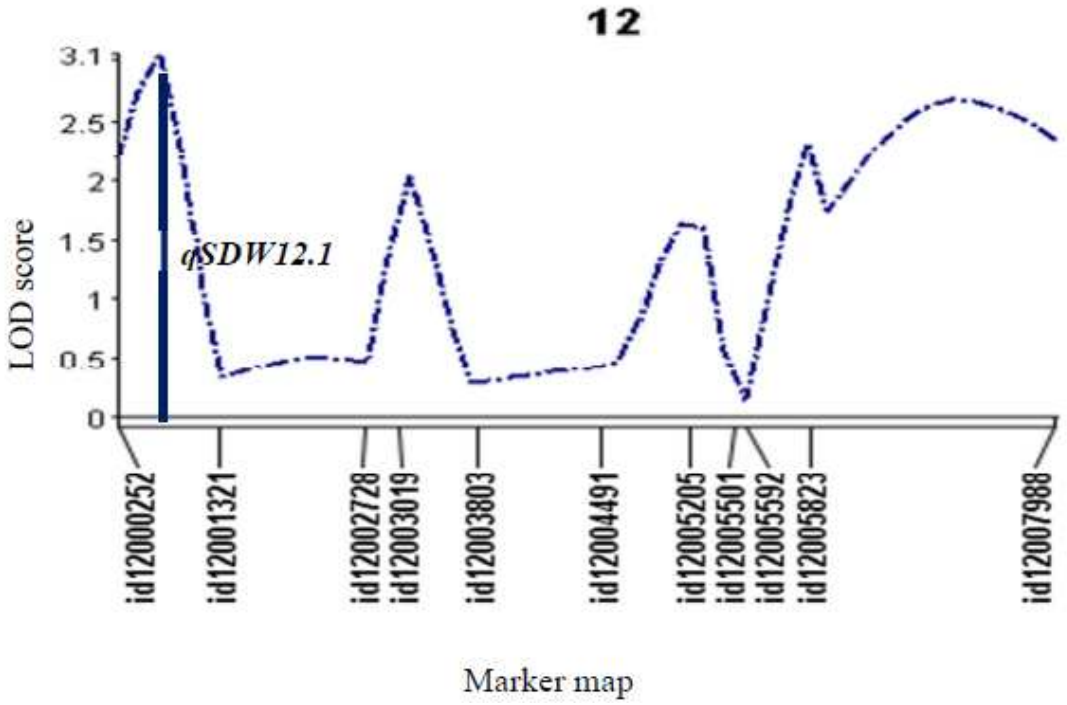

Figure 25: LOD plots showing position of putative QTL for shoot dry weight (SDW) on chromosome 12

4. 7. 2 Final salinity tolerance scores

Two putative QTLs, *qSESF2.1* and *qSESF12.1*, conferring salt tolerance after 25 days of salt stress (12dSm^{-1}) at the young seedling stage were mapped on chromosome 2 and 12 respectively. *qSESF2.1* was flanking to id2004774 and id2007526, and *qSESF12.1* toid12000252 and id12001321. The phenotypic variation explained by *qSESF2.1* and *qSESF12.1* were 11.1 and 10.6 % respectively. The parental additive effects of the two QTLs were -2.145 and -1.332 respectively. The IR29 allele increased the salinity injury scores at both loci. In other words, *qSESF2.1* contributed an additional value of 2.145 SES to the final salinity injury scores; and *qSESF12.1* contributed 1.332 SES (Table 12 and Fig. 19).

4. 7. 3 Root length

Two putative QTLs, *qRL6.1* and *qRL12.1,* conferring RL at the young seedling stage were mapped on chromosome 6 and 12 respectively. The *qRL6.1* which its location coincided with id6003318 marker was flanking to fd13 and id6004343, while *qRL12.1* was flanking to id12000252 and id12001321.The phenotypic variation explained by *qRL6.1* and *qRL12.1* were 12.1 and 11.5% respectively. The parental additive effects of the two QTLs were 1.496 and 2.378 respectively. The Hasawi allele increased the root length at both loci. In other words, *qRL6.1* contributed an additional value of 1.496cm to root length; and *qRL12.1* contributed 2.378cm (Table 12 and Fig.19).

4. 7. 4 Shoot length

Four putative QTLs; *qSL1.1* and *qSL1.2* (chromosome 1), *qSL6.1* (chromosome 6), *qSL12.1* (chromosome 12), conferring SL at the young seedling stage were mapped. The flanking markers were id1023892 and id1024836, id1024972 and id1025983, fd13 and id6004343, and id12000252 and id12001321 for *qSL1.1, qSL1.2, qSL6.1,* and *qSL12.1* respectively. The phenotypic variation explained by *qSL1.1, qSL1.2, qSL6.1,* and *qSL12.1* were 20.6, 11.8, 12.1, and 9.8 % respectively. The alleles' contribution levels from the parent were 5.262, 4.049, 3.631, and 5.34 respectively. The Hasawi allele increased the shoot length at all four loci. In other words, *qSL1.1* contributed an additional value of 5.262 cm to shoot length; *qSL1.2* contributed 4.049 cm; *qSL6.*1 contributed 3.631 cm; and *qSL12.1* contributed 5.34 cm (Table 12 and Fig.19).

For all QTLs identified, *qSL1.1* was unique because it contributed to the phenotypic variation explained with a high R^2 (20.6) and high LOD (7.21).The peak (a) of LOD of this QTL is illustrated on Fig. 26. The *qSL1.1* was flanking to *qSL1.2* which contributed to the phenotypic variation explained with R^2 =11.8 with a LOD of 3.93 (peak b) (Table12).

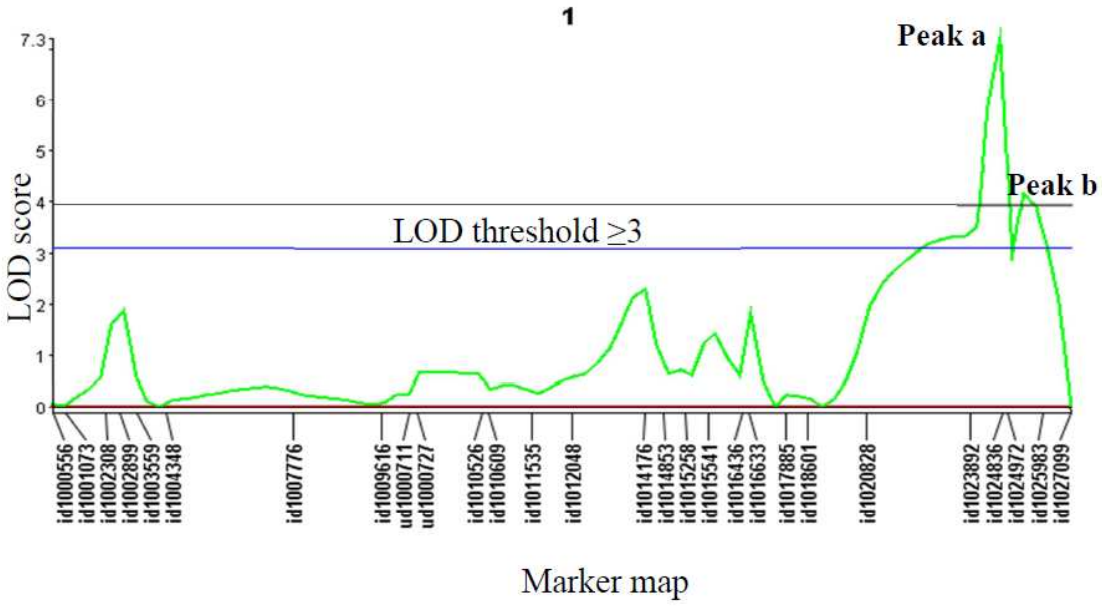

Figure 26: LOD plots showing position of two putative QTLs for shoot length on chromosome 1

The height of peaks is measured on a LOD scale along Y-axis.

4. 7. 5 Root fresh weight

Two putative QTLs, *qRFW4.1* and *qRFW9.1,* conferring RFW at the young seedling stage were mapped on chromosome 4 and 9.The flanking markers were id4007444 and id4008092, and id9001614 and id9002014 for *qRFW4.1* and *qRFW9* respectively. The phenotypic variation explained by *qRFW4.1* and *qRFW9.1* was 10.3 and 10.1% respectively. The alleles' contribution levels from the parent were 0.100 and 0.281 respectively. The Hasawi allele increased the root fresh weight at both loci. In other words, *qRFW4.1* contributed an additional value of 0.100g to the root fresh weight; and *qRFW9.1* contributed 0.281g (Table 12 and Fig.19).

4. 7. 6 Shoot fresh weight

Three putative QTLs; *qSFW4.1*, *qSFW9.1* and *qSFW12.1*, conferring SFW at the young seedling stage were mapped on chromosome 4, 9 and 12. The flanking markers were id4001113 and id4001932, id9001614 and id9002014, andid12000252 and id12001321

for*qSFW4.1, qSFW9.1* and *qSFW12.1* respectively. The phenotypic variation explained by *qSFW4.1, qSFW9.1* and *qSFW12.1* were 12.8, 12.6 and 9.5% respectively. The alleles' contribution levels from the parent were 0.571, 0.684 and 0.343 respectively. The Hasawi allele increased the shoot fresh weight at all three loci. In other words, *qSFW4.1* contributed an additional value of 0.571g to the shoot weight; *qSFW9.1 1* contributed 0.684g; and *qSFW12.1* contributed 0.684g (Table 12 and Fig.19).

4. 7. 7 Root dry weight

One putative QTL, *qRDW4,* conferring RDW at the young seedling stage was mapped on chromosome 4. The flanking markers for this QTL were id4001113 and id4001932. The phenotypic variation explained by *qRDW4* was10.0%. The alleles' contribution level from the parent was 0.022. The Hasawi allele increased the root dry weight at this locus. In other words, *qRDW4* contributed an additional value of 0.022g to the root dry weight (Table 12 and Fig.19).

4. 7. 8 Shoot dry weight

Three putative QTLs; *qSDW2.1, qSDW8.1* and *qSDW12.1*, conferring SDW at the young seedling stage were mapped on chromosome 2, 8 and 12. The flanking markers were id2004774 and id2007526, id8006485 and wd8004122, and id12000252 and id12001321 for *qSDW2.1, qSDW8.1* and *qSDW12.1* respectively. The phenotypic variation explained by *qSDW2.1, qSDW8.1* and *qSDW12.1* were 11.6, 9.4 and 9.3% respectively. The alleles' contribution levels from the parent were 0.132, 0.072 and 0.071 respectively. The Hasawi allele increased the shoot dry weight at all three loci. In other words, *qSDW2.1* contributed an additional value of 0.132g to shoot dry weight; *qSDW8.1* contributed 0.072g; and *qSDW12.1* contributed 0.071g (Table 12 and Fig.19).

4. 8 SSR Markers Corresponding to the Position of SNP Markers Used

For the purpose of getting an idea on SSR markers which could be at the same position as the SNP markers used during this study, a comparison of SNP and SSR markers position for the same QTL identified at specific position was made. Appendix 8 shows the position of the QTL identified flanking SNP and SRR markers and their position in cM . The positions in cM of the SSR markers referred to were found in the McCouch *et al.* (2002) publication.

CHAPTER FIVE

5. 0 DISCUSSION

Salt tolerance is a complex trait and understanding of its molecular basis is essential for breeding and makeover in crop plants (Chinnusamy *et al*., 2005). The field screening is the most ideal method for identifying adapted and tolerant genotypes; however spatial variability in the field allows the possible escapes. As such, field screening may have limitations if proper care is not taken. Therefore, salinized nutrient solution culture was used to screen the $F_{5:6}$ RILs population derived from cross IR29/Hasawi for salinity tolerance at seedling stage. Recent progress and technical advances in molecular/DNA markers technology facilitate the mapping of major genes for abiotic and biotic stresses in rice. They have advantages as well as disadvantages in terms of implementation, cost, polymorphism, dominance, abundance, reproducibility, and throughput. Single nucleotides polymorphism (SNP) markers were used in the present study.

5. 1 Parental Diversity

The parents of mapping populations must have sufficient variation for the traits of interest at both the DNA sequence and the phenotypic level (Meksem and Kahl, 2005; Semagn *et al*., 2006c). The significant differences were found between Hasawi and IR29 for the morphological studied traits and their relative percentage reduction were due to the salinity stress. This confirmed the sensitivity of IR29 and tolerance of Hasawi under salt stress, and therefore IR29 and Hasawi were significantly different.

The polymorphism level (50.52%) found between the parents in this study was much better than in some earlier studies. Therefore, it was good enough to be used for QTL analysis and linkage map as well. For example, Ammar *et al.* (2007) when mapping QTLs for salinity tolerance at seedling stage, among 471 STMS and EST markers, surveyed only

89 (18.88 %) polymorphism. Islam *et al*. (2011), also mapping QTL for salinity tolerance at seedling stage, over 260SSR and two EST markers, only 90 markers (34.35%) were polymorphic. Only 65 over 395 SSR markers (16.45%) were clearly polymorphic on polyacrylamide gels when Alam *et al.* (2011) investigated seedling-stage salinity tolerance QTLs using backcross lines derived from Pokkali.

Genetically and phenotypically, the results obtained during the present study showed significant difference between Hasawi and IR29. This confirms that the RILs mapping population derived from the cross between IR29xHasawi were suitable for mapping of the QTLs for salinity tolerance traits. These results fit to the suggestion of Flowers (2004) to use the variation which is already present in existing crops to enhance salinity tolerance.

5. 2 Phenotypic Variation of Genotypes and Salt Effect at Seedling Stage

In general growth and morphology of rice seedlings were greatly affected by salt stress, as reflected by injury symptoms such as leaf tip burning and slower formation of new leaves, whitish leaf tips, and ceasation of growth and dying of some plants (Singh and Flowers, 2010). The differences were highly significant ($P<0.001$) among RILs for RL, SL, RFW, SFW, RDW, and SDW. Similar results were obtained by Hosseini *et al*. (2012). The mean distributions for these traits showed transgressive segregation but was skewed to lower values and continuous variation. The frequency distribution of RL and SL of the RILs fitted the normal distribution.

Apparently, most of these traits were skewed towards IR29 (sensitive) parent; but mostly with negative effects on seedling vigour under salt stress, except for RL and SL. This finding agrees with the results of Haq (2009), who suggested that alleles from the salt sensitive parent decreased shoot fresh weight, when studying salinity tolerance of F_9 recombinant inbred lines (RILs) from the Co39/Moroberekan cross.

Genetically, transgression is defined as the appearance of individuals in segregating population that fall beyond the parental phenotypes (Tanksley, 1993). In this mapping population, lines having phenotypic values greater than the higher parent and lesser than the lower parent were observed for all these traits. It was observed that, for the traits studied including RL, SL, RFW, SFW, RDW, and SDW, there was a transgressive segregation in both directions on different extents. The observation of transgressive segregation with extreme salt tolerance under salt stress is promising for the prospect of developing rice cultivars or potential donors with salt tolerance greater than that of Hasawi. The transgressive segregants might have accumulated the positive genes/QTLs conferring salt tolerance from both parents. The distribution for each trait indicated also that the traits are controlled by multiples genes and that Hasawi and IR29 are both contributing for these traits. Furthermore, Akbar *et al.* (1985) reported that at seedling stage, dry biomass of rice under salt stress is affected by at least two groups of genes with additive effects.

Rick and Smith (1953) proposed three reasons for the occurrence of interspecific transgression: (a) *de novo* mutation induced by the wide cross itself, (b) complementary action of genes from the two parental species, and (c) unmasking of recessive genes normally held heterozygous in the wild species. For this study, the transgression could be due to the first reason. First, the results of SNP genotyping results showed genetic difference between the sorted RILs and secondly, the QTL analysis showed the contribution of the two parents (Hasawi and IR29) to the variation of the phenotypes. This observation confirmed that, even though the percentage of homozygosity increases with the number of generation (for this study RIL inbreeding generation were $F_{5:6}$) and therefore the percentage within lines (homozygosity were 92.25 % in this study), there was a certain percentage of segregation.

5. 2. 1 Salt tolerance of recombinant inbred lines through SES

The results of phenotypic response of rice genotypes to salinity stress at the seedling stage indicated that there were varied genotypic responses. The salt tolerance scoring discriminated the highly sensitive and sensitive from the tolerant and the moderately tolerant genotypes. At the same time, the transgressive segregation (offspring were outside the range of the parental phenotypes) was observed on both parental sides. But after 25 days under salt stress (SESF), the trend changed and the frequency distribution for the salinity tolerance scores among the genotypes were skewed towards the sensitive parent. This transgressive segregation in RILs confirmed that the salinity tolerance is a polygenic character. Flowers and Flowers (2005) stated that transgressive segregation in offspring is one of the characteristics of multiple gene inheritance, an polygenic inheritance.

The final average salinity tolerance score of Hasawi was low, i.e. 1.9, which indicated and confirmed the high tolerance at EC=12dSm^{-1}while IR29 was scored 9, the highest score in system of evaluation as described by Gregorio *et al*. (1997). These results confirmed the results of several previous and recent researches (Gregorio, 1997; Mohammadi-Nejad *et al*., 2010; Kanjoo, 2011; Kanawapee *et al*., 2011; Hosseini *et al*., 2012; Saeedipour, 2012; and Mansuri *et al*., 2012) suggesting the susceptibility of IR29 to salt stress.

These findings suggested that, during screening for salinity tolerance at seedling stage, one must consider salinity level and duration of the stress since the results showed that the distribution of SES shifted from normal, after 12 days of stress, to skewed behaviour towards the sensitive parent after 25 days of salt stress. Combining together the traits including SESF (from high tolerant to moderate tolerant), RL, SH, RFW, SFW, RDW and SDW, 67 lines drew more attention and therefore could be tested in field conditions to check their performance.

5. 2. 2 Growth reduction due to salinity and correlation between evaluated traits stress

The estimation of percentage of reduction of the traits (RL, SL, RFW, SFW, RDW, and SDW) was estimated in order to see at which extent salinity stress affected these traits at seedling stage. According to Yadav *et al*. (2011), the salinity level between EC 6 to15dSm^{-1}is classified as high. Consequently, the salinity level (EC 12 dSm^{-1}) used in the study was high. The morphological traits assessed, generally, were severely affected by the salinity and showed significant reduction. This confirmed the previous findings of several authors (Munns and Tester, 2008; Yadav *et al*., 2011 and Rad *et al*., 2012). The high salinity affects on rice plants by disturbing the capacity of roots to extract water and by inhibition of many physiological and biochemical processes and thereafter reduction of plant growth, development and survival.

Surprisingly, at the same time root had strange reaction vis-à-vis to salinity stress which was expressed by RL, RFW, and RDW for salinity tolerance (SESF) being lesser than 4.3, lesser than 1.7 and equal to 1 respectively. These findings were similar with the argument of Läuchli and Grattan (2007) who stated that with an adequate supply of calcium, salinity stress reduces shoot growth, particularly leaf area, more than root. This is true because the phenotyping was done in hydroponic system with adequate supply of calcium through nutrient solution. The effect of salt concentration on root growth was comparatively less severe than that observed on shoots growth (Munns, 2002; Shereen *et al*., 2007). In this study even IR29, the sensitive parent, showed lower reduction in RL (32.65%) which confirmed that salt affects the roots less than shoots. According to Munns and Tester (2008), in drying soils shoot growth is more sensitive than root growth probably because a reduction in the leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent salt concentration

in the soil. But, during this study the nutrient solution was used, there was no reason to the plant to conserve the moisture and prevents salt concentration in the solution. Thus, there may be other unknown reasons to explain our findings.

The correlation between traits was computed by regressing phenotypic values of one trait on those of other traits. The results showed highly significant and positive correlations between RL, SL, RFW, and SFW (at 1% level of probability). Mansuri*et al*. (2012) when using a population of 40 genotypes (20 Iranian landraces, 19 improved rice cultivars and 2 foreign genotypes) with Pokkali (tolerant check) and IR29 (sensitive check) and tested in salt stress (0, 6, 8 and 12 dSm^{-1}) found significant correlations detected between height of seedling and root dry weight.Similarly, Haq (2009) found significant and positive correlation between SFW and SDW. The inverse and highly correlation between final salinity injury scores (SESF), and others viz. RL, SL, RFW, SFW, RDW, and SDW implied that salt tolerant genotypes (having lower salt tolerance score) exhibited higher root and shoot length, fresh and dry root weight, and higher fresh and dry shoot weight. But, the percentage of reduction of each trait is significant and positively correlated with SESF, implies that salt sensitive genotypes (having higher salt tolerance score) exhibited higher percentage of reduction of root and shoot length, fresh and dry root weight, and higher percentage of reduction of fresh and dry shoot weight. The significant correlation between morphological parameters including RL, SL, RFW, and SFW at seedling stage suggested that these traits were appropriate to be used for identification of tolerant genotypes of rice in salt stress.

Highly significant difference among the RILS for the percentage of reduction of RL, SL, RFW, SFW, RDW, and SDW observed in this study was similar with the results obtained by Shereen *et al*. (2007).

5. 3 Single Nucleotide Polymorphism Marker Survey and Construction of Genetic Linkage Map

In this study, the 384 SNP chip tried to cover the whole genome evenly. Some marker intervals were relatively large due to the lack of sufficient polymorphic markers within these intervals. Thus, the large gaps in these chromosomes, where there was no marker (polymorphic or monomorphic) could result into missing some probable QTLs for traits related to salt tolerance.

The density of the current linkage map (194 SNP markers) is slightly higher than the density of some maps published earlier. To construct a linkage map, Koyama *et al.* (2001) used only 28 RFLPs during their study. Masood *et al.* (2004) mapped QTL for salt tolerance by using only 74 RFLP markers. Ammar *et al.*, (2007) used 89 STMS and EST markers to map QTLs for salinity tolerance at seedling stage, while only 65 SSR markers were used by Alam *et al.* (2011).

It is also important to note that for the RILs used for this study, 92.3% of homozygosity was observed. This result agreed with the theoretical expectation percentage of homozygosity in $F_{5:6}$ RILs generation (92.25%) which was observed by Vinod (2006).

5. 4 Quantitative Trait Loci in $F_{5:6}$ Recombinant Inbred Lines Grown Under Stressed Conditions

Composite interval mapping (CIM) method was chosen because it combines interval mapping with linear regression andincludes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Collard *et al*., 2005). The main advantage of CIM is that it is more precise and effective at mapping QTLs compared to single-point analysis and interval mapping, especially when linked QTLs are involved. This study allowed identification of 20 QTLs linked to RL, SL, RFW,

RDW, SFW, SDW, SESI, and SESF. They were located on chromosomes number 1, 2, 4, 6, 8, 9, and 12.

Both parents were found to possess QTL alleles which increased phenotypic values. For this mapping population (142 RILs) used to identify the QTLs associated to salinity tolerance at seedling stage, the results suggested that the increases of initial and final salinity tolerance scores were contributed (ranged from 1.333 to 2.145 SES) by the alleles from the sensitive parent (IR29). This parental contribution values were high compared with the results of Lee *et al*. (2006). The authors, for the two QTLs identified, found the parental contribution equal to 0.89 and 0.57 when they were mapping QTLs for salinity tolerance at seedling stage using 164 $F_{18:19}$ recombinant inbred lines derived from a cross between Milyang23 (indica/japonica) and Gihobyeo (japonica). The increases of RL, SL, RFW, SFW, RDW, and SDW under salt stress were contributed by the alleles from the tolerant parent. This was confirmed by the additive effect observed during identification of QTLs affecting these traits.

Several studies related to identification of QTLs for salinity tolerance in rice at seedling stage and using different mapping population and markers in different locations, have been undertaken (Gregorio, 1997; Masood *et al*., 2004; Lee *et al*., 2006, Ming-zhe *et al*., 2005; Ammar *et al*., 2007; Haq, 2009; Thomson *et al*., 2010; Islam *et al*., 2011, Kanjoo *et al*., 2011). But it is difficult to compare the chromosomal locations of QTLs directly because different materials and molecular markers were used. During the present study, contrary to what one might expect, there were no QTLs found in *Saltol* region located between 14.7-18.6 cM (Singh *et al*., 2007) on short arm of chromosome 1.

5. 4. 1 QTLs associated with the initial and final salinity scores

Two QTLS associated with initial salinity scores were identified on chromosome 1 and 12 while the two QTLs identified for final salinity scores were located on chromosome 2 and 12. QTLs associated with initial and final salinity scores were located at same position on chromosome 12. This means that the genes controlling both traits are pleiotropic on other hand. Thomson *et al*. (2010) found two QTLs associated with final SES tolerance scores on chromosome 4 and 9. Six QTLs linked to salinity injury scores were identified on chromosome 1, 3, 4, and 5 by Ammar *et al*. (2007). The two QTLS which were located on chromosome 1, one was located on short arm close to RM84 at 0.11 cM position while the other one was on long arm at 14 cM from RM572. These results suggested that these traits were controlled by multiple genes (polygenes) under salt stress. In addition to that, these previous findings suggested that *qSESI2.1*, *qSESF2*, *qSESF12.1*, and *qSESI12.1* were new QTLs for the salt tolerance.

5. 4. 2 QTLs associated with the root length

The two QTLs associated with root length were identified on chromosome 6 and 12. One QTL associated to root length with $R^2 = 23.0$ was found on chromosome 2 by The (2010) when using Kalarata/Azucena crosses mapping population. Sabouri and Sabouri (2008) found one QTL and two QTLs on chromosome 7 and 9 respectively. These findings showed that the previous works found QTLs which controlled root length at different chromosomes comparing with the results of this study and therefore confirmed that *qRL6.1* and *qRL12.1* were also novel QTLs. Also, the results suggest that root length were controlled by different genes.

5. 4. 3 QTLs associated with the shoot length

In this study, four QTLS associated with shoot length were identified on chromosome 1, 6 and 12. Two QTLs were located at long arm of chromosome 1, while Sabouri and Sabouri

(2008) identified one QTL on chromosome 3 and another was on chromosome 10. The (2010) found one QTL on short arm of chromosome 1. Singh *et al*. (2007) indicated that some traits based QTLs associated with shoot length have been identified on short arm of the chromosome 3 and on long arm of chromosome 7. Thomson *et al*.(2010) found two QTLs associated with seedling height on chromosome 2 and 4. These findings suggested that there were several genes controlling shoot length under salinity stress. Furthermore, since *qSL1.1*, *qSL1.2*, *qSL6.1*, and *qSL12.1* were located at different positions compared with the QTLs identified during previous studies, they were therefore novel QTLs associated with shoot length. In addition, with regards to the contribution of 20.6 % in total phenotypic variation explained by *qSL1.1*, this QTL had main-effect on expression of salt tolerance for root length.

5. 4. 4 QTLs associated with the root fresh weight

Two QTLS associated with root fresh weight were identified on chromosome 4 and 9. This means that these traits were controlled by at least two different genes. The (2010) found one QTL on chromosome 4 and 11, however the position of the QTL identified on chromosome 4 was different with the one identified in the present study. Singh *et al*. (2007) indicated that two QTLs for root weight were identified on chromosome 3 and 9. These results confirmed that root fresh weight was controlled by many genes. Comparing the above previous findings with the results of the present work, *qRFW4.1* and *qRFW9.1* were new QTLs associated to root fresh dry weight.

5. 4. 5 QTLs associated with the shoot fresh weight

The two QTLs identified for shoot fresh weight suggested that this trait were controlled by at least two different genes since they were identified on two different chromosomes 4 and 12. Singh *et al*. (2007) indicated that one QTL for shoot weight were identified on chromosome 11. One QTL was identified on chromosome 1 (The, 2010), two QTL were

identified on chromosome 1 and 3 (Sabouri and Sabouri, 2008), and Haq (2009) identified one QTL on chromosome 1 at 44.1 cM position. Thus, shoot fresh weight was controlled by multiples genes and also the associated QTL to that trait were new since they were located on different chromosomes comparing with the mentioned previous findings.

5. 4. 6 QTLs associated with the root dry weight

During this study, a single QTL was identified on chromosome 4 to be associated with root dry weight. But in previous studies, one QTL was identified on chromosome 3, two QTLs were identified on chromosome 5, and two QTLs on chromosome 9 (Sabouri and Sabouri, 2008). The (2010) identified two QTLs on chromosome 1 and 5. Based on regression ANOVA, eight QTLs were identified on chromosome 1, 3, 8 and 11 by Masood *et al.* (2004). These findings suggest that this QTL was new and could be controlled by many genes since the previous works shown that many QTLs have been identified on different chromosomes.

5. 4. 7 QTLs associated with the shoot dry weight

The two QTLs identified for shoot dry weight suggested that this trait were controlled by at least two different genes since they were identified on two different chromosomes 2 and 12. For the same trait, Masood *et al.* (2004) identified 14 QTLs distributed through different chromosomes viz. chromosome1, 3, 6, 8, 9, 10, and 11; The (2010) found one QTL on chromosome 1. Whereas, Sabouri and Sabouri (2008) identified two QTLs located on different chromosomes (on 3 and 7) compared with the two identified in this study. Even though Haq (2009) identified one QTL on chromosome 1, and its position was 120.10 cM while in this study, the QTL identified was located at 62.8 cM. Therefore these findings suggested that this trait were controlled by many genes and the linked QTLs were new.

Over the 20 QTLs identified, 11 QTLs were linked to the shoots either to length or fresh and dry weight. Singh *et al.(*2007) in their overview of the progress made towards linkage mapping to salt tolerance-QTL mapping for salinity tolerance in Rice- showed that QTLs for seedling length and dry weight have been identified on chromosome 6, salt tolerance at vegetative and reproductive stage on chromosome 8 at 6.3cM position, and root weight on chromosome 9 at 16.1cM. These findings are very encouraging since it is known that the shoot growth is severely affected with the salt stress than roots (Läuchli *et al*., 2007) and paved the chances of discovering the unknown genes candidate (Munns and Tester, 2008) for shoot growth.

Interestingly, among all 20 QTLs identified, six were located on chromosome 12; five QTLs were located at 6.9 cM position while only one was located at 84.9cM. Two QTLs were located at the same position (18.1cM) on chromosome 4; two QTLs at same position (18.5cM) on chromosome 6; and also two QTLs were located at the same position (27.0cM) on chromosome 9. The fact that these QTLs were located at the same position, suggested that one or a group of genes control the assessed traits.

The traits viz. SESI, SESF, RL, SL, RFW, SFW, RDW and SDW which were controlled with these identified QTLs were significantly correlated. According to Veldboom *et al*. (1994) and Xiao *et al*. (1996), often the correlated traits have QTLs mapping to the same chromosomal location. For example, in this study, RL and SESF was highly correlated (r= -0.581**), similarly were SDW and SFW (r= 0.891**). Trait correlation may result from either pleiotropic effects of genes or from tight linkage of several genes controlling the traits. This suggests that QTL affecting RL (*qRL12.1*) is closely linked with the QTL (*qSESF12.1*) affecting final salinity tolerance scores (SESF) in that region of chromosome12. Likewise, the QTLs affecting SDW (*qSDW12.1*) and SFW (*qSFW12.1*) are closely linked.

The identified QTLs certainly do not comprise the entire set of genes which affect the traits under study, but only a subset of genes, mainly because of the limited number of polymorphic markers scored through the genome. For instance, the interval between the flanking markers of the QTL identified on chromosome 12 (*qSESI12.1*) was high which indicated that within this interval there should be more QTLs. Consequently, QTLs occur in chromosomal gaps where additional markers are needed to be added to increase the precision of QTLs detection. Thomson *et al.* (2010) identified two QTLs on chromosome 12 affecting initial and final salinity tolerance scores when they were characterizing *Saltol* region. Also, the linkage map showed that the interval between the flanking markers of these two QTLs were high. These two studies suggested that in this region of chromosome12 there were QTLs affecting salinity tolerance scores. Furthermore, the SSR markers which are comprise between RM7018 to RM3331 (75.8 to 89.5cM) where the QTL *qSESI12.1* was detected during this study, should be surveyed for polymorphism and used in genotyping of QTLs for salt tolerance. Haq (2009) found a QTL affecting shoot dry weight on chromosome 2 like in this study but at 120.10 cM position of their genetic map with RM1287 as the nearest markers.

The findings of this study were in agreement with the statement of previous authors who found that salinity tolerance is controlled by multiples genes (Singh *et al.*, 2007; Baby *et al*., 2010; Singh *et al.*, 2010). Furthermore, the results of the previous works revealed that most of putative QTL/genes for salinity tolerance were located at different positions compared with the QTLs identified during this study which revealed new position for QTLs linked to salinity tolerance at seedling stage. This could be due to the use of novel source of salinity tolerance which has not been used so far in any salt-tolerance related studies. The novelty of QTL could also be attributed to the use of SNP markers in this study with novel source of salinity tolerance. Previous studies used different kind of

molecular markers from RFLP, AFLP, CAPs and SSRs while this study employed SNPs to identify the precise relationship between phenotypic variance for the studied traits and polymorphism. This suggested, in addition to numerous genetic markers used to-date, the SNP markers can be used to explore and identify more novel QTLs for salinity tolerance at seedling stage and at later stages of rice growth. The study also suggested that novel sources for salinity tolerance should be explored to identify novel QTL so that degree of salinity tolerance in the improved genotypes could be enhanced by deploying multiple QTLs for different component mechanism in desired genetic background.

CHAPTER SIX

6. 0 CONCLUSIONS AND RECOMMENDATIONS

6. 1 Conclusions

Development in molecular marker assisted selection permit the rapid and accurate selection of individuals that contain QTLs/genes for salt tolerance. In this regard several molecular markers have been developed. Two experiments were conducted to identify the big effect QTLs associated with salinity tolerance using 300 RILs developed from crosses between contrasting parents i.e. sensitive parent IR29 and a novel salinity tolerant genotype Hasawi under salinity stress conditions. Therefore, the results showed that:

i. The two parents as well as the RILs showed significant differences for all the assessed traits and 67 RILs had a good performance under salinity stress;
ii. The mapping population developed from cross between Hasawi and IR29 was suitable to genotype QTLs for salinity tolerance. Consequently, Hasawi, tolerant check and parent is really a novel source of salinity tolerance;
iii. The polymorphism survey of 384 SNP markers done out of which 50.52 % were polymorphic and distributed with different extent through the 12 chromosomes of rice, helped in construction of genetic linkage map;
iv. Contrary to what one might expect, no QTL was found in *Saltol* region of chromosome 1, meanwhile a QTL with relatively high R^2 and high LOD was identified at the distal region of the long arm of the same chromosome. In addition to that, chromosome 12 deserved more attention;
v. A total of 20 putative QTLs were identified on chromosome 1, 2, 4, 6, 8, 9, and 12 using phenotypic traits of 142 RILs sorted based on SES and percentage of reduction of root and shoot dry weight;

vi. All the QTLs occupied new positions in the rice genome compared with the results of the previous studies. These QTLs could be useful to enhance the level of salinity tolerance through marker assisted selection for the pyramiding of different QTLs in one background.

6. 2 Recommendations

Thus, in order to complete this study, the following research topics are recommended to be undertaken:

i. Evaluation of the phenotypic response of this population (IR29×Hasawi) under saline field conditions, up to maturity;
ii. Conduct a fine mapping of these novel QTLs using the same population as well as in a different genetic background.

REFERENCES

Abrol, I. P., Yadav, J. S. P. and Massoud, F. I. (1988). Salt-Affected Soils and their Management. Soil Resources Management and Conservation Service. *FAO soils bulletin 39*. [http://www.fao.org/docrep/x5871e/x5871e00.htm] site visited on 22/10/2011.

Acquaah, G. (2007). *Principles of Plant Genetics and Breeding*. Blackwell Publishing, Oxford. 498pp.

Ahmadi, J. and Fotokian, M. H. (2011). Identification and mapping of quantitative trait loci associated with salinity tolerance in rice (*Oryza Sativa*) using SSR markers. *Iranian journal of biotechnology* 9(1): 21 - 30.

Ahmed, M. A., Islam, M. M., Begum, S. N., Halder, J. and Ahmed, M. H. (2010). Evaluation of F_3 rice lines of JANGLIBORO × BRRI Dhan40 for salt tolerance using SSR markers. *International Journal of Bioresearch* 1(1): 01 - 05.

Akbar, M., Khush, G. S. and Hillerislambers, D. (1985). Genetics of salt tolerance in rice. In: *Rice Genetics.Proceeding of International Rice Genetic Symposium.* (*Editing, design, and production by: Island Publishing House, Inc.*), Manila, Philippines, IRRI. pp. 399 - 409.

Alam, R., Sazzadur Rahman, M., Seraj, Z. I., Thomson, M. J., Ismail, A. M., Tumimbang-Raiz, E. and Gregorio, G. B. (2011). Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. *Plant Breeding* 130: 430 - 437.

Ammar, M. H. M., Singh, R. K., Singh, A. K., Mohapatra, T., Sharma, T. R. and Singh, N. K. (2007). Mapping QTLs for salinity tolerance at seedling stage in rice (*Oryza sativa* L.). *African Crop Science Conference Proceedings* l(8): 617 - 620.

Angaji, S. A. (2009). QTL Mapping: A Few Key points.*International Journal of Applied Research in Natural Products* 2(2): 1 - 3.

Australian Centre for Plant Functional Genomics (2010). Identification of genes that help wheat and barley maintain yield in high saline environments.Salt group PhD Research Areas.[http://www.sciences.adelaide.edu.au/pgprograms/research/Salt%20Group%20PhD%20Portfolio.pdf] site visited on 02/10/2011.

Baby, J., Jini, D. and Sujatha, S. (2010). Biological and physiological perspectives of specificity in abiotic salt stress response from various rice plants. *Asian Journal of Agricultural Sciences* 2(3): 99 - 105.

Bennet, M. D. and Smith, J. B. (1976). Nuclear DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society* B272: 227 - 274.

Bhowmik, S. K., Islam, M. M., Emon, R. M., Begum, S. N., Siddika, A. and Sultana, S. (2007). Identification of salt tolerant rice cultivars via phenotypic and marker-assisted procedures. *Pakistan Journal of Biological Sciences* 10: 4449 - 4454.

Bhowmik, S. K., Titov, S., Islam, M. M., Siddika, A., Sultana, S. and Haque, M. D. S. (2009). Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *African Journal of Biotechnology* 8(23): 6490 - 6494.

Bonilla, P., Dvorak, J., Mackill, D., Deal, K. and Gregorio, G. (2002). RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philippine agricultural scientist* 85: 68 - 76.

Chen, H., He, H., Zou, Y., Chen, W., Yu, R., Liu, X., Yang, Y., Gao, Y. M., Xu, J. L., Fan, L. M., Li, Y., Li, Z. K. and Deng, X. W. (2011). Development and application of a set of breeder-friendly SNP markers for genetic analyses and molecular breeding of rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 123: 869 - 879.

Chinnusamy, V., Jagendorf, A. and Zhu, J. K. (2005). Understanding and improving salt tolerance in plants. *Crop Science* 45:437 - 448.

Churchill, G. A. and Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963 - 971.

Collard, B. C. Y. and Mackill, D. J. (2007). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society* B363: 557 - 572.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B. and Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142: 169 - 196.

Cooper, D. N., Smith, B. A., Cooke, H. J., Niemann, S. and Schmidtke, J. (1985). An estimate of unique DNA sequence heterozygosity in the human genome. *Human Genetics* 69(3): 201 - 205.

Fairhurst, T. H., Witt, C., Buresh, R. J. and Dobermann, A. (2007). *Rice: A Practical Guide to Nutrient Management* (2nd edition). International Rice Research Institute. Metro Manila, Philippines. 89pp.

Farooq, S. and Azam, F. (2002). Molecular Markers in Plant Breeding-II. Some Prerequisites for Use. *Pakistan Journal of Biological Sciences* 5(10): 1141 - 1147.

Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany* 55(396): 307 - 319.

Flowers, T. J. and Flowers, S. A. (2005). Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management* 78: 15 - 24.

Food and Agricultural Organization (2005). Management of irrigation-induced salt-affected soils. [ftp://ftp.fao.org/agl/agll/docs/salinity_brochure_eng.pdf] site visited on 01/09/2011.

Food and Agricultural Organization (2010). *Rice Market Monitor*. Trade and Markets Division, Volume XIII - Issue (3), Rome, 37pp.

Gregorio, G. B. (1997). Tagging salinity tolerance genes in rice using Amplified Fragment Length Polymorphism (AFLP). Thesis for Award of PhD degree at University of Philippines Los Baños, Laguna, Philippines, 118pp.

Gregorio, G. B., Senadhira, D. and Mendoza, R. D. (1997). Screening rice for salinity tolerance. IRRI discussion paper series N°.22, IRRI, DAPO Box 7777, Metro Manila, *The Philippines*: 3 - 19.

Gupta, P. K., Roy, J. K. and Prasad, M. (2001). Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Current science* 80(4): 524 - 535.

Haq, T. U. (2009). Molecular mapping of Na^+ accumulation quantitative trait loci (QTLs) in rice (*Oryza sativa* L.) under salt stress.Thesis for Award of PhD degree at University of Agriculture, Fasialabad, Pakistan, 281pp.

Hosseini, S. J., Sarvestani, Z. T. and Pirdashti, H. (2012). Analysis of tolerance indices in some rice (*Oryza sativa* L.) genotypes at salt Stress condition. *International Research Journal of Applied and Basic Sciences* 3(1): 1 - 10.

International Rice Research Institute (2006). Stress and disease tolerance. In: *Rice Breeding Course*. [http://www.knowledgebank.irri.org/ricebreedingcourse/ Breeding_for_salt_tolerance.htm] site visited on 01/09/2011.

Islam, M. R., Salam, M. A., Hassan, L., Collard, B. C. Y., Singh, R. K. and Gregorio, G. B. (2011). QTL mapping for salinity tolerance at seedling stage in rice.*Emirates Journal of Food and Agriculture* 23(2): 137 - 146.

Islam, M. Z., Baset Mia, M. A., Islam, M. R. and Akater, A. (2007). Effect of different saline levels on growth and yield attributes of mutant rice. *Journal of Soil and Nature* 1(2): 18 - 22.

Jeremy, D. E. and McCouch, S. R. (2007). Molecular markers for use in plant molecular breeding and germplasm evaluation. In *Marker-Assisted selection, current status and future perspectives in crop, livestock, forestry and fish (Edited byGuimarães, E. P. Ruane, J. Scherf, B. D. Sonnino, A. Dargie, J. D.).* Food and Agriculture Organization of the United Nations, Rome. pp. 29-49.

Joehanes, R. and Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics* 24: 2788 - 2789.

Kanawapee, N., Sanitchon, J., Srihaban, P. and Theerakulpisut, P. (2011). Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers.*Electronic Journal of Biotechnology* 14: 0717 - 3458.

Kanjoo, V., Jearakongman, S., Punyawaew, K., Siangliw, J. L., Siangliw, M., Vanavichit, A. and Toojinda, T. (2011). Co-location of quantitative trait loci for drought and salinity tolerance in rice. *Thailand Journal of Genetics* 4(2): 126 - 138.

Kosambi, D. D. (1944). The estimation of map distance from recombination values. *Annals of Eugenics* 12: 172-175.

Koyama, M. L., Levesley, A., Koebner, R. M. D., Flowers, T. J. and Yeo, A. R. (2001). Quantitative Trait Loci for Component Physiological Traits Determining Salt Tolerance in Rice. *Plant Physiology* 125: 406 - 422.

Kshirod, K. J. (2010). The species of the genus Oryza and transfer of useful genes from wild species into cultivated rice (*O. Sativa). Breeding Science* 60(5): 518 - 523.

Kurata, N., Nonomura, K. I. and Harushima, Y. (2002). Rice Genome Organization: the Centromere and Genome Interactions. *Annals of Botany* 90: 427 - 435.

Läuchli, A. and Grattan, S. R. (2007). Plant growth and development under salinity stress. In: *Advances in molecular breeding toward drought and salt tolerant crops.* (*Edited by Jenks, M. A.; Hasegawa, P. M and Jain, S. M.*), Springer, Netherlands. pp.1 - 32.

Lee, S. Y., Ahn, J. H., Cha, Y. S., Yun, D. W., Lee, M. C., Ko, J. C., Lee, K. S. and Eun, M. Y. (2006). Mapping of Quantitative Trait Loci for Salt Tolerance at the Seedling Stage in Rice. *Molecules and Cells* 21(2): 192 - 196.

Li, C. B., Zhang, D. M., Ge, S., Lu, B. R. and Hong, D. Y. (1997). Identification of genome constitution of *Oryza malampuzhaensis, O. minuta,* and *O. punctata* by multicolor genomic in situ hybridization. *Theoretical and Applied Genetics* 103: 204 - 211.

Li, Z. K. (2001). QTL mapping in rice: a few critical considerations. In: *Rice Genetics IV (Edited by Khush, G. S. Brar, D. S. and Hardy, B.).* Science Publishers, Inc. International Rice Research Institute: pp. 153 - 171.

Mansuri, S. M., Jelodar, N. B. and Bagheri, N. (2012). Evaluation of rice genotypes to salt stress in different growth stages via phenotypic and random amplified polymorphic DNA (RAPD) marker assisted selection. *African Journal of Biotechnology* 11(39): 9362 - 9372.

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

Matsuo, T., Futsuhara, Y., Kikuchi, F. and Yamaguchi, H. (1997). *Science of Rice plant, Genetics* Volume 3, Food and Agriculture policy Research Center, Tokyo. 1003pp.

McCouch, S. R., Teytelman, L., Xu, Y., Lobos, K. B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y., Zhang, Q., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D. and Stein, L. (2002).

Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.). *Research* 9: 199 - 207.

McCouch, S. R., Zhao, K., Wright, M., Chih-Wei, T., Ebana, K., Thomson, M., Reynolds, A., Wang, D., DeClerck, G., Ali, M. L., McClung, A., Eizenga, G. and Bustamante, C. (2010). Development of genome-wide SNP assays for rice. *Breeding Science* 60: 524 - 535.

Meksem, K. and Kahl, G. (2005). *The Handbook of Plant Genome Mapping, Genetic and Physical Mapping.* Wiley-Vch Verlag GmbH and Co. KGaA, Weinheim. 380pp.

Milne, I., Shaw, P., Stephen, G., Bayer, M., Cardle, L., Thomas, W. T. B., Flavell, A. J. and Marshall, D. (2010). Flapjack-graphical genotype visualization. *Geneticsand population analysis* 26(24): 3133 - 3134.

Ming-zhe, Y., Jian-fei, W., Hong-you, C., Hu-qu, Z. and Hong-sheng, Z. (2005). Inheritance and QTL Mapping of Salt Tolerance in Rice. *Rice Science* 2(1): 25 - 32.

Mohammadi-Nejad, G., Arzani, A., Rezai1, A. M., Singh, R. K. and Gregorio, G. B. (2008). Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *Saltol* QTL. *African Journal of Biotechnology* 7(6): 730 - 736.

Mohammadi-Nejad, G., Singh, R. K., Arzani, A., Rezaie, A. M., Sabouri, H. and Gregorio, G. B. (2010). Evaluation of salinity tolerance in rice genotypes. *International Journal of Plant Production* 4(3): 1735 - 8043.

Mondini, L., Noorani, A. and Pagnotta, M. A. (2009). Assessing Plant Genetic Diversity by Molecular Tools.*Diversity* 1(1): 19 - 35.

Moorby, J. and Besford, R. T. (1983). Mineral nutrition and growth. In *Encyclopedia of Plant Physiology New Series Vol. 15 B: Inorganic Plant Nutrition.(Edited by Lauchi, A. and. Bieleski, R. L.*). Springer-Verlag, Berlin. pp. 481 - 515.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Environment* 25: 239 - 250.

Munns, R. and Tester, M. (2008). Mechanisms of Salinity Tolerance. *Annual Review of Plant Biology* 59: 651 - 81.

Park, Y. J., Lee, J. K. and Kim, N. S. (2009). Simple Sequence Repeat Polymorphisms (SSRPs) for Evaluation of Molecular Diversity and Germplasm Classification of Minor Crops. *Molecules* 14: 4546 - 4569.

Rad, H. E., Aref, F. and Rezaei, M. (2012). Evaluation of Salinity Stress Affects Rice Growth and Yield Components in Northern Iran. *American Journal of Scientific Research* 54: 40 - 51.

Rajendran, K., Tester, M. and Roy, S. J. (2009). Quantifying the three main components of salinity tolerance in cereals. *Plant, Cell and Environment* 32: 237 - 249.

Randhawa, G. J., Verma, D. D., Bhalla, S., Hota, M., Chalam, V. C. and Tyagi, V. (2006). *Document on Biology of Rice (Oryza sativa L.) in India. National Bureau of Plant Genetic Resources*. National Bureau of Plant Genetic Resources, New Delhi. 79pp.

Ray, P. K. S. and Islam, M. A. (2008). Genetic analysis of salinity tolerance in rice. *Bangladesh Journal of Agricultural Research* 33(3): 519 - 529.

Rick, C. M. and Smith, P. G. (1953). Novel variation in tomato species hybrids. *The American Naturalist* 87: 359 - 373.

Sabina, A., Bhat, M. A., Shafiq, A. W., Bhat, K. A., Chalkoo, S., Mir, M. R. and Shabir. A. W. (2010). Marker assisted selection in rice. *Journal of Phytology* 2(10): 66 - 81.

Sabouri, H. and Sabouri, A. (2008). New evidence of QTLs attributed to salinity tolerance in rice. *African Journal of Biotechnology* 7(24): 4376 - 4383.

Saeedipour, S. (2012). Is salinity tolerance of rice lines concerned to endogenous ABA content or to the cellular ability for ABA synthesis under stress? *African Journal of Biotechnology* 11(49): 10938 - 10943.

Sasaki, T., Matsumoto, T., Baba, T., Yamamoto, K., Wu, J., Katayose, Y. and Sakata, K. (2001). QTL mapping in rice: a few critical considerations. In: Rice Genetics IV (*Edited by Khush, G. S. Brar, D. S. and Hardy, B.*), International Rice Research Institute. pp. 189 - 196.

Semagn, K., Bjørnstad , Å. and Ndjiondjop, M. N. (2006c). Principles, requirements and prospects of genetic mapping in plants. *African Journal of Biotechnology* 5(25): 2569 - 2587.

Semagn, K., Bjørnstad, Å. and Ndjiondjop, M. N. (2006a). Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. *African Journal of Biotechnology* 5(25): 2588 - 2603.

Semagn, K., Bjørnstad, Å. and Ndjiondjop, M. N. (2006b). An overview of molecular marker methods for plants. *African Journal of Biotechnology* 5(25): 2540 - 2568.

Sharma, S. R. and Gupta, I. C. (1986). *Saline Environment and plant growth*. Agro botanical Publishers, India, 172pp.

Shereen, A., Ansari, R.U., Yasmin, S., Raza, S., Mumtaz, S., Khan, M. A. and Mujtaba, S. M. (2007). Physiological responses of rice (*Oryza sativa* L.) to saline stress. *Pakistan Journal of Botany* 39(7): 2527 - 2534.

Singh, R. K. and Flowers, T. J. (2010). The physiology and molecular biology of the effects of salinity on rice. In: Third Edition of "*Handbook of Plant and Crop Stress*" (*Edited by Pessarakli, M.)*, Florida, USA. pp. 901 - 942.

Singh, R. K., Gregorio, G. B. and Jain, R. R. (2007). QTL mapping for salinity Tolerance in Rice. *Physiology and Molecular Biology of Plants* 13(2): 87 - 99.

Singh, R. K., Redoña, E. and Refuerzo, L. (2010). Varietal Improvement for Abiotic Stress Tolerance in Crop Plants: Special Reference to Salinity in Rice. In: *Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation. (Edited by Pareek, A. Sopory, S. K. Bohnert, H. J. and Govindjee)*, Springer. pp. 387 - 415.

Tanksley, S. D. (1993). Mapping polygenes. *Annual Review of Genetics* 27: 205 - 233.

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. (1989). RFLP mapping in plant breeding: new tools for an old science. *Biotechnology* 7: 257 - 264.

The, H. V. (2010). QTL mapping and Marker-Assisted backcrossing for developing salinity tolerant rice (*Oryza sativa* L.). Thesis for Award of MSc degree at University of Philippines Los Baños, Laguna, Philippines, 121pp.

Thomson, M. J. (2009). Marker-assisted breeding for abiotic stress tolerance in rice: progress and future perspectives. *International Symposium, Rice Research in the Era of Global Warming*: 43 - 55.

Thomson, M. J., De Ocampo, M., Egdane , J., Rahman, M. A., Sajise, A. G., Adorada, D. L., Tumimbang-Raiz, E., Blumwald, E., Seraj, Z. I., Singh, R. K., Gregorio, G. B. and Ismail, A. M. (2010). Characterizing the *Saltol* Quantitative Trait Locus for Salinity Tolerance in Rice. *Rice*, doi:10.1007/s12284-010-9053-8.

Veldboom, L.R., Lee, M. and Woodman, W. L. (1994). Molecular-marker facilitated studies in an elite maize population: I. Linkage analysis and determination of QTLs for morphological traits. *Theoretical and Applied Genetics* 88: 7 - 16.

Vinod, K. K. (Ed.)(2006). *Genome mapping in plant population*. Proceedings of training programme on "Modern approaches in Plant Genetics resources-collection, conservation and utilization", Tamil Nadu Agricultural University, Coimbatore, India. 1 to 24 November, 2006. 12pp.

Wang, D. L., Zhu, J., Li, Z. K. and Paterson, A. H. (1999). Mapping QTLs with epistatic effects and genotype x environment interactions by mixed linear model approaches. *Theoretical and Applied Genetics*99:1255 - 1264.

Wang, S., Basten, C. J. and Zeng, Z. B. (2011). *Windows QTL Cartographer 2.5 User Manual.* State University, Bioinformatics Research Center, Carolina. 89pp.

Xiao, J., Li, J., Yuan, L. and Tanksley, S. D. (1996). Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a sub specific rice cross. *Theoretical and Applied Genetics* 92: 230 - 244.

Yadav, S., Irfan, M., Ahmad, A. and Hayat, S. (2011). Causes of salinity and plant manifestations to salt stress: A review. *Journal of Environmental Biology* 32: 667 - 685.

Yoshida, S., Forno, D. A., Cock, J. H. and Gomez, K. A. (1976). *Laboratory manual for physiological studies of rice*, 3rd edition. International Rice Research Institutes, Manila, Philippines, 83pp.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics* 136: 457 - 1468.

Zhou, S., Bechner, M. C., Place, M., Churas, C. P., Pape, L., Leong, S. A., Runnheim, R., Forrest, D. K., Goldstein, S., Livny, M. and Schwartz, D. C. (2007) .Validation of rice genome sequence by optical mapping. *BMC Genomics* 8:278 doi: 10.1186/1471-2164-8-278.

APPENDICES

Appendix 1: Key features of common molecular marker technologies

Markers	RFLPs	RAPDs	AFLPs	SSRs	ISSRs	SNPs	DArTs
PCR-based	N	Y	Y	Y	Y	Y	Y
Uses Restriction enzymes	Y	N	N	N	N	N	Y
Abundance	H	H	H	H	H	H	H
Polymorphism	M	M	M	H	M	H	H
Locus-specific	Y	N	N	Y	N	Y	Y
Co-dominant	Y	N	N	Y	N	N	N
Reproducibility	H	L	H	H	M	H	H
Labour intensity	H	L	L	L	L	M	M
Technical demand	H	L	L	L	L	H	H
Throughput	L	M	M	M	M	H	H
Development cost	M	L	L	H	L	H	M
Operational cost	H	L	M	L	L	L	L
DNA quality/ quantity	H	L	M	L	L	M	M
Automation	N	N	N	Y	N	Y	Y
Loci per assay	1 to few	Many	Many	1 to about 20	Many	1 to thousands	Many
Specialized Equipment	Radioactive isotope	Agarose gels	Polyacrylamide gels/capillary	Polyacrylamide gels/capillary	Polyacrylamidegels	Variable	Microarrays

H: High, M:Medium, N: No, Y: Yes, L: Low, RFLPs: Restriction Fragment Length Polymorphisms, RAPDs: Randomly Amplified Polymorphic DNAs, AFLPs: Amplified Fragment Length Polymorphism, SSRs: Single Sequence Repeats, ISSR: Inter-simple sequence repeat, SNPs: Single Nucleotide Polymorphisms, DArTs: Diversity Array Technologies, CAPS: Cleaved Amplified Polymorphic Sequences.

Source: Farooq and Azam, 2002; Jeremy *et al.*, 2007 and Mondini *et al*., 2009

Appendix 2: List of recombinant inbred lines used in this study

Genotype Number	Lines	Selected	Genotype Number	Lines	Sorted	Genotype Number	Lines	Sorted
P1	Hasawi	*	34	IR 91477-34-1-1		69	IR 91477-69-1-1	*
P2	IR 29	*	35	IR 91477-35-1-1		70	IR 91477-70-1-1	
1	IR 91477-1-1-1		36	IR 91477-36-1-1	*	71	IR 91477-71-1-1	*
2	IR 91477-2-1-1		37	IR 91477-37-1-1	*	72	IR 91477-72-1-1	*
3	IR 91477-3-1-1	*	38	IR 91477-38-1-1		73	IR 91477-73-1-1	
4	IR 91477-4-1-1		39	IR 91477-39-1-1	*	74	IR 91477-74-1-1	*
5	IR 91477-5-1-1	*	40	IR 91477-40-1-1		75	IR 91477-75-1-1	
6	IR 91477-6-1-1	*	41	IR 91477-41-1-1	*	76	IR 91477-76-1-1	*
7	IR 91477-7-1-1		42	IR 91477-42-1-1	*	77	IR 91477-77-1-1	*
8	IR 91477-8-1-1		43	IR 91477-43-1-1	*	78	IR 91477-78-1-1	
9	IR 91477-9-1-1		44	IR 91477-44-1-1	*	79	IR 91477-79-1-1	*
10	IR 91477-10-1-1	*	45	IR 91477-45-1-1	*	80	IR 91477-80-1-1	*
11	IR 91477-11-1-1		46	IR 91477-46-1-1		81	IR 91477-81-1-1	*
12	IR 91477-12-1-1	*	47	IR 91477-47-1-1	*	82	IR 91477-82-1-1	
13	IR 91477-13-1-1	*	48	IR 91477-48-1-1		83	IR 91477-83-1-1	
14	IR 91477-14-1-1		49	IR 91477-49-1-1	*	84	IR 91477-84-1-1	*
15	IR 91477-15-1-1	*	50	IR 91477-50-1-1		85	IR 91477-85-1-1	
16	IR 91477-16-1-1	*	51	IR 91477-51-1-1	*	86	IR 91477-86-1-1	*
17	IR 91477-17-1-1		52	IR 91477-52-1-1		87	IR 91477-87-1-1	*
18	IR 91477-18-1-1		53	IR 91477-53-1-1	*	88	IR 91477-88-1-1	*
19	IR 91477-19-1-1	*	54	IR 91477-54-1-1	*	89	IR 91477-89-1-1	*
20	IR 91477-20-1-1		55	IR 91477-55-1-1	*	90	IR 91477-90-1-1	*
21	IR 91477-21-1-1		56	IR 91477-56-1-1		91	IR 91477-91-1-1	
22	IR 91477-22-1-1		57	IR 91477-57-1-1	*	92	IR 91477-92-1-1	*
23	IR 91477-23-1-1	*	58	IR 91477-58-1-1	*	93	IR 91477-93-1-1	*
24	IR 91477-24-1-1		59	IR 91477-59-1-1	*	94	IR 91477-94-1-1	
25	IR 91477-25-1-1	*	60	IR 91477-60-1-1	*	95	IR 91477-95-1-1	*
26	IR 91477-26-1-1		61	IR 91477-61-1-1	*	96	IR 91477-96-1-1	*
27	IR 91477-27-1-1	*	62	IR 91477-62-1-1		97	IR 91477-97-1-1	*
28	IR 91477-28-1-1	*	63	IR 91477-63-1-1		98	IR 91477-98-1-1	*
29	IR 91477-29-1-1		64	IR 91477-64-1-1	*	99	IR 91477-99-1-1	*
30	IR 91477-30-1-1		65	IR 91477-65-1-1	*	100	IR 91477-100-1-1	
31	IR 91477-31-1-1		66	IR 91477-66-1-1	*	101	IR 91477-101-1-1	*
32	IR 91477-32-1-1		67	IR 91477-67-1-1	*	102	IR 91477-102-1-1	
33	IR 91477-33-1-1	*	68	IR 91477-68-1-1		103	IR 91477-103-1-1	*

*: Genotypes selected for genotyping, in total 144 (142 RILs +2 parents: Hasawi and IR29); All genotypes were phenotyped

Appendix 2: (Continued)

Genotype Number	Lines	Sorted	Genotype Number	Lines	Sorted	Genotype Number	Lines	Sorted
104	IR 91477-104-1-1		141	IR 91477-141-1-1		177	IR 91477-177-1-1	
105	IR 91477-105-1-1		142	IR 91477-142-1-1		178	IR 91477-178-1-1	*
106	IR 91477-106-1-1	*	143	IR 91477-143-1-1		179	IR 91477-179-1-1	*
107	IR 91477-107-1-1		144	IR 91477-144-1-1		180	IR 91477-180-1-1	
108	IR 91477-108-1-1	*	145	IR 91477-145-1-1		181	IR 91477-181-1-1	*
109	IR 91477-109-1-1	*	146	IR 91477-146-1-1		182	IR 91477-182-1-1	*
110	IR 91477-110-1-1	*	147	IR 91477-147-1-1		183	IR 91477-183-1-1	*
111	IR 91477-111-1-1	*	147	IR 91477-147-1-1		184	IR 91477-184-1-1	
112	IR 91477-112-1-1		148	IR 91477-148-1-1	*	185	IR 91477-185-1-1	
113	IR 91477-113-1-1	*	149	IR 91477-149-1-1		186	IR 91477-186-1-1	*
114	IR 91477-114-1-1		150	IR 91477-150-1-1	*	187	IR 91477-187-1-1	*
115	IR 91477-115-1-1	*	151	IR 91477-151-1-1		188	IR 91477-188-1-1	
116	IR 91477-116-1-1	*	152	IR 91477-152-1-1		189	IR 91477-189-1-1	
117	IR 91477-117-1-1	*	153	IR 91477-153-1-1		190	IR 91477-190-1-1	*
118	IR 91477-118-1-1	*	154	IR 91477-154-1-1		191	IR 91477-191-1-1	
119	IR 91477-119-1-1	*	155	IR 91477-155-1-1		192	IR 91477-192-1-1	
120	IR 91477-120-1-1	*	156	IR 91477-156-1-1		193	IR 91477-193-1-1	
121	IR 91477-121-1-1	*	157	IR 91477-157-1-1	*	194	IR 91477-194-1-1	
122	IR 91477-122-1-1	*	158	IR 91477-158-1-1		195	IR 91477-195-1-1	*
123	IR 91477-123-1-1	*	159	IR 91477-159-1-1		196	IR 91477-196-1-1	*
124	IR 91477-124-1-1	*	160	IR 91477-160-1-1	*	197	IR 91477-197-1-1	
125	IR 91477-125-1-1	*	161	IR 91477-161-1-1		198	IR 91477-198-1-1	
126	IR 91477-126-1-1	*	162	IR 91477-162-1-1		199	IR 91477-199-1-1	*
127	IR 91477-127-1-1		163	IR 91477-163-1-1	*	200	IR 91477-200-1-1	
128	IR 91477-128-1-1		164	IR 91477-164-1-1		201	IR 91477-201-1-1	
129	IR 91477-129-1-1		165	IR 91477-165-1-1		202	IR 91477-202-1-1	*
130	IR 91477-130-1-1	*	166	IR 91477-166-1-1	*	203	IR 91477-203-1-1	*
131	IR 91477-131-1-1		167	IR 91477-167-1-1	*	204	IR 91477-204-1-1	
132	IR 91477-132-1-1		168	IR 91477-168-1-1		205	IR 91477-205-1-1	
133	IR 91477-133-1-1	*	169	IR 91477-169-1-1		206	IR 91477-206-1-1	
134	IR 91477-134-1-1		170	IR 91477-170-1-1	*	207	IR 91477-207-1-1	
135	IR 91477-135-1-1		171	IR 91477-171-1-1		208	IR 91477-208-1-1	*
136	IR 91477-136-1-1	*	172	IR 91477-172-1-1		209	IR 91477-209-1-1	
137	IR 91477-137-1-1	*	173	IR 91477-173-1-1		210	IR 91477-210-1-1	
138	IR 91477-138-1-1	*	174	IR 91477-174-1-1		211	IR 91477-211-1-1	
139	IR 91477-139-1-1		175	IR 91477-175-1-1		212	IR 91477-212-1-1	
140	IR 91477-140-1-1		176	IR 91477-176-1-1	*	213	IR 91477-213-1-1	

Appendix 2: (Continued)

Genotype Number	Lines	Sorted	Genotype Number	Lines	Sorted	Genotype Number	Lines	Sorted
214	IR 91477-214-1-1	*	251	IR 91477-251-1-1	*	288	IR 91477-288-1-1	
215	IR 91477-215-1-1		252	IR 91477-252-1-1		289	IR 91477-289-1-1	
216	IR 91477-216-1-1		253	IR 91477-253-1-1		290	IR 91477-290-1-1	
217	IR 91477-217-1-1		254	IR 91477-254-1-1	*	291	IR 91477-291-1-1	
218	IR 91477-218-1-1		255	IR 91477-255-1-1		292	IR 91477-292-1-1	
219	IR 91477-219-1-1		256	IR 91477-256-1-1		293	IR 91477-293-1-1	
220	IR 91477-220-1-1	*	257	IR 91477-257-1-1		294	IR 91477-294-1-1	*
221	IR 91477-221-1-1	*	258	IR 91477-258-1-1		295	IR 91477-295-1-1	
222	IR 91477-222-1-1		259	IR 91477-259-1-1	*	296	IR 91477-296-1-1	
223	IR 91477-223-1-1		260	IR 91477-260-1-1		297	IR 91477-297-1-1	
224	IR 91477-224-1-1		261	IR 91477-261-1-1		298	IR 91477-298-1-1	
225	IR 91477-225-1-1		262	IR 91477-262-1-1		299	IR 91477-299-1-1	*
226	IR 91477-226-1-1	*	263	IR 91477-263-1-1		300	IR 91477-300-1-1	
227	IR 91477-227-1-1		264	IR 91477-264-1-1				
228	IR 91477-228-1-1	*	265	IR 91477-265-1-1				
229	IR 91477-229-1-1	*	266	IR 91477-266-1-1	*			
230	IR 91477-230-1-1	*	267	IR 91477-267-1-1	*			
231	IR 91477-231-1-1	*	268	IR 91477-268-1-1				
232	IR 91477-232-1-1		269	IR 91477-269-1-1				
233	IR 91477-233-1-1	*	270	IR 91477-270-1-1				
234	IR 91477-234-1-1		271	IR 91477-271-1-1				
235	IR 91477-235-1-1		272	IR 91477-272-1-1	*			
236	IR 91477-236-1-1		273	IR 91477-273-1-1				
237	IR 91477-237-1-1	*	274	IR 91477-274-1-1				
238	IR 91477-238-1-1	*	275	IR 91477-275-1-1	*			
239	IR 91477-239-1-1		276	IR 91477-276-1-1				
240	IR 91477-240-1-1	*	277	IR 91477-277-1-1	*			
241	IR 91477-241-1-1	*	278	IR 91477-278-1-1				
242	IR 91477-242-1-1	*	279	IR 91477-279-1-1	*			
243	IR 91477-243-1-1		280	IR 91477-280-1-1	*			
244	IR 91477-244-1-1		281	IR 91477-281-1-1				
245	IR 91477-245-1-1	*	282	IR 91477-282-1-1				
246	IR 91477-246-1-1		283	IR 91477-283-1-1	*			
247	IR 91477-247-1-1		284	IR 91477-284-1-1				
248	IR 91477-248-1-1		285	IR 91477-285-1-1	*			
249	IR 91477-249-1-1	*	286	IR 91477-286-1-1				
250	IR 91477-250-1-1	*	287	IR 91477-287-1-1				

Appendix 3: Rice culture solution as modified from the Yoshida *et al.* (1976) solution

Stock		Common name	Reagent	g l^{-1}
Major nutrients				
1		Ammonium nitrate	NH_4NO_3	91.400
2		Potassium sulphate	K_2SO4	97.800
3	a	Potassium dihydrogen orthophosphate	KH_2PO_4	29.000
	b	Potassium dihydrogen phosphate dibase	K_2HPO_4	8.000
4		Calcium chloride dihydrate	$CaCl_2.2H_2O$	175.000
5		Magnesium sulphate,7-hydrate	$MgSO_4.7H_2O$	324.000
6 Minor nutrients				
	a	Manganous chloride, 4-hydrate	$MnCl_2.4H_2O$	1.500
	b	Ammonium molybdate, 4-hydrate	$(NH_4)Mo_7O_{24}.4H_2O$	0.074
	c	Zinc sulphate, 7-hydrate	$ZnSO_4.7H_2O$	0.035
	d	Boric acid	H_3BO_3	0.934
	e	Cupric sulphate,5-hydrate	$CuSO_4.5H_2O$	0.031
7		Ethylenediaminetetraacetic acid iron (III) sodium salt hydrate (FeNaEDTA)	$C_{10}H_{12}FeN_2NaO_8Na_2.H_2O$	10.500

Source: Singh *et al*., 2010

Appendix 4: Normal field temperature and relative humidity observation points during the experiment

Date	Min %RH	Max %RH	Min T°	Max T°
16/03/2012	57.4	63.8	22.2	31.4
17/03/2012	40.2	80	22.2	27.3
18/03/2012	41.6	85.2	20.0	27.1
19/03/2012	53.6	72.3	26.0	32.6
20/03/2012	55.5	70.7	26.3	31.4
21/03/2012	67.2	70.7	30.6	31.4
22/03/2012	69.2	72.3	29.5	30.8
23/03/2012	65.7	72.8	25.3	31.3
24/03/2012	69.7	75.3	29.4	31.5
25/03/2012	72.8	74.3	29.3	30.3
26/03/2012	68.2	74.3	26.5	31.5
27/03/2012	60.3	70.2	24.1	36.5
28/03/2012	64.2	68.7	27.8	40.4
29/03/2012	64.4	70.1	25.4	42.2
30/03/2012	64.8	74.9	26.4	40.8
31/03/2012	58.2	72.6	26.4	41.5
01/04/2012	66.4	69.3	24.3	44.1
02/04/2012	65.7	75.3	25.4	42.1
03/04/2012	64.7	70.1	25.9	42.4
04/04/2012	63.4	71.3	24.9	43.9
05/04/2012	57.5	72.1	23.8	43.2
06/04/2012	59.3	70.6	24.2	43.0
07/04/2012	60.8	71.1	24.0	41.7
08/04/2012	58.4	73.8	24.5	42.3
09/04/2012	58.3	73.1	24.3	42.7
10/04/2012	59.9	73.9	24.7	43.5
11/04/2012	56.4	70.3	23.0	38.5
12/04/2012	61.2	72.8	22.1	41.0
13/04/2012	60.7	74.9	24.0	40.8
14/04/2012	61.9	74.7	23.9	34.1
15/04/2012	46.1	83.3	23.7	41.0
19/04/2012	55.5	87.9	24.2	32.8
20/04/2012	17.0	93.3	23.9	39.0
21/04/2012	20.6	90.6	23.2	37.6

Max: Maximum, Min: minimum, T°: temperature, RH : relative humidity

Appendix 4: (continued)

Date	Min %RH	Max %RH	Min T°	Max T°
22/04/2012	21.4	87.4	23.4	39.0
23/04/2012	20.2	89.5	23.4	38.5
24/04/2012	24.7	85.2	25.2	38.7
25/04/2012	56.9	86.9	22.4	32.0
26/04/2012	28.9	90.6	23.9	37.3
27/04/2012	48.4	83.1	25.1	31.6
28/04/2012	46.1	96	24.4	35.7
29/04/2012	52.6	96	24.2	35.2
30/04/2012	66.2	99.9	23.3	29.7
01/05/2012	50.3	96.6	24.2	35.2
02/05/2012	41.6	92.2	23.5	36.7
03/05/2012	46.1	96	23.6	36.3
04/05/2012	31.4	98.8	23.4	35.8
05/05/2012	29.3	98.3	24.1	37.6
06/05/2012	32.3	93.3	24.7	34.8
07/05/2012	32.3	98.3	21.4	37.4
08/05/2012	29.8	95.5	22.8	36.7
09/05/2012	27.2	96	22.6	36.8
10/05/2012	42	95.5	22.6	40.4
11/05/2012	59.3	100	23.6	42.7
12/05/2012	71.2	100	22.9	37.1
13/05/2012	0	100	21.4	40.4
14/05/2012	54.1	100	23.7	40.8
15/05/2012	71.2	100	23.9	35.8
16/05/2012	54.1	100	23.4	43.0
17/05/2012	50.8	100	22.8	46.9
18/05/2012	48.4	100	21.5	45.4
19/05/2012	48.4	100	21.6	45.3
AVERAGE	**51**	**84**	**24**	**37**

Appendix 5: Modified standard evaluation score of visual salt injury at seedling stage

Score	Observation	Tolerance
1	Normal growth, only the old leave show white tips while no symptoms on young leaves	Highly tolerant
3	Near normal growth, but only leaf tips burn, few older leaves become whitish partially and rolled	Tolerant
5	Growth severely retarded; most leaves severely injured, few young leaves elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dried; only few young leaves still green	Susceptible
9	Almost all plants dead or dying	Highly sensitive

Source: Gregorio *et al*., 1997

Appendix 6: Salt-induced injuries and salinity injury scores

SES1, SES3, SES5, SES7 and SES9: Salt injury scores

Appendix 7: Procedure of genomic DNA extraction from rice leaves

1° Freezer dried rice leaves were cut into pieces and put inside 2 ml autoclaved tubes. 2 steel balls have been placed in each tube. The tube have been closed and then arranged in the rack. A couple of rack has been placed onto cryogenic container where the liquid nitrogen has been poured over the tube followed by grinding using the GenoGrinder. Grinding took 1.5 minutes under 1500 rpm;

2° 600 µl 2X CTAB buffer warmed to 65°C have been added to each tube using micropipette. After mixing thoroughly, the mixture has been incubated at 65°C for 30 minutes in water bath and spun gently after first 15 minutes;

3° From the water bath, the mixture has been briefly cooled down and thereafter 600 µl of chloroform-isoamyl (24:1) have been added. After shaking at room temperature, pair rack containing those tubes have been place into centrifuge and then spun at 3500 rpm for 10 minutes at 22°C;

4° The aqueous phase has been decanted into new tube;

5° 600 µl isopropanol has been added to each tube and incubated at -20°C overnight;

6° From freezer, the racks containing tubes have been spun at 3500 rpm for 10 minutes at 22°C. Then, isopropanol has been decanted and the pellet washed with 600 µl of 70% EtOH. The proper washing has been performed by spinning at 3500 rpm for 5 minutes at 4°C and then the EtOH has been drained;

7° The dried pellets have been dissolved into 200 TE. 2 µl RNase (10 mg/ml) have been incubated at 37°C at for 30 minutes;

8° 20 µl sodium acetate (1/10 volume solution) has been added and 400 µl of absolute EtOH have been added. The mixture has been incubated at -20° C for 1 hour;

9° After, the tubes have been spun at at 3500 rpm for 5 minutes at 4°C, drained and pellets rinsed with 600 µl of 70% EtOH. Air dried, the pellets have been dissolved in 100 µl TE;

10° The DNA quality and concentration have been checked on agarose gel and using spectrophotometer.

Appendix 8: Comparison of SNP and SSR markers position for the same QTL identified at specific position

Chromosome number	QTL name		SNP MARKERS		SSR Markers*	
		Position (cM)	Flanking markers	Flanking markers position (cM)	Flanking markers	Flanking markers position (cM)
1	*qSL1.1*	162.6	id1023892 - id1024836	158.1 – 163.5	RM529 -RM8088	161.5 -163.5
	qSL1.2	168.6	id1024972- id1025983	164.49 -170.32	RM3681- RM8048	166.9 - 170.4
2	*qSESI2.1*	60.8	id2004774 - id2007526	40.1 – 88.3	RM6844 -RM1038	58.4-62.2
	qSDW2.1	62.8	id2004774 - id2007526	40.1 – 88.3	RM7413 -RM8254	62.2 - 77.8
	qSESF2.1	64.8	id2004774 - id2007526	40.1 – 88.3	RM7413 -RM8254	62.2 - 77.8
4	*qSFW4.1*	18.1	id4001113 - id4001932	9.8 – 18.2	RM3471 - RM4835	16.7 - 18.3
	qRDW4.1	18.1	id4001113 - id4001932	9.8 – 18.2	RM3471 - RM4835	16.7 - 18.3
	qRFW4.1	98.1	id4007444 - id4008092	91.4 - 99.16	RM6507 - RM3474	97.7 - 99.3
6	*qSL6.1*	18.5	fd13 - id6004343	12.3 – 27.0	RM8258 - RM2126	15.8 - 32.7
	qRL6.1	18.5	fd13 - id6004343	12.3 – 27.0	RM8258 - RM2126	15.8 - 32.7
	qSESI6.1	52.5	ud6000572 - id6009055	52.1 – 65.6	RM3431A - RM5850	52.0 - 53.0

*: Source, McCouch *et al.* (2002) ; cM: Centimorgan

Appendix 8: (Continued)

Chromosome number	QTL name	SNP MARKERS			SSR Markers*	
		Position (cM)	Flanking markers	Flanking markers position (cM)	Flanking markers	Flanking markers position (cM)
8	*qSDW8.1*	98.6	id8006485 - wd8004122	90.4 – 103.4	RM8058 - RM6542	96.6 - 106.1
9	*qRFW9.1*	27	id9001614 - id9002014	26.4 – 30.1	RM8206 - RM1817	3.2 - 34.4
	qSFW9.1	27	id9001614 - id9002014	26.4 – 30.1	RM8206 - RM1817	3.2 - 34.4
12	*qSESI12.1*	84.9	id12005823 - id12007988	70.2 – 93.9	RM7018 - RM3331	75.8 - 89.5
	qSESF12.1	6.9	id12000252 - id12001321	2.9 – 12.8	n - RM6335	n - 7.4
	qRL12.1	6.9	id12000252 - id12001321	2.9 – 12.8	n - RM6335	n - 7.4
	qSL12.1	6.9	id12000252 - id12001321	2.9 – 12.8	n - RM6335	n - 7.4
	qSFW12.1	6.9	id12000252 - id12001321	2.9 – 12.8	n - RM6335	n - 7.4
	qSDW12.1	6.9	id12000252 - id12001321	2.9 – 12.8	n - RM6335	n - 7.4

n: there is no SSR marker at lower position