MOLECULAR CHARACTERIZATION OF ELITE GENOTYPES FOR SALINITY TOLERANCE IN RICE (*Oryza sativa* L.)

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B.Sc. (Ag.)

MASTER OF SCIENCE IN AGRICULTURE
(GENETICS AND PLANT BREEDING)

2019

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By

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B.Sc. (Ag.)

THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE
(GENETICS AND PLANT BREEDING)

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GUNTUR, ANDHRA PRADESH

2019

DECLARATION

I, **Mr. K. HRUDAYA RAJ,** hereby declare that the thesis entitled **"MOLECULAR CHARACTERIZATION OF ELITE GENOTYPES FOR SALINITY TOLERANCE IN RICE (*Oryza sativa* L.)",** submitted to **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** in the major field of **Genetics and Plant Breeding** is the result of original research work done by me. I also declare that any material in the thesis has not been published earlier.

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CERTIFICATE

Mr. K. HRUDAYA RAJ has satisfactorily prosecuted the course of research and that the thesis entitled **"MOLECULAR CHARACTERIZATION OF ELITE GENOTYPES FOR SALINITY TOLERANCE IN RICE (*Oryza sativa* L.)"** submitted is the result of original research work and is of sufficiently high standard to warrant it's presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **"MOLECULAR CHARACTERIZATION OF ELITE GENOTYPES FOR SALINITY TOLERANCE IN RICE (*Oryza sativa* L.)"** submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture in the major field of **Genetics and Plant Breeding** of the Acharya N. G. Ranga Agricultural University, Guntur, is a record of the bonafied research work carried out by **Mr. K. HRUDAYA RAJ** under our guidance and supervision. The subject of the thesis has been approved by the student's advisory committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been duly acknowledged by the author of the thesis.

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 # ACKNOWLEDGEMENTS

*First and foremost, I would like to put on record my heartful gratitude to **Dr.T. Srinivas,** Professor & Head , Agricultural college, Bapatla and esteemed chairperson of my advisory committee for his generous help, guidance, timely advice, constant supervision, constructive criticism and vivid encouragement.*

*With respectful regards and immense pleasure, I wish to express my sincere gratitude to **Dr.P.Venkata Ramana Rao,** Senior Scientist (Plant Breeding), RARS, Maruteru for his constant attention, worthy counsel, and constructive criticism during the work and affectionate dealing throughout the period of investigation & during the preparation of manuscript.*

*I Wish to express my esteem gratitude, my deepest admiration and heartful thanks to my advisory committee , **Dr.V.Satyanarayana Rao,** Associate Director of Research, RARS,Lam farm, Guntur for his immense help, constant inspiration and fruitful advice during the course of investigation and my post-graduation studies.*

*I express my deep sense of gratitude to **Sk. Nafeez Umar,** Assistant Professor, Department of Statistics and Mathematics, Agricultural College, Bapatla and member of my advisory committee for his cooperation and guidance during my research work.*

*I Wish to express my esteem gratitude, my deepest admiration and heartful thanks to **Dr.M.Girija Rani**, Senior Scientist (Plant Breeding), RARS, Maruteru, for her constant critical supervision, sustained interest, caring nature, immense help, constant inspiration and fruitful advice during the course of investigation.*

*I extend my effusive thanks to **Dr. P. V. Rama Kumar,** Professor and Head (Retd.), Department of Genetics and Plant Breeding, **Dr.J.Dayal Prasad Babu**, Senior Scientist, DSA Office, ANGRAU, **Dr. Lal Ahamed Mohammed,** Associate Professor, APGC, Lam, Guntur and **Dr. D. Ratna Babu,** Associate Professor, APGC, Lam,Guntur for their valuable suggestions and help rendered during my research work.*

*I equally owe my sense of gratitude to **Dr. P.V. Satyanarayana,**Principal Scientist (Rice),RARS, Maruteru, **Dr. Y. Suneetha,** Scientist (Plant Breeding), ARS, Bapatla. Dr. **B.N.V.S.R. Ravi Kumar**, Senior Scientist (Plant Breeding), RARS, Maruteru, **Dr. N. Chamundeswari**, Senior Scientist (Plant Breeding), RARS, Maruteru.*

I also acknowledge ***Associate Dean****, Agricultural College, Bapatla for his valuable suggestions. I am also thankful to the staff of Agricultural College, Bapatla for their co-operation during the period of my study.*

I allocate my highest respect and heartful regards from my inner core of hearts to my ever loved parents ***K. Anthoni Raj (Late)*** *and* ***K. Elizabeth Rani*** *who have given me life and taught the concepts of life and their dedicated efforts to educate me to this level and without whose valuable moral support, the thesis would not have seen the light of the day.*

I shower my heartiest affection on my beloved friends ***Sudhakar, Sandeep, Avinash, Ramaraju, Rajasekhar, Srikanth, Srinu, Muniraju, Manikanta, Santosh.M, santosh.T, Srilakshmi.M, Srilakshmi.P, Kusuma*** *for their support and extended to boost up my morale in carrying out this thesis research work and my warmest thanks are to my colleagues* ***M.Srilakshmi chaitanya, Anamika, , Mounika, Dada Azmi, Sudheer, Jai Shankar*** *and* ***Ram Prabhu****for their memorable friendship and cooperation.*

On a personal account, I would like to pour my heartfelt thanks to my seniors Prabhakar, Bharathi, Shiva, Poornima, Borraiah, Shalini, Sudeepthi,Shanthi, Hari Ram, Ravikanth, Kalyan, Kavya, Sruthi and my juniors Vamsi, Rama Krishna, Sharath, Venugopal, Sunil, Shalem Raju, Anisha for their cooperation and encouragement during my study period.

I am very much thankful to Deepika, Pavani, Kumari and all the team of Biotechnology Laboratory RARS, Maruteru , Suchandranath babu, Raghu, Sai Kumar for their constant help and support during my work period.

I am thankful to ***Acharya N. G. Ranga Agricultural University*** *for providing financial assistance in the form of stipend during my course of study.*

I am very much thankful to Ramulamma, Bhaskar Rao, Srinivasa Reddy, Siva Parvathi for their help during my PG programme.

Finally I frankly admit that it is not possible to remember all the faces that stood behind the facade of this juncture & omission of any names does not mean lack of gratitude.

Place:

Date: ***(K. Hrudaya Raj)***

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LIST OF SYMBOLS AND ABBREVIATIONS

$\overline{X}$	:	Grand mean
µl	:	Micro litre
µg	:	Micro gram
nm	:	Nano metre
pM	:	Pico mole
σ^2	:	Variance
%	:	per cent
^0C	:	Degree centigrade
BC_1	:	Back cross 1 with parent 2
BC3	:	Back cross 3 with parent 3
BPT	:	Bapatla
Conc.	:	Concentration
CD	:	Critical difference
cm	:	Centimeter
CTAB	:	Cetyl Tris Methyl Ammonium Bromide
CV	:	Coefficient of variation
DNA	:	Deoxy Ribo Nucleic acid
DAS	:	Days after sowing
dNTP	:	Deoxy nucleotide triphosphate
df	:	Degrees of freedom
et al.	:	and coworkers
EDTA	:	Ethidium diamino tri acetate
ESS	:	Error sum of squares
F1	:	First filial generation of a cross
F2	:	Second filial generation of a cross
F3	:	Third filial generation of a cross
F_4	:	Fourth filial generation of a cross
Fig	:	Figure
g	:	Grams
HCl	:	Hydrochloric acid
H_2SO_4	:	Sulphuric acid
i.e.	:	That is
kg	:	Kilogram
m	:	Meter
m^2	:	Square meter

M	:	Molar
mM	:	milli molar
mg	:	milli gram
mg g^{-1}	:	milligram per gram
mha	:	million hectares
ml	:	millli litre
mt	:	million tons
$MgCl_2$	:	Magnesium chloride
MSS	:	Mean sum of squares
MTU	:	Maruteru
ng	:	Nano grams
N	:	Normality
NS	:	Non-significant
NaCl	:	Sodium chloride
Per se	:	As such with mean
PCR	:	Polymerase Chain Reaction
PIC	:	Polymorphic information content
rpm	:	Revolutions per minute
SS	:	Sum of squares
SSR	:	Simple sequence repeats
TE	:	Tris EDTA
TAE	:	Tris acetate EDTA
TSS	:	Total sum of squares
TrSS	:	Treatment sum of squares
V	:	Volts
viz.,	:	Namely

ABSTRACT

Name of the Author	:	**K. HRUDAYA RAJ**
Title of the thesis	:	**MOLECULAR CHARACTERIZATION OF ELITE GENOTYPES FOR SALINITY TOLERANCE IN RICE (*Oryza sativa* L.)**
Faculty	:	**AGRICULTURE**
Department	:	**GENETICS AND PLANT BREEDING**
Major Advisor	:	**Dr. T. SRINIVAS**
University	:	**ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY**
Year of Submission	:	**2019**

The present investigation was carried out to evaluate and characterize 80 rice genotypes for seedling and reproductive salinity tolerance and also to study character association for reproductive salinity tolerance parameters, namely, grain yield and yield components and salinity score at seedling stage under saline conditions. The investigation was undertaken at Regional Agricultural Research Station, Maruteru during *kharif* 2016. The analysis of variance revealed significant differences among the genotypes for all characters studied.

Phenotyping of the genotypes for reproductive and seedling salinity tolerance revealed the superiority of Nonabokra and Pokkali. Hence, these genotypes are identified as promising salinity tolerant lines with both seedling and reproductive salinity tolerance. The results on character associations between reproductive salinity tolerance parameters, namely, grain yield and yield components and seedling salinity scores revealed positive and significant association of grain yield with panicle length, number of filled grains per panicle, ear bearing tillers per plant and 100-seed weight under saline conditions.

The study of 80 genotypes for their molecular diversity with 14 SSR markers revealed that banding pattern of MCM-41, MCM-48, MCM-100, MCM-223, MLT-5, MLT- 7, MTU-1064, MTU-2077, MTU-3626, MTU-1078, MTU-1153, MTU-1156, FL-478 was analogous to Pokkali (wild donor) and Nonabokara (salinity tolerant variety) at 180bp with RM10793on chromosome 1. The genotypes, MCM-41, FL-478, CST-9, MTU-1078, Pokkali and Nonabokra showed similar amplicon size of 210 bp and were distinct from banding pattern of remaining genotypes with the SSR marker, RM10964.

Among the markers used for study of diversity, marker RM 10793 reported highest PIC value of 0.838 followed by RM 10694 (0.763), RM 20224 (0.637) and RM 518 (0.603). RM 10793 showed maximum number of four alleles along with RM 10694 (4) followed by RM 20224 (3), RM 492 (3). Cluster analysis and dendrogram for the 80 genotypes by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) revealed distribution of the 80 genotypes into two major clusters. Cluster I comprised of three sub clusters, while, Cluster II comprised of two sub clusters. Most of the salt tolerant varieties *viz.,*Pokkali, Nonabokra, CSR -27 were grouped in sub cluster II B. It is concluded that Pokkali and Nonabokra, genotypes can be used as donor for varietal improvement or for introgression of salinity tolerance into a susceptible variety.

Chapter – I

Introduction

Chapter I

INTRODUCTION

Rice (*Oryza sativa* L.) is principle food for more than three billion people world wide. Asia alone contributes to more than 90 per cent of the world's rice production (Mitin, 2009). In Asia, 90 per cent of rice is produced by small and marginal farmers. Rice provides almost 50–80 per cent of daily calorie intake amongst the poor class of the society. The total rice cultivated area in India is about 43.5 m. ha. but the production and productivity of the crop is very low, compared to China. The dismal state of rice production in our country is due to major abiotic and biotic stresses. Abiotic factors like drought, salinity and floods affect rice production adversely in more than 50 per cent of the crop area.

Salinity is the second most wide spread problem in rice growing countries next only to drought and is considered as a serious limitation in increasing rice production worldwide (Gregorio *et al.,* 1997). Salt tolerance is generally defined as sustained growth of the plant in the soil environment impregnated with NaCl or other salt combinations. The available three categories of alkali, saline and saline-sodic soils are considered as problem soils and hence are generally termed as salt affected soils. In India, about 8.6 m. ha (18%) of rice cultivated area is affected by either inland or coastal salinity. Of the two saline situations, inland salinity (mainly sodicity) is increasing in the irrigated areas either due to defective irrigation management or poor drainage system. It causes yield reduction and also shrinks caloric and nutritional potential of agricultural products (Yokoi *et al.,* 2002).

Several studies have clearly indicated that rice is sensitive to salt stress not only at early seedling stage but also during pollination and fertilization (Lutts *et al*. 1995). Earlier studies have revealed that the correlation between seedling stage tolerance and reproductive stage tolerance is low and that the gene(s) governing the tolerance to salinity at early seedling and reproductive stages are independent. Hence, exposing the genotypes for salt stress separately both at

early seedling and reproductive stages is desired to identify the tolerant lines. Seedling stage screening is rapid and simple but very efficient method. However, appropriate stage for salt stress screening is reproductive stage because grain yield is vital factor in rice. Salinity during reproductive stage causes sterility and thereby decreases grain yield. Hence, salt-tolerant varieties with tolerance at both, early seedling and reproductive stages are preferred in breeding programmes.

Screening under field conditions for salinity is however, difficult due to stress heterogeneity, presence of other soil-related stresses, and the significant influence of environmental factors such as temperature, relative humidity, and solar radiation. So screening under controlled condition has the benefit of reduced environment effects and in this context, the hydroponic system has been identified to be free of difficulties associated with field screening.

Marker technology for characterization of the genotypes and study of the molecular diversity is more reliable than the morphological diversity because environment has strong influence on the latter. SSRs are highly effective in molecular characterization of the genotypes and also in assessing the genetic diversity existing in the genotypes as well as in genetic mapping studies. At seedling stage '*Saltol*' a major QTL located on chromosome 1of rice genome was identified against salt stress. Mapping Quantitative trait loci for salt tolerance in rice have been reported by many scientists (Sankar *et al.,* 2011). In recent studies QTL's were identified using 50K SNP chip for salinity tolerance at reproductive stage.

Characterization of the genotypes and study on the extent of variability among the genotypes is also essential to incorporate such genotypes in the salinity tolerance breeding programmes. In this context, the present investigation is proposed with the following objectives:

1. Screening of the varieties and advanced breeding lines for seedling stage salinity tolerance.

2. Screening of the varieties and advanced breeding lines for reproductive stage salinity tolerance.

3. Molecular characterization and study of diversity analysis using microsatellite markers.

4. Character associations of yield components under salinity conditions

Chapter – II

Review of Literature

Chapter II

REVIEW OF LITERATURE

A brief review of literature related to the salinity in general and the objectives of the present investigation in particular is presented in this chapter under the following subheads.

2.1 Salinity stress in rice

2.2 Screening methodology and characterization for salinity tolerance

2.3 Genetics of salinity tolerance in rice

2.4 Molecular diversity

2.5 Correlation

2.1 SALINITY STRESS IN RICE

Salinity is one of the major impediments for enhancing production in rice growing areas world wide. One - fifth of irrigated arable land in the world have been reported to be adversely influenced by high soil salinity. Over 800 million ha of worldwide land is severely salt affected and approximately 20 per cent of irrigated area (about 45 million ha.) suffers from salinization problems by various degrees (FAO, 2010). It is a major threat since irrigated areas are responsible for one-third of world's food production. Salt stress is a major problem in coastal areas because of seawater intrusion during high tide and the rising shallow saline ground water, particularly during the dry season. In Asia, 21.5 million ha. of rice area is affected by salinity and estimated to cause the loss upto 50 per cent fertile land by the 2050.

Millions of hectares of land suitable for rice production in India is currently unexploited because of salinity and other related soil problems. An accurate assessment of the extent of salt affected soil in India is difficult due to the dynamic nature and seasonal fluctuation of the degree of salt stress, particularly in coastal areas. Broadly there are two types of salt affected areas

based on their vicinity to the sea *i.e.,* coastal salinity and inland salinity. Based on the chemical nature of the salt stress these soils can be divided into either saline soils or sodic soils.

Salt stress is a major constraint to cereal production worldwide. Salinity is one of the major obstacles in increasing rice production worldwide. Accumulation of salt has deleterious effects and there by hampers rice production. Rice is a salt-sensitive crop, but it is the only cereal that has been recommended as a desalinization crop because of its ability to grow well under flooded conditions. Rice is comparatively tolerant to salt stress during germination, active tillering, and towards maturity and is highly sensitive during early seedling and reproductive stages.

About 9.5 million ha. of saline soils can be managed by large-scale irrigation and drainage schemes and by chemical treatment of soil, but the scale of the problem renders these solutions too costly (Gregorio *et al.*, 2002). Use of salt-tolerant variety is considered to be the most economical, ecofriendly and most effective way of increasing rice production and productivity in saline soils. Therefore, development of salt tolerant varieties is considered as one of the best strategies to increase rice production in saline prone coastal areas.

2.2 SCREENING METHODOLOGY AND CHARACTERIZATION FOR SALINITY TOLERANCE

2.2.1 Seedling stage

Pearson *et al.,* (1966) conducted an experiment with 15 varieties at different salinity levels *i.e.,* 0.3, 10.7, 20.5, 30.4 and 40.3 mmho per cm at 25°C in order to identify the stage which is tolerant to salinity. They concluded that it is sensitive to salinity at early seedling stage and also during pollination and fertilization but it gains tolerance at vegetative stage and also at maturity stage.

Nejad *et al.* (2008) grouped 36 diverse rice genotypes including IR29 (salt sensitive) and FL 478 (salt tolerant). Using thirty three polymorphic SSR markers located on chromosome 1 they determined the impact of these markers

associated with salt tolerance in rice. Based on the clustering pattern, the genotypes were grouped into three groups, each one having 12, 8 and 16 genotypes respectively. Highly salt-tolerant IRRI elite lines were grouped in cluster I, while moderately tolerant lines and the checks *viz.,* Pokkali, FL 478 and Micha were grouped in cluster II and susceptible genotypes were included in cluster III.

Mahmood *et al.* (2009) studied the effect of salt tolerance on four commercial varieties and 17 breeding lines of Basmati rice by assessing growth at early stage and at maturity in field plots artificially salinized with NaCl and $CaCl_2$ (1:1 by weight) and finally concluded that significant variability was present for salt tolerance in Basmati rice and hence selection for salinity tolerance can be carried out at early stages of growth. Also, there is a relationship between Na exclusion and salt tolerance in respect of grain yield and reduced grain yield under saline conditions is mainly due to reduction in grain number per panicle.

Bhowmik *et al.* (2009) evaluated eleven rice germplasm lines which includes six Bangladeshi landraces, four BINA developed mutants and one salt tolerant Indian variety, Pokkali. Under saline conditions, these genotypes showed wide variation in phenotypes ranging from score 1 (highly tolerant) to score 9 (highly susceptible) and finally concluded that Pokkali, THDB and TNDB-100 genotypes were tolerant to salinity and RD-2586, PNR-519, DholKochuri and Bara Dhan genotypes were moderately tolerant and Kaliboro139-2 and Kaliboro 109-4 genotypes were extremely susceptible to saline conditions.

Tambhale *et al.* (2011) studied effect of salinity by evaluating two local rice cultivars *viz.,* Indrayani and Ambemohar based on germination, growth and biochemical parameters. In this experiment, the effect of increasing salt stress was observed on germination, biomass production and biochemical parameters including total protein content, proline accumulation, starch content, polyphenol levels, reducing and non-reducing sugars and observed continuous decrease in

starch content with increased salt stress in Indrayani. On the other hand, an increase in starch content was evident in Ambemohar under the influence of NaCl induced salt stress. Thus, finally concluded that higher salt tolerant nature was observed in Ambemohar comparatively than Indrayani which might be attributed to higher proline, protein and starch content under salt stress.

Lodha *et al.* (2011) studied 17 rice genotypes which includes eight West Bengal traditional rice lines, two exotic lines, six improved rice lines and one wild rice relative (*Oryza rufipogon*) using six polymorphic microsatellite markers associated with *Saltol QTL* mapped on rice chromosome 1. DNA fingerprint profiles of each of these 17 rice genotypes were constructed unequivocally which were used for assessing the genetic diversity present within rice lines and identification of the individual genotypes and concluded that these genotypes showed considerable amount of genetic diversity based on which they are classified into two major clusters and one minor cluster.

Song *et al.* (2012) studied two putative salt tolerant lines (ST-87 and ST-301) which were exposed to gamma-irradiation followed by salinity tolerance screening under *in vitro* conditions. These two lines were subjected to physiological analysis and the traits *viz.,* electrolyte leakage (EL), malondialdehyde (MDA), antioxidant, chlorophyll, total amino acid and Na^+/K^+ ratio were analyzed and observed that these ST-lines had lower levels of enzymatic catalase activity and higher peroxidise activity under the salt condition.

IAEA (2012) screened six genotypes *viz.,* Pokkali, Nona Bokra, Bicol, STDV, IR 20 and Taipei using hydroponics for salinity tolerance at seedling stage. In this method stress was subjected to salt stress at 2-3 leaf stage *i.e.,* after 1-2 weeks of seedling establishment Seedlings were treated with Yoshida solution having salt (Salt concentration was taken as 10 dS/m corresponds to 6.4 g of NaCl in one litre of medium). Among the genotypes screened Pokkali and Nona Bokra showed slight damage at leaf tips becoming brown, Bicol and

STDV exhibited more leaf damage with death of older leaves while younger leaves are green only at their leaf bases whereas IR20 and Taipei were dead.

Dhar *et al.* (2012) screened 26 germplasm lines for salt tolerance at seedling stage in hydroponic system. In addition to phenotypic observations, data on traits *viz.,* plant height, root length and total dry matter was recorded at salinized and non-salinized conditions and wide variation was observed among the phenotypes under salt stress and concluded that among the 26 germplasm lines, 16 were moderately salt tolerant and 10 were susceptible.

Sudharani *et al.* (2013) studied genetic diversity of eight rice genotypes based on different adaptation to salinity stress and characterized the genotypes using microsatellite markers. These eight genotypes clustered into three distinct groups according to their similarity levels. Two susceptible varieties, RP Bio-226 and Swarna, were in one group, three moderately tolerant varieties CSR-27, CSR-30 (aromatic basmati type), CST-7-1 were in second group and three tolerant cultivars CSRC(S) 7-1-4, SR26B and CSRC(S) 5-2-2-5 were included in the third group.

Joseph and Mohanan (2013) evaluated yield performance of seven native cultivars of rice including five cultivars (Orthadian, Orkazhama, Kuthiru, Kuttusan and Chovvarian) collected from saline rice habitats of Kerala and two native rice cultivars namely Kunhutty and Veliyan collected from the non-saline rice habitats of Kerala. Yield per plant was drastically reduced in all the cultivars except in Kuttusan and Veliyan which did not show significant yield reduction even under higher salt concentration and concluded some of the cultivars like Veliyan which is traditionally cultivated under non-saline conditions in Kerala have inherent capability of growing and performing well under moderately saline conditions.

Amin *et al.* (2013) screened twenty eight genotypes for salinity tolerance at seedling stage in hydroponics following international rice research institute (IRRI) standard protocol. Genotypes were evaluated based on the scale 1-9 for

salinity tolerance on the basis of seedling growth parameters and on the basis of SES and total dry matter (TDM) reduction of the genotypes *viz.,* PBSAL-614, PBSAL-613, PBSAL-730, Horkuch, S-478/3, Pokkali and PBSAL (STL)-15 were found to be salt tolerant; while the genotypes Iratom-24, S-653/32, S-612/32, S-604/32, S-633/32, Charnock(DA6), BINA dhan -6 and S-608/32 were identified as salt susceptible.

Talesha *et al*. (2014) screened eight modified rice cultivars under four levels of irrigation water salinity (1, 2, 4 and 6 dS m^{-1}) with three replications for evaluating eight screening indices (for salinity tolerance). The results showed that Khazar cultivar was the most salt-sensitive cultivar in all salinity levels. In the irrigation salinity levels of 2 and 4 dS m^{-1} Neda cultivar and in the level of 6 dS m^{-1} Dasht cultivar were the most salt-resistant cultivars.

Rubel *et al.* (2014) studied the effect of salinity on 27 traditional improved rice genotypes which includes one salt tolerant cultivar *viz*., BINA Dhan 8, nine high yielding varieties, 16 advanced breeding lines and one land race (Kashrail) of Bangladesh. These selected genotypes were screened for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol and classified the 27 genotypes as tolerant (eight), moderately tolerant (four) and susceptible (fifteen).

Mehede *et al*. (2014) carried out phenotypic screening at seedling stage for 27 rice genotypes at EC 12 dS/m and 6 dS/m. Based on modified standard evaluation score for visual salt injury at seedling stage, eight genotypes were salt tolerant, four were moderately tolerant and the rest fifteen were susceptible.

Harimansis *et al.* (2014) developed a non- destructive image based phenotyping protocol to identify salinity tolerance traits for IR 64 and Fatmawati. In this experiment these two varieties were imposed to three levels of stress conditions *i.e.,* 50mM, 75 mM and 100 mM Nacl. At initial phase the effect of Nacl on growth of rice was small however after 20 days of treatment shoot area of stressed plants was reduced compared with non-stressed plants.

The response of these cultivars to different levels of salt stress was quantified overtime based on total shoot area and senescent shoot area and then calculated from visible red-green- blue and fluorescence images. These images are useful to discriminate between the different aspects of salt stress *i.e.,* shoot ion-independent stress and shoot ion- dependent stress and also to identify the tolerant cultivar.

Yuda *et al.* (2015) focused on the response of the six genotypes taken for study and these were subjected to five different salinity levels i.e., 0 mM, 50 mM, 100 mM, 150 mM and 200 mM of NaCl. All six were tolerant for 100mM of Nacl, four found to be sensitive around 150 mM of Nacl and death occurred at 200 mM of Nacl. The growth response was observed by measuring the total leaf area per plant, height and electrophysiology by measuring potential differences (PD's) of leaves on a weekly basis. significant difference was observed between the genotypes.

Teresa *et al.* (2015) screened 49 rice genotypes at seedling stage for salinity tolerance and concluded that effective identification and selection for high tolerance can be achieved by the accumulation of multiple favourable traits under salt stress.

Syed *et al.* (2015) evaluated for different morpho-physiological traits *viz.,* germination percentage, root length and shoot length, seedling fresh and dry weight, Na^+ and K^+ uptake after 15 days of seedling emergence under control as well as saline conditions and observed significant difference between control and salt stress conditions.

Mukta *et al*. (2017) collected 80 germplasm lines from coastal areas of Bangladesh and IRRI and subjected to phenotypic screening. Based on phenotypic analysis using SES scoring and there was reduction of plant height, root length and dry matter weight in susceptible lines.

Lang *et al*. (2017) developed a population of ninety three lines of BC_3 F_2 by crossing OM 1490 as female parent and Pokkali as male parent. Screening

was done at two levels of EC @ 8 $dsm^{-1}$and 15 dsm^{-1} and score was recorded as per IRRI standards. At 8 dsm^{-1} 35 lines are with level of moderately tolerant to susceptible, 14 lines with a level of tolerant to moderately tolerant and only one with a level of highly tolerant to tolerant but at 15 dsm^{-1} 23 lines with a level of moderately tolerant to susceptible, 5 with a level of tolerant to moderately tolerant indicating moderate salt tolerance.

Ling *et al*. (2018) tested 46 genotypes under saline conditions during seed germination stage and observed that water content, cholorophyll, superoxide dismutase were higher in tolerant line with lower incidence of sodium ion accumulation whereas ascorbate peroxidase and catalase was higher in susceptible lines.

Kumari *et al.* (2018) screened 30 genotypes including two tolerant (Pokkali and CSR -36) and two susceptible (IR 29 and IR 64) was assessed under different levels of salinity (0, 4, 8 and 16 dS m^{-1}) created by salt mixture of NaCl, $CaCl_2$, Na_2SO_4 in 7:2:1 ratio. Overall salinity tolerance indices clearly reflected that 17 varieties including the two tolerant checks were highly tolerant, seven varieties exhibited moderately tolerant, whereas six varieties including the two susceptible checks had highly susceptible response to salt stress.

Banumathy *et al.* (2018) introgressed *saltol* loci from FL 478 to 51 Backcross inbred lines (BIL's) of ADT -31 and 17 lines of CR 1009 Sub 1 were evaluated for their salinity tolerance along with recurrent parents (ADT-31and CR 1009). All 68 BILs were subjected to genotyping using marker RM 3412 which led to the identification of 20 lines in the genetic background of ADT 37 and 12 lines in the genetic background of CR 1009 harboring saltol loci from FL478. Seedlings of all 32 positive progenies were subjected to phenotypic evaluation followed by scoring as per IRRI Protocol. Out of 32 selected BILs, 14 lines were found to be tolerant and remaining 18 lines were identified to be moderately tolerant. Saline tolerant lines viz., BIL 33, BIL 1094, BIL 1096 and BIL 1101 have recorded maximum uptake of K+ in both root and shoot which resulted in low Na^+/k^+ ratio.

2.2.2 Tissue Culture Studies

Saleem *et al.* (2005) tested basmati seeds induced calli on MS medium and the calli were irradiated with gamma rays using ^{60}Co for creating genetic variability against salinity. Irradiated and non-irradiated calli were screened *in vitro* through three consecutive proliferation phases at 4.0, 6.0, 8.0 and 10.0 dSm^{-1} electrical conductivity of NaCl and concluded that *in vitro* technique may be used as a versatile approach to improve the level of salt tolerance in Basmati rice for saline environment.

Shanthi *et al.* (2010) studied seven genotypes which includes salt tolerant (Pokkali, CSR 10, TRY 1 and TRY2) and moderately tolerant (White Ponni and BPT 5204) and susceptible (IR 29) types. For callus induction frequency under different salt concentrations they noticed that callus growth was rapidly reduced with relative increase of NaCl concentration. Further the study also revealed that NaCl had an inhibitory effect on the growth of callus and concluded that all the genotypes showed better regeneration frequency in control regeneration media but the regeneration frequency decreased with increased salt concentration. Out of seven genotypes, Pokkali registered the highest level of callus development (35%) even at high level of NaCl stress (150 mM).

Rawal *et al.* (2010) evaluated six rice genotypes *viz.,* CARI DHAN 5, NDRK 11-1, NDRK 11-2, NDRK 11-3, NDRK 11-4, NDRK 11-5 against salt tolerance. Out of the six genotypes, the genotype NDRK 11-1, had expressed highest yield per plant of 13.6g under saline condition.

Nejad *et al.* (2010) studied the performance of 30 rice genotypes including land races, pure lines and improved cultivars at reproductive stage against three levels of salt stresses (0, 60, 100 mM NaCl) and classified the selected genotypes into five groups from highly tolerant (score 1) to highly sensitive (score 9). Fourteen genotypes were highly tolerant, three were tolerant, six were moderately tolerant, three were sensitive and four were highly sensitive for salinity.

Zinnah *et al.* (2013) cultured *in vitro* two genotypes, BRRI Dhan 38 and Chini Kanai and callus was induced. These calli were transferred to the regeneration medium supplemented with different concentrations of NaCl to check the inherent capacity of calli to regenerate on medium under salt stress condition and reported that plant regeneration of BRRI Dhan 38 was 80% at 0 mM (control) NaCl, but decreased to 20% at 100 mM NaCl and 0% at 150 mM NaCl. The genotype Chini Kanai showed 60% regeneration on no-stress medium and regeneration was reduced to 20% at 150 mM NaCl. Thus the study repeated these two genotypes, Chini Kanai, is more salt tolerant then BRRI Dhan 38.

Rudra *et al.* (2013) utilized Mature Seed Scutellum (MSS) as explants of three rice varieties *viz.,* Rajashail, Katicota and BRRI-22 and cultured them under *in vitro* conditions (MS medium supplemented with different concentrations of NaCl 0.2 to 1.5%) for assessing the effect of salt stress on germination rate, length of shoot and root, callus induction, fresh weight of callus and finally regeneration and concluded that upon increasing the NaCl concentration, the rate of germination and callus proliferation were decreased. All three varieties responded at 0.2% and 0.5% NaCl but at 1.0% level, regeneration was not observed. Among the three varieties,Rajashail and Katicota, responded well in regeneration than BRRI-22.

2.2.3 Reproductive Stage

Aref and Rad (2012) studied the effect of salinity by taking saline water at four levels (2, 4, 6, and 8 dSm^{-1}) at reproductive stages of crop growth (tillering, panicle initiation, panicle emergence and ripening) and the results revealed that the effect of different levels of salinity on all the yield components was significant. Resistance at final growth stages *i.e.,* panicle emergence and ripening stages against salinity was more than primary growth stages *i.e.,* tillering and panicle initiation. Panicle initiation was the most sensitive stage to salinity followed by tillering, ripening and panicle emergence stages.

Diana *et al*. (2013) conducted experiments in order to standardize rice screening for reproductive-stage salinity tolerance. Four different experiments was conducted – in experiment A, all the leaves were cut except the flag leaf. Meanwhile, in experiment B all leaves were trimmed except flag leaf and the penultimate leaf while plants in experiment C were pruned to have the top three leaves (including the flag leaf). The plants in the control setup remained untrimmed. Data on number of filled grains/plant, panicle length, and 100-seed weight were taken for each plant. The stage-specific effect of salt stress was observed in salt-sensitive (IR64) and salt-tolerant (IR4630-22-2-5-1-3) genotypes, along with 201 F_2 plants derived from their cross. Leaf cutting before the booting stage efficiently directed the salt concentration to the reproductive stage and helped in discriminating the tolerant genotypes from the sensitive ones as evidenced by the low pollen viability and higher accumulation of toxic ions in the flag leaf of the sensitive genotype (IR64). The opposite was found true for the tolerant genotype (IR4630-22-2-5-1-3).

Hossain *et al*. (2014) developed F_2 mapping population from two rice genotypes contrasting in tolerance: Cheriviruppu and Pusa Basmati 1 (PB1). Cheriviruppu is highly tolerant at the reproductive stage, while PB1 is highly sensitive at both seedling and reproductive stages. One hundred and thirty-one microsatellite markers polymorphic between the parents were used to construct a linkage map of 1458.5 cM (Kosambi), with a mean intermarker distance of 11.1cM. Sixteen QTLs with LOD values ranging from 3.2 to 22.3 were identified on chromosomes 1, 7, 8 and 10, explaining 4–47 % of the phenotypic variation. The results suggest that pollen fertility, Na^+ concentration and Na/K ratio in the flag leaf are the most important mechanisms controlling salt tolerance at the reproductive stage in rice.

Mostafa *et al.* (2016) standardized a methodology (by cutting old leaf) that allows salt translocation to the reproductive organs as quickly as possible just at the initiation of booting which was growth stage-specific rather than growth duration-dependent. The results showed cutting the old leaves of the rice plant, leaving only the flag leaf and penultimate leaf, had no significant effect on

yield components and that way salt reached to the reproductive parts very quickly after 2 or 3 days of stress treatment in the leaf pruning method. Whereas in untrimmed plants, there is no high Na^+ accumulation of in the top two leaves even after 9 days of stress treatment. Saline stress (10 dSm^{-1}) was imposed for 20 days at the time of the first appearance of the flag leaf for the reproductive-stage phenotyping. The aim of the phenotyping is to discriminate the genotypes as sensitive, tolerant or intermediate rather than killing or making all the plants sterile.

2.3 GENETICS OF SALINITY TOLERANCE IN RICE

2.3.1 Seedling Stage

Jiming *et al.* (2001) studied the performance of 127 lines of doubled haploid (DH) population obtained from the cross ZYQ8, an *indica* variety and JX17, a *japonica* variety under salt stress and control conditions and reported that JX17 was more tolerant to salt stress than the ZYQ8. These findings also showed ZYQ8 performed well for all the five traits investigated, particularly for more number of effective tillers than that of JX17 under salt stress condition.

Lin *et al.* (2003) evaluated 133 F_3 lines against salt stress along with their parental lines Nonabokra, (salt tolerant) and Koshihikari (salt-susceptible) and studied physiological traits associated with salt tolerance is a shoot and root traits of rice and concluded that salinity affects almost all processes of the plant. The osmotic effects by high ionic concentrations leads to competitive interference with nutrient uptake and causes toxic effects within the plant tissue that ultimately hinders plant growth and even causes death of plant tissues.

Zhong *et al*. (2005) derived F_1 plants from the cross between Nonabokara (tolerant variety) and Koshihikari (susceptible variety & recurrent parent) repetitive backcross and marker assisted back cross was done and selected near isogenic line for *SKC1* (which contain a small chromosome segment of Nonabokara containing *SKC* locus) from BC_5F_2 generation. *SKC1* gene found that it encodes a member of HKT-type transporters. *SKC1* is preferentially expressed in the parenchyma cells surrounding the xylem vessels. Voltage-clamp

analysis showed that *SKC1* protein functions as a Na^+-selective transporter. Physiological analysis suggested that *SKC1* is involved in regulating K^+/Na^+ homeostasis under salt stress, providing a potential tool for improving salt tolerance in crops.

Akhtar *et al.* (2010) evaluated a total of 29 lines of F_4 population of rice along with their parents Binadhan-5 (high yielding and salt susceptible) and Harkuch (salt tolerant landrace) for salt tolerance at the reproductive stage. In this experiment, high heritability coupled with high genetic advance was observed for plant height and number of filled grains/plant in saline condition and hence concluded that these characters were under additive gene control and selection for salt tolerance might be effective. Analysis of genetic variability, correlation and path coefficient analysis clearly indicated that the number of filled grains/plant is the determining trait for grain yield under salt stress and this trait can serve as indicator in selecting rice lines for salt tolerance with higher yield.

Michael *et al*. (2010) characterized Pokkali derived QTL at seedling salinity tolerance and used in marker assisted breeding. A study was undertaken for detecting the location of *SALTOL* QTL on chromosome 1 by using 100 SSR markers in IR 29/pokkali recombinant lines and confirmed the location. In addition to this, additional QTL associated with tolerance was identified. Analysis of a series of backcross lines and near-isogenic lines (NILs) developed to better characterize the effect of the *Saltol* locus revealed that *Saltol* mainly acted to control shoot Na^+/K^+ homeostasis. Multiple QTLs were required to acquire a high level of tolerance. Multiple Pokkali alleles at *Saltol* were detected within the RIL population and between backcross lines and representative lines were compared with seven Pokkali accessions to better characterize this allelic variation.

Aliyu *et al.* (2011) studied two F_2 populations (population one with 180 plants and population two with 150 plants) along with parental lines IR88399-B and IR88317-B using four *Saltol* markers and reported that the percentage of

tolerant lines (17.5%) and moderately tolerant lines (33.33%) in population two were higher than the percentage of tolerant (10.6%) and moderately tolerant lines (28.9%) in population one. The study finally concluded that in rice, important traits such as salt tolerance was controlled by polygenes with additive and dominant effects. When two populations originated from distinct germplasm were evaluated then the DNA markers identified in one population may not be useful in the second population.

Islam *et al.* (2011) evaluated three different F_2 populations against salt stress. Population I consists of 288 plants obtained from the cross BRRI Dhan40 (susceptible variety) and IR61920-3B-22-2-1 (tolerant variety) where as population II consists of 281 plants obtained from the cross BRRI Dhan28 (susceptible variety) and IR50184-3B-18-2B-1 (tolerant variety). Population III consists of 292 plants obtained from the cross Kajalshail and IR52713-2B-8-2B-1-2 and in this population both the parents were tolerant to salinity and reported that among the 288 plants in population I, 125 were classified as tolerant to highly tolerant and remaining plants were scored as susceptible to highly susceptible to salinity. In population II, among the 281 plants studied, 185 plants were scored as susceptible to highly susceptible to salinity and only few were scored as tolerant. In population III, among 292 plants, more than 250 plants were highly tolerant and the remaining were tolerant. Almost none were susceptible to salinity.

Moniruzzaman *et al.* (2012) studied 40 BC_1F_1 progenies of the cross Binadhan-5 x FL-478. Three markers *viz.*, RM336, RM510 and RM585 were used to identify salt tolerant lines. They observed some genotypically salt tolerant rice lines using SSR markers. In respect of primer RM336, 12 lines were found as salt tolerant and 25 lines were heterozygous and three lines were susceptible. Primer RM510 identified two tolerant, 14 heterozygous and 22 susceptible lines while primer RM585 identified four lines as tolerant and 35 lines as susceptible.

Ramana *et al.* (2017) performed cross between a high yielding elite salt susceptible *japonica* rice cultivar Jupiter and a salt tolerant *indica* landrace Nonabokra and derived 138 ILs (Introgression lines). These lines were evaluated for screening at seedling stage and were also genotyped using 126 SSR markers. By composite interval mapping for 8 morphophysiological traits, 33 additive QTLs were detected. From phenotypic responses, genomic composition and QTLs identified they concluded that Na/K ratio is the key factor for salinity tolerance.

Quan *et al*. (2018) crossed between Dongxiang (wild rice) /Ningjing 16 (cultivated rice variety) and identified a salt tolerant line DJ (15). By QTL mapping studies they identified 9 QTL's for salt tolerance (*qST*) located on chromosome number 1, 3, 4, 5, 6, 8 & 10 at seedling stage.

Ramana *et al.* (2018) studied 112 introgression lines (ILs) of a salt-tolerant donor 'Nonabokra' in the genetic background of a US cultivar 'Cheniere' to elucidate the genetic basis of seedling stage salinity tolerance. These introgressed lines were evaluated under saline conditions, control conditions and were also genotyped by using 116 SSR markers. In this study 18 QTLs were detected for salt tolerance indices while 32 QTLs were identified for eight morpho-physiological traits. They finally concluded that shoot Na^+/K^+ homeostasis, Na^+ exclusion and compartmentation as possible salt tolerance mechanisms in 'Nonabokra' in addition to this Candidate gene identification and gene ontology analysis revealed that the genes involved in ion transport, ion homeostasis and signaling may have important role in improving salinity tolerance.

2.3.2 Reproductive Stage

Hien *et al*. (2012) applied marker assisted backcrossing system on foreground selection, recombinant selection followed by background selection for development of Vietnamese rice variety that can tolerate salinity. The results showed that, the best plants of BC_3F_1 generation carry segments of the donor (11.16 - 12.6 Mb), which had 96.8% - 100 % of the recipient genome.

Le Hung *et al*. (2012) used marker assisted backcrossing to improve salt tolerance in rice cultivar BT7 cultivar, FL478 was used as a donor parent to introgress the *Saltol* QTL conferring salt tolerance into BT7. Three backcrosses were conducted and successfully transferred positive alleles of *Saltol* from FL-478 into BT7. The plants lines IL-30 and IL-32 in BC_3F_1 population expected recurrent genome recovery of up to 99.2% and 100%, respectively. The improved lines were screened for agronomic traits in the field. All improved lines had *Saltol* allele similar to the donor parent FL478, whereas their agronomic performances were the same as the original BT7.

2.4 MOLECULAR DIVERSITY

Aliyu *et al*. (2011) used SSR markers associated with *SALTOL* QTL and studied 150 diverse rice genotypes. Result of phenotypic response of rice genotypes to salinity stress at seedling stage indicated varied genotypic response. Alleles ranged from 3 in RM493 and RM3412 to 4 in RM10793. Polymorphic information content value ranged from 0.6 to 0.73. RM10793 with a resolving power of 0.96 was most informative primer for genetic diversity in this study. Cluster analysis of the allelic data obtained clustered some tolerant genotypes with Pokkali.

Amin *et al.,* (2013) screened 28 genotypes genotypically by using 10 SSR markers *viz.,* RM443, RM140, RM169, RM18, RM21, RM152, RM9, RM127, OSR17 and OSR140. With respect to RM127, 7 lines were found to salinity tolerance, 11 were found moderately tolerant and 10 were susceptible. Nine tolerant, nine moderately tolerant and ten susceptible lines were found when the primer RM 140 was used and primer RM 443 identified 8 lines as tolerant, 9 lines as moderately tolerant and 11 lines as susceptible.

Mehede *et al.* (2014) selected three SSR markers for identification of salinity tolerant genotypes in rice *viz.,* RM 10772, RM 7075, RM 296. Of 27 genotypes, seven genotypes as tolerant but ten of them were susceptible for all three markers.

Mukta *et al.* (2017) conducted studies on 80 germplasm and identified 25 genotypes for molecular studies. Six random primers were used for parental polymorphism of them three markers were used to evaluate the genotypes (RM510, RM585, RM336). Score was given as 1 for the presence of allele and 0 was given for absence of allele. Number of alleles ranged from 10-12/ locus with an average of 11. Among the three primers highest number of alleles was shown by RM 510. Polymorphic information content (PIC) was calculated by using Anderson method and average PIC value was 0.88. Highest PIC value was observed with primer RM 510 (0.89) and lowest with primer RM336 (0.85). Analysis was done using NTSYS-PC 2.1 version and genotypes were clustered into 5 major clusters.

Kumari *et al.* (2018) screened 30 genotypes including two tolerant (pokkali and CSR -36) and two susceptible (IR 29 and IR 64) by using 24 SSR markers and results revealed that 8.5 alleles per primer with altogether 114 shared and 91 unique allelic variants. Considering the allele number, polymorphism information content and polymorphism percent, SSR primers RM302, RM8094, RM10665, RM10694, RM10748 and RM10825 appeared to be highly polymorphic and comparatively more informative.

2.5 CORRELATION OF THE YIELD TRAITS AND SALT STRESS

Zeng *et al.* (2003) studied correlation between leaf area index and grain yield under saline conditions in twelve rice genotypes of rice in green house conditions and observed high genotypic diversity for LAI and shoot ion concentration among the genotypes studied, LAI contributed the most to the variation in grain yield under salt stress. A high significant correlation between grain yield and leaf area index indicated that leaf area played a significant role in the salt tolerance as defined by grain yield.

Sexicon *et al.* (2009) evaluated Sixteen rice genotypes for salinity tolerance. The genotypes were subjected to 12 dS m^{-1} salt stress during seedling stage. Prior to salinization relative vigor was measured while visual evaluation

of salinity stress symptoms, leaf chlorophyll concentration, shoot and root growth, and sodium and potassium concentrations were measured 25 days after salinization. Among the genotypes Pokkali, Cheriviruppu, FL478, and IR651 consistently expressed salt tolerance characters *viz.,* higher vigor, lower standard evaluation system (SES) scores, higher total chlorophyll concentration, higher shoot and root biomass, lower shoot Na^+ accumulation, and lower shoot Na^+/K^+ ratio compared to the salt-sensitive genotypes. Cultivars IR29, CSR11, IR74, Kalimekri, and Bhirpala had high shoot Na^+ concentration, high shoot Na^+/K^+ ratio, high SES scores, low shoot and root biomass, low leaf chlorophyll concentration, and low vigor, indicating sensitivity to salt stress. These morpho-physiological traits were strongly correlated with one another.

Haq *et al.* (2009) evaluated morpho-physiological response of seven rice varieties (Moroberekan, Co39, Azucena, Bala, IR64, Kalinga-III and Nipponbare) and recorded physiological and growth parameters after 21 or 42 days of their exposure to salt stress and observed that physiological parameters showed significant higher accumulation of Na^+ in Moroberekan (52.9 mol m^{-3}) and lowest (14.1 mol m^{-3}) in IR64, followed by Co39 (14.6 mol m^{-3}) and Azucena (14.7 $molm^{-3}$). The study revealed that there was a negative correlation between leaf Na^+ and K/Na ratio in varieties under salt stress. Growth parameters were also negatively correlated with salinity stress and no significant correlations were observed between leaf K^+ and any of the growth traits.

Bhowmik *et al.* (2009) conducted phenotyping of 11 genotypes under hydroponic system using salinized (EC 12 dSm^{-1}) nutrient solution. Large variation for salinity tolerance among the rice germplasm was observed and reported that plant height and total dry matter of tolerant lines were reduced by 19.0% and 40.6% respectively while the susceptible lines were reduced by 46.0% and 73.5%, respectively under salt stress (EC 12 dSm^{-1}).

Mansuri *et al.* (2012) screened 40 rice genotypes at vegetative growth stage in saline soil with electrical conductivity (EC) 4.0, 8.0 and 12.0 dSm^{-1}. Tolerant genotypes selected were tested against salt stress initially at young

seedling stage in hydroponic system and then at reproductive stage as per IRRI standard protocol (Gregorio *et al.,* 1997) and finally, 15 genotypes were selected. These selected genotypes were further analysed for correlation among the physiological traits under saline conditions. Correlation analysis showed that grain yield had very significant correlation with panicles/plant at 6.0 and 12.0 dSm^{-1} and concluded that harvest index had negative and very significant correlation with spikelet sterility in both salinity levels. Further, length of panicles had negative and significant correlation with spikelet sterility. Correlation between biomass with panicles/plant, straw weight and grain weight was significant in 6 and 12 dSm^{-1}.

Khadija *et al.* (2013) studied effect of salinity on rice and salinity relieving role of GA_3 (150 ppm) using two salt tolerant rice varieties *viz*., Pokkali and MR219. Both these cultivars were grown at various salt concentrations (0, 50, 100, 150 and 200 mM) in glass house condition and observed that there was a correlation between number of panicles/plant and GA_3 treatment under saline conditions. A decreasing trend in number of panicles/plant with a unit increase in salt concentration was observed without GA_3 treatment. Both genotypes MR219 and Pokkali, had moderate by tolerance to salinity (50 and 100 mM) and application of GA_3 performed better in recording higher grain yield. Plants of MR219 grown in severe salinity stress (150 and 200 mM) could not initiate/form panicles and thus grains. However, Pokkali showed tolerance but recorded low number of panicles and filled grains. Thus, the study indicated that without GA_3 treatment negative correlation between panicles per plant and salinity was observed where as positive correlation was seen after the GA_3 treatment.

Khan *et al.* (2014) evaluated 24 rice varieties under natural saline condition (salinity block) and normal field conditions to know the effect of salinity on various agronomic traits and observed positive correlation of morphological traits such as number of total tillers/plant, number of effective tillers/plant, panicle length, panicle weight, number of spikelets/panicle, number of grains/panicle, panicle fertility (%) with grain yield under saline conditions.

Under normal field conditions, negative correlation was observed between days to 50% flowering and days to maturity with grain yield/plant, whereas under saline conditions these traits showed non significant negative correlation. Thus, the study concluded that these cultivars had better balance between vegetative and reproductive phase, resulting in better grain yield/plant under stress conditions.

Vijayadurga (2015) evaluated eight families of BC_3F_2 population of the cross MTU 1010 x FL 478 for salinity screening at seedling stage and also studied association between salinity tolerance at seedling stage and yield components. Results revealed that association between grain yield/plant, initial and final salinity scores were negatively significant. But yield parameters *viz.,* ear bearing tillers, panicle length and spikelet fertility showed positive and significant association with grain yield.

Lang *et al.* (2017) crossed OM 1409 and Pokkali and generated a population of BC_3 F_2 with ninety three lines and screening was done at seedling stage at two EC levels *i.e.,* 8 ds/m and 15 ds/m and certain parameters were taken for the study and observed that survival time was highly correlated with plant height, dry weight of the stem, dry weight of root and level of salinity tolerance.

Chapter – III

Material and Methods

Chapter III

MATERIAL AND METHODS

3.1 MATERIAL

The present study was conducted during *kharif* 2016 at Regional Agricultural Research Station (RARS), Maruteru, West Godavari district of Andhra Pradesh State located at 81.44^0 E longitude and 26.38^0 N latitude in Godavari Zone of Andhra Pradesh.

Eighty rice genotypes comprising of rice varieties and advanced breeding lines from RARS, Maruteru; Agricultural Research Station, Machilipatnam; and Agricultural Research Station, Bapatla presented in Table 3.1 were used in the current studies.

3.2 METHODS

3.2.1 Molecular Characterization Using Microsatellite Markers

3.2.1.1 DNA Extraction

The DNA was extracted from leaf tissue from all the genotypes using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Dellaporta *et al.* (1983) with certain modifications. The stock solutions required for the preparation of CTAB were prepared as per the procedure given below:

a) 1 M TrisHcl

Trizma base (30.28 g) dissolved in 200 ml of distilled water and the pH of the solution adjusted to 8.0 using concentrated hydrochloric acid. The solution was allowed to cool at room temperature. Final volume adjusted to 250 ml distilled water and the solution was sterilized by autoclaving.

Table 3.1 List of rice genotypes studied in the present investigation

1	AST - 56	11	CSR -27	21	CST - 10	31	MCM-100	41	MLT - 10	51	MTU-1075	61	MTU-1194	71	MTU5182
2	AST - 57	12	CST – 1	22	E 456	32	MCM-223	42	MLT - 11	52	MTU-1078	62	MTU-1210	72	MTU-5249
3	AST - 60	13	CST – 2	23	E460	33	MCM-225	43	MTU-1001	53	MTU-1112	63	MTU-1224	73	MTU-5293
4	AST - 62	14	CST – 3	24	E 467	34	MLT - 1	44	MTU-1006	54	MTU-1121	64	MTU-1226	74	MTU-7029
5	AST - 64	15	CST – 4	25	FL -478	35	MLT - 2	45	MTU-1010	55	MTU-1140	65	MTU-1229	75	NONABOKRA
6	AST - 66	16	CST – 5	26	IR64	36	MLT - 4	46	MTU -1031	56	MTU-1153	66	MTU-2067	76	PLA 1100
7	BPT - 2231	17	CST – 6	27	IR76393-2B-7-1	37	MLT - 5	47	MTU- 1032	57	MTU-1156	67	MTU-2077	77	POKKALI
8	BPT-2270	18	CST – 7	28	MCM-27	38	MLT - 6	48	MTU-1061	58	MTU-1166	68	MTU-2716	78	STBN-12-1
9	BPT-3291	19	CST – 8	29	MCM-41	39	MLT - 7	49	MTU-1064	59	MTU-1184	69	MTU-3626	79	STBN-12-7
10	BPT-5204	20	CST – 9	30	MCM-48	40	MLT - 8	50	MTU-1071	60	MTU-1187	70	MTU4870	80	STBN-12-10

b) 0.5 M EDTA

Disodium Ethylene Diamine Tetra Acetate (EDTA) with $2H_2O$ weighed to 46.35 g and added to 200 ml of distilled water. The solution was dissolved using magnetic stirrer by adding 4 g of NaOH pellets. The pH was adjusted to 8.0 with NaOH. The final volume made up to 250 ml with distilled water and the prepared stock was sterilized by autoclaving.

c) 5 M Nacl

NaCl (73.05 g) dissolved in 200 ml of distilled water and the final volume adjusted to 250 ml with distilled water and it was sterilized by autoclaving.

d) 2% CTAB

A quantity of 2 g of CTAB (Cetyl Tri Methyl Ammonium Bromide) was dissolved in 100 ml of sterile distilled water and stored at room temperature. The reagents required for the preparation of extraction buffer by using the chemicals.

Composition and concentrations of CTAB extraction buffer

S. No.	Components	Stock concentration	Working concentration	Volume for 500 ml
1	Tris HCL	1 M	100 mM	100 ml
2	EDTA	0.5 M	20 mM	20 ml
3	NACL	5 M	1.4 mM	140 ml
4	CTAB	2%	2 g	10 g
5	PVP	2%	2 g	10 g
6	DDW	To make up final volume		

e) *Ribonuclease*A

Approximately 10 mg of *Ribonuclease*A powder was dissolved in 8 ml of double distilled water taken in a sterilized 15 ml centrifuge tube and the contents were boiled in a boiling water bath for 15 minutes. The solution cooled to room temperature and the final volume made up to 10 ml using double distilled water. Solution was stored at -20ºc.

f) Ethidium bromide solution

The 10 mg ml^{-1} of ethidium bromide solution was prepared by dissolving 1 g of ethidium bromide in 100 ml of distilled water.

g) 50 X TAE

The 50 X TAE buffer was prepared by dissolving 242 g of Tris Base in 400 ml double distilled water, to which 57.1 ml glacial acetic acid was added and mixed well by continuous stirring on magnetic stirrer. Then 100 ml of 0.5 M EDTA (pH 8.0) was added and the solution was made up to 1000 ml using double distilled water. Later, the solution was sterilized by autoclaving at 121° C temperature, 15 psi pressure for 20 minutes. Later, 1 X TAE was prepared by diluting 20 ml of 50 X TAE to 1000 ml by the addition of sterile distilled water.

3.2.1.2 Procedure of genomic DNA isolation

Healthy leaf samples (2-3 cm) from 20-25 days old seedlings were taken and cut into small pieces and placed in the well of spot test plate (M/s. Thomas Scientific, USA). About 400 µl of extraction buffer (50 Mm Tris HCl, pH 8.0; 25 mM EDTA; 300 mM NaCl and 1% SDS) was added and the leaf sample was ground with the help of alcohol sterilized blunt end glass rod till it was completely homogenized. Another 400 µl of extraction buffer was added to the well containing the homogenized leaf sample. Using a micropipette of 1 ml capacity (the tip was cut at the bottom using a sterile scissor), the entire contents from the well were transferred to a fresh 1.5 ml capacity micro centrifuge tube.

About 400-500 µl Chloroform: Isoamyl alcohol (24:1) mix was then added to the micro centrifuge tube and the contents were mixed well for about 10 minutes by inversion and centrifuged at 13,000 rpm for about 15 minutes. After centrifugation, the supernatant was aspirated from the micro centrifuge tube, without disturbing the intermediate layer, into a fresh 1.5 ml micro- centrifuge tube. To the clear supernatant, 5-10 µl of RN*ase* (10 mg/ml) was added and incubated for about 45-60 minutes at room temperature. After incubation, about 500 µl of chloroform: Isoamyl alcohol (24:1) was added to the micro-centrifuge tube and mixed well by inversion for 10 minutes. The contents were centrifuged at 13000 rpm for 10 minutes and the supernatant was transferred to another fresh 1.5 ml micro centrifuge tube.

To this clear supernatant, an equal volume (~ 500-600 µl) of chilled isopropanol was added and kept at 4° C for 10 minutes. The sample was then mixed gently and centrifuged at 13,000 rpm for 10 minutes. The supernatant was drained gently without disturbing the DNA pellet. About 300 µl of 70 per cent ethanol was added to the pellet and the centrifuge tube was tapped in order to dispense the pellet in ethanol. The contents were centrifuged at 13,000 rpm for 5 minutes. The supernatant was drained and 70 per cent ethanol wash was repeated again. Finally, after centrifugation and draining out the supernatant, the pellet was left for overnight air drying at room temperature. After complete drying of the pellet, depending on the size of the pellet, about 50-100 µl of 1X TE buffer was added for dissolving the pellet. About 3 µl of DNA sample was loaded in 0.8 per cent ethidium bromide stained agarose-TBE gel, electrophoresed for about an hour and the bands of genomic DNA were visualized and documented in a UV gel documentation system (Gene Flash; SYNGENE, U.K).

3.2.1.3 Procedure for quality and quantity estimation of DNA

The purity and concentration of the isolated genomic DNA samples were estimated by loading in 0.8 percent agarose gel and as well with Nanodrop (Thermo Scientific, Nanodrop Technologies, U.S.A).

Quantification of DNA was done by analyzing the purified DNA on 0.8 percent agarose gel with diluted 100 bp DNA ladder (New England Biolabs, UK) as standard. Based on the intensity and thickness of genomic DNA bands compared to λ DNA, the concentration and quality of DNA in individual samples was determined.

The loading gel was prepared by weighing 0.8 g of agarose in 100 ml of 1X TAE buffer. The agarose was melted in a microwave oven until a clear, transparent solution was obtained. The appropriate gel tray was fixed into the gel cast and the combs were placed on the gel tray. The melted agarose was allowed to cool to room temperature for 5 minutes and then 2 µl of ethidium bromide (10 mg ml^{-1}) was added, mixed well by swirling and poured into the gel tray with prefixed combs, carefully avoiding the formation of air bubbles. The gel was allowed to solidify at room temperature for 1 hr. Then the solidified gel was transferred to electrophoresis unit containing the running buffer, 1X TAE buffer. The DNA samples were mixed with $1/6^{th}$ volume of gel loading dye (40% Sucrose: 0.25% Bromo phenol blue) and loaded onto the gel. The electrophoresis was carried out at 100 volts at room temperature for

about 1hr. After that, the gel was visualized in a UV light transmitted gel documentation system (SYNGENE Gene flash, U.K.). After checking the concentration of the DNA, based on the intensity, the DNA samples were diluted to 50 ng μl^{-1} for further PCR (Polymerase Chain Reaction) analysis.

Quantification using Nano drop

1. Opened the sampling arm and wiped the upper and lower pedestals using double distilled water and loaded 1 μl of double distilled water to calibrate the instrument zero.
2. Pipette 1 μl of DNA sample onto the lower measurement pedestal.
3. Closed the sampling arm and initiated a spectral measurement using the operating software in the personnel computer (PC).
4. When the measurement is completed, opened the sampling arm and wiped the sample from both the upper and lower pedestals using a soft wipe.

3.2.1.4 Genomic DNA amplification in PCR using SSR-markers

The PCR was carried out using a programmable thermalcycler (Eppendorf, Germany). The PCR plates were taken and 2 μl of 50 ng template DNA was pipetted into each of the wells in the PCR plate wells after proper labelling and kept the PCR plate at 4° C. Then, the master mix was prepared by taking each 0.5 μl of 10 pmol primer (both forward and reverse primer), 0.75 μl of 2.5 mM deoxyribo nucleotides (dNTPs), 1 μl of Genei 10X assay buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$,) and 0.2μl of 3 U/μl *Taq* DNA polymerase (Bangalore Genei Private Limited, Bangalore) and 5.05 μl of sterile distilled water was added to make up the volume to 8 μl. The master mix was centrifuged for short duration of about 10 sec for thorough mixing of the components. Subsequently, 8 μl of master mix was added to each of wells in the PCR plate well having 2 μl of template DNA to make the final volume to 10 μl. PCR plate was covered and kept in a thermal cycler for the reaction to take place as given below:

STEP-I	Initial denaturation	94° C for 5 minutes	
	Denaturation	94° C for 30 seconds	35 cycles
STEP-II	Primer annealing	55° C for 30 seconds	
	Elongation	72° C for 1 minute	
STEP-III	Final elongation	72° C for 7 minutes	

After completion, the PCR plate was stored at 4° C and the amplified products were later resolved on 3% agarose gel.

3.2.1.5 Agarose gel electrophoresis for resolution of SSR markers

The PCR products were electrophoresed in 3% agarose gel in a gel electrophoresis unit (CBS Scientific, USA). About 9.0 g of agarose was weighed and transferred to a reagent bottle containing 300 ml of 1X TAE buffer and mixed well. The contents were then boiled gently in a microwave oven with intermittent mixing. The process was followed until complete melting of agarose was achieved and the solution became crystal clear. The gel-casting tray was washed with water and wiped with 70% ethanol. The boiled agarose was cooled to room temperature, 2 µl of ethidium bromide (10 mg ml^{-1}) was added to the melted agarose, mixed thoroughly and poured into the gel cast tray prefixed with the appropriate gel combs and was allowed to solidify at room temperature for 20-30 minutes. Later, the gel was transferred to electrophoresis unit containing 1 X TAE buffer. Before loading, PCR amplified products were mixed with $1/6^{th}$ volume of gel loading dye (40% sucrose and 0.25% bromophenol blue) and loaded into the wells. 100 bp ladder was added in one well to determine the size of amplified fragments. The DNA fragments were then visualized under UV-transilluminator and documented using gel documentation system (SYNGENE Gene flash, U.K.)

3.2.1.6 Genotyping using SSR markers

Qualitative multistate traits that depict an array of characters were converted into binary characters (Sneath and Sokal, 1973) based on the variations present. Only the clear and unambiguous bands of SSR markers were scored. Markers were scored for the presence or absence of the corresponding

band among the genotypes. The score 1 and 0 indicates the presence and absence of the bands respectively. A data matrix comprising of '1' and '0' were formed depending upon the character and this data matrix was subjected to further analysis.

Marker Polymorphism

To measure the informativeness of the markers, the polymorphism information content (PIC) (http://www.agri.huji.ac.il/-Weller/Hayim/parent/PIC) for each SSR marker was calculated according to the formula given by Anderson *et al.* (1993)

$$PICi=1-\sum_{f=1}^{n} P{ij}^2$$

Where P*ij* is the frequency of the jth allele for the ith marker, and is summed over n alleles. The calculation was based on the number of alleles per locus. A high degree of polymorphism of microsatellite markers allows rapid and efficient identification of rice breeding lines. In the present study, a total of 14 markers were used, which covered most of the genome of rice (chromosomes 1, 2, 4, 6, 8) and the microsatellite markers used in the present study are presented in Table 3.2.

3.2.1.7 Cluster analysis

Cluster analysis and dendrogram construction was done by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) clustering algorithm using Darwin 6.0.14 version (Perrier and Jacquemond, 2006).

3.2.2 Screening for Salinity Tolerance at Seedling Stage

Phenotyping of ANGRAU varieties and advanced breeding lines for salinity tolerance at seedling stage using hydroponic study was performed as per protocol of Gregorio *et al.* (1997) with certain modifications. Sterilized seeds were placed in petri dishes with moistened filter papers and incubated at 30°C

Table 3.2 Details of Salinity markers used for diversity study

S.No	Marker	Forward Sequence	Reverse Sequence	Chromosome number
1	RM10793	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC	1
2	RM10694	TTTCCCTGGTTTCAAGCTTACG	AGTACGGTACCTTGATGGTAGAAAGG	1
3	RM562	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC	1
4	RM493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	1
5	RM1287	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG	1
6	RM10711	GCTTCGATCGATGAGAAAGTAGAGG	GAATCTCCCATCCTTCCCTTCC	1
7	RM174	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG	2
8	RM423	AGCACCCATGCCTTATGTTG	CCTTTTTCAGTAGCCCTCCC	2
9	RM518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	4
10	RM551	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG	4
11	RM253	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC	6
12	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC	6
13	RM20224	AGTATGAAAGTCGGTGACGATGG	GAGATGTCACGTCTTCACTTAGGG	6
14	RM210	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	8

for 48 hr for germination. One pre-germinated seeds per hole were placed on the styrofoam seedling float**.** The radicle was inserted through the nylon mesh. The Styrofoam seedling float was placed on the tray filled with distilled water (plate 3.1). There are adequate nutrients in the endosperm for the seedlings to grow normally for three to four days. After three days, when seedlings were well established, distilled water was replaced with nutrient solution. The components of nutrient solution are presented below:

Preparation of stock solution

Element	Reagent (AR grade)	Preparation (g/250ml solution)
MACRO NUTRIENT		
N	Ammonium nitrate (NH_4NO_3)	22.85 g
P	Sodium phosphate, monobasic monohydrate ($NaH_2PO_4.H_2O$)	8.9 g
K	Potassium sulphate (K_2SO_4)	17.85 g
Ca	Calcium chloride, dehydrate ($CaCl_2.2H_2O$)	29.33 g
Mg	Magnesium sulphate, 7-hydrate($MgSO_4.7H_2O$)	81 g
MICRO NUTRIENT		
Mn	Manganous chloride, 4-hydrate ($MnCl_3.4H_20$)	0.375 g
Mo	Ammonium molybdate, 4-hydrate [$(NH_4)_6Mo7O_2.4H_20$]	0.0185 g
Zn	Zinc sulphate,7-hydrate ($ZnSO_4.7H_2O$)	0.00875 g
B	Boric acid (H_3BO_3)	0.233 g
Cu	Cupric sulphate, 5-hydrate ($CuS0_4.5H_2O$)	0.00770 g
Fe	Ferric chloride,6-hydrate ($FeCl_3.6H_2O$)	1.975 g
	Citric acid, monohydrate ($C_6H_8O_7.H_2O$)	

Initial salinity stress was imposed with EC=6 dSm^{-1} by adding NaCl to the nutrient solution. The pH was monitored daily and was maintained at 5.0. After eight days of initial salinization, the EC was increased to12 dSm^{-1}. The solution was renewed after eight days. Initial scoring of the selected individual plants was recorded at 10 days after initial salinization, while the final score was recorded 16 days after initial salinization. After recording the final score the plants were transferred to normal distilled water and kept for three days followed by transferring to the pots. The initial and final scores were recorded as per SES of IRRI (1997) and description of the SES scale is presented below:

Plate 3.1 Screening of genotypes at Seedling Stage

Standard evaluation score (SES) of visual salt injury at seedling stage

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plants drying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

3.2.3 Screening for Reproductive Stage Salinity Tolerance

Screening of the genotypes for salinity tolerance at reproductive stage was performed as per protocol of Diana *et al.* (2013) with certain modifications. The experimental setup was kept in the poly house at RARS, Maruteru.

The experimental design adopted was completely randomized design. A plastic tray filled with ordinary tap water that could house the replicates and serve as a water bath to maintain the same water level was used for the study. Six pots were placed in each tray. Perforated pots of 15 cm height, 11 cm diameter, and 3-4 mm diameter holes spaced 2 cm apart at the bottom were used as planting pots. Each perforated pot consisted of a cloth bag filled with fertilized soil. The level of soil was about 1 cm above the top most circle of holes. The soil was fertilized with 50, 25, and 25 mg N, P, and K, respectively, per kilogram of soil used. The water level in the plastic tray was kept level with the soil in the pots (plate 3.2).

Three pre germinated seeds were placed on the soil surface of each pot. Two weeks after seeding, the rice seedlings were thinned to one per pot. Hence, each replicate involved a single plant grown until maturity. The water level in each of the plastic trays was raised to about 1 cm above the potted soil after thinning and this water level was maintained daily. Proper crop protection measures were employed as and when needed.

All plants were grown in regular water until the appearance of the flag leaf. All leaves of the plants were pruned immediately after shifting to saline water, leaving only flag leaf and the penultimate leaf to direct the salt stress to the reproductive parts of the rice plant quickly and effectively rather than to compartmentalize the salt in older leaf tissues. Salinization was done by dissolving NaCl into the water to raise the electrical conductivity up to 10 dSm^{-1}.

Recording of Observations

Data on plant height (cm), number of tillers $plant^{-1}$, panicle length (cm), number of filled grains $panicle^{-1}$, grain yield $plant^{-1}$ (g) and test weight (g) were taken for each plant. The details of the observations recorded and methods followed are presented here under character wise.

YIELD AND YIELD COMPONENTS

3.2.3.1 Plant Height (cm)

The plant height was measured in centimetres from ground level in the pot to the tip of the panicle of the mother tiller excluding awns at the time of harvest.

3.2.3.2 Number of Ear Bearing Tillers $Plant^{-1}$

Total number of ear bearing tillers in each plant in each pot was counted at maturity.

3.2.3.3 Panicle Length (cm)

The length from base of the panicle to the tip was measured in centimetres.

3.2.3.4 Number of Filled Grains $Panicle^{-1}$

The number of filled grains per panicle were counted and recorded.

Plate 3.2 Reproductive Screening of Genotypes

3.2.3.5 100 Seed Weight (g)

Hundred well filled grains were counted at random and the weight was recorded in grams.

3.2.3.6 Grain Yield Plant^{-1} (g)

The matured panicles from each plant in each pot were harvested, threshed, cleaned and seed dried to 12% moisture level. The grain yield per plant was recorded in grams.

3.3 STATISTICAL ANALYSIS

Analysis of variance for completely randomized design

For analysis of variance on completely randomized design was computed separately as per standard statistical procedure (Panse and Sukhatme, 1985).

$$Y_{ij} = \mu + g_i + e_{ij}$$

Where,

Y_{ij} = Phenotypic observation of ith genotype and j^{th} replication

μ = General mean

g_i = Effect of ith genotype

e_{ij} = Random error associated with i^{th} genotype and j^{th} replication

ANOVA for completely randomized design

Source of variation	d.f	SS	MSS	Expected MSS	'F' calculated
Treatments (Genotypes)	(t-1)	TrSS	M_t	$\sigma^2 e + r\, \sigma^2 g$	M_t / M_e
Error	(r-1)(t-1)	ESS	M_e	$\sigma^2 e$	
Total	(tr-1)	TSS	TMSS		

The analysis of variance for each character was carried out as indicated below

Where,

g = Number of genotypes

M_t = Mean sum of square of treatment

M_e = Mean sum of square of error

$\sigma^2 e$ = Environmental variance

$\sigma^2 g$ = Variance due to genotypes

Test of significance for these was carried out against the corresponding error degrees of freedom using 'F' table values given by Fisher and Yates (1963).

Correlations

Phenotypic and genotypic correlations were worked out by using the formulae suggested by Falconer (1964).

Phenotypic coefficient of correlation (r_p)

$$r(x_i.x_j)p = \frac{Cov(x_i.x_j)p}{\sqrt{V(x_i)p.V(x_j)p}}$$

Where,

$r(x_i.x_j)p$ = Phenotypic correlation between i^{th} and j^{th} character

$Cov(x_i.x_j)p$ = Phenotypic covariance between i^{th} and j^{th} character

$V(x_i)p$ = Phenotypic variance of ith character

$V(x_j)p$ = Phenotypic variance of jth character

Genotypic coefficient of correlation (r_g)

$$r(x_i.x_j)g = \frac{Cov(x_i.x_j)g}{\sqrt{V(x_i)g.V(x_j)g}}$$

Where,

r $(x_i.x_j)g$	=	Genotypic correlation between ith and jth character
$Cov\ (x_i.x_j)g$	=	Genotypic covariance between ith and jthcharacter
$V\ (x_i)g$	=	Genotypic variance of ith character
$V\ (x_j)g$	=	Genotypic variance of jth character

Test of significance

Significance of correlation coefficients was tested by comparing phenotypic correlation coefficients with the table values (Fisher and Yates, 1963) at (n-2) degrees of freedom at 5% and 1% level where 'n' denotes the number of paired observations used in the calculation.

Chapter – IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

The present investigation was carried out to evaluate and characterize 80 rice genotypes for salinity tolerance and also to study character association for yield, yield parameters and salinity score of the genotype at seedling stage under saline conditions. The results are presented as follows:

4.1 Analysis of variance

4.2 Screening for reproductive and seedling salinity tolerance

4.3 Correlation analysis

4.4 Molecular diversity

4.1 ANALYSIS OF VARIANCE

The analysis of variance for plant height, ear bearing tillers per plant, panicle length, filled grains, grain yield, test weight, initial (10 days) and final (16 days) seedling salinity score is furnished in Table 4.1. The results revealed significant differences among the genotypes for all characters studied.

Table 4.1. Analysis of variance for yield, yield components and salinity score at seedling stage in rice (*Oryza sativa* L.) under saline conditions

Source of variance	Degrees of freedom	Plant height (cm)	Panicle length (cm)	Ear bearing tillers per plant	Filled grains per panicle	Grain yield per plant (g)	100 seed weight (g)	Initial Score (10 days)	Final score (16 days)
Treatments	79	215.88**	13.86**	1.26**	459.04**	0.37**	0.44**	0.04**	0.03**
Error	80	12.08	3.38	0.09	3.65	0.004	0.04	0.01	0.01

4.2 SCREENING FOR REPRODUCTIVE AND SEEDLING TOLERANCE FOR SALINITY

The mean performance of 80 genotypes evaluated for yield and yield components in pot culture under saline conditions till maturity, representing reproductive salinity tolerance and tolerance at seedling stage are presented in Table 4.2.

Table 4.2. Screening of 80 rice (*Oryza sativa* L.) genotypes for reproductive and seedling salinity tolerance

		Reproductive stage						Seedling stage	
S. No.	Genotype	Plant height (cm)	Panicle length (cm)	Ear bearing tillers per plant	Filled grains per panicle	Grain yield per plant (g)	100 Seed weight (g)	Initial score (10 days)	Final score (16 days)
1	AST -56	80.50	20.50	3.50	42.00	0.94	2.29	5	7
2	AST – 57	60.00	22.50	4.50	63.50	1.45	2.38	5	5
3	AST – 60	81.50	20.50	4.00	34.00	0.87	2.71	5	3
4	AST – 62	78.20	22.00	3.00	32.55	0.86	2.80	3	5
5	AST – 64	72.00	20.00	5.00	52.00	1.52	2.80	3	5
6	AST – 66	72.50	20.25	6.00	43.00	1.17	2.59	5	5
7	BPT – 2231	52.50	13.75	3.50	34.00	0.66	2.05	7	9
8	BPT – 2270	80.00	21.00	3.00	18.50	0.30	1.64	7	9
9	BPT – 3291	68.00	17.00	4.00	42.50	0.80	1.99	7	7
10	BPT – 5204	64.30	17.00	3.00	41.00	0.67	1.64	7	7
11	CSR – 27	66.20	18.45	5.50	85.50	2.34	2.71	5	7
12	CST – 1	75.20	17.00	3.50	35.00	1.02	2.90	5	7
13	CST – 2	80.00	19.50	2.00	40.00	1.02	2.60	3	5
14	CST – 3	75.00	19.00	4.00	42.50	1.16	2.56	5	5
15	CST – 4	63.00	17.00	4.00	46.50	1.11	2.48	5	5

S. No.	Genotype	Plant height (cm)	Panicle length (cm)	Ear bearing tillers per plant	Filled grains per panicle	Grain yield per plant (g)	100 Seed weight (g)	Initial score (10 days)	Final score (16 days)
16	CST – 5	65.50	15.00	4.00	52.50	1.62	3.27	5	5
17	CST – 6	71.50	18.50	4.50	49.00	1.37	2.96	5	5
18	CST – 7	68.00	18.00	4.00	58.50	1.48	2.61	5	3
19	CST – 8	63.50	18.00	4.00	42.50	1.07	2.57	5	3
20	CST – 9	52.40	12.10	4.00	36.00	1.11	3.20	3	3
21	CST – 10	61.15	21.00	4.00	48.50	1.33	2.80	5	5
22	E – 456	76.50	19.50	4.00	63.00	1.69	2.72	7	9
23	E – 460	75.50	19.00	4.00	53.50	1.13	2.16	9	9
24	E – 467	58.00	17.75	4.00	59.00	1.21	2.11	7	9
25	FL – 478	72.50	20.50	4.00	50.50	1.73	3.30	3	5
26	IR - 64	66.50	21.50	4.00	58.00	1.71	2.91	5	3
27	IR – 76393 – 2B – 7 – 1 – 1 – 3 – 1	70.00	20.50	4.00	67.50	1.51	2.31	5	3
28	MCM – 27	72.00	18.50	2.00	61.50	1.54	2.42	3	5
29	MCM – 41	69.50	22.00	4.00	67.00	1.46	2.12	3	3
30	MCM – 48	61.25	16.90	5.00	56.00	1.19	2.20	5	5
31	MCM – 100	56.50	16.50	5.00	53.00	1.09	2.11	5	3
32	MCM – 223(S)	92.50	23.00	4.50	57.00	1.28	2.26	5	5

S. No.	Genotype	Plant height (cm)	Panicle length (cm)	Ear bearing tillers per plant	Filled grains per panicle	Grain yield per plant (g)	100 Seed weight (g)	Initial score (10 days)	Final score (16 days)
33	MCM – 225	97.50	30.68	5.00	57.00	1.44	2.54	3	5
34	MLT -1	65.75	15.25	4.00	59.00	1.28	2.17	7	7
35	MLT -2	87.00	19.50	4.50	51.00	1.24	2.43	7	9
36	MLT -4	84.00	21.00	4.00	58.00	1.15	1.98	9	9
37	MLT -5	80.50	25.28	6.00	63.00	1.20	1.99	7	9
38	MLT -6	66.70	19.00	4.00	56.50	1.08	2.01	7	7
39	MLT -7	72.00	19.75	4.50	56.50	1.09	2.06	9	7
40	MLT -8	61.90	21.00	3.00	57.50	0.73	1.30	7	7
41	MLT -10	86.85	21.25	4.00	64.00	1.23	1.92	7	5
42	MLT -11	66.85	19.00	4.00	56.50	1.01	1.82	9	7
43	MTU – 1001	82.20	21.50	4.00	64.50	1.76	2.76	5	7
44	MTU – 1006	72.00	22.20	4.50	62.00	1.77	2.88	9	9
45	MTU – 1010	75.45	21.00	6.00	64.50	1.81	2.82	9	9
46	MTU – 1031	61.40	18.15	4.50	67.00	1.49	2.13	7	9
47	MTU – 1032	71.50	19.50	6.00	110.50	2.39	2.11	7	9
48	MTU – 1061	78.00	22.50	4.00	61.00	1.24	2.12	5	5
49	MTU – 1064	55.50	18.75	5.00	84.00	1.93	2.31	7	7
50	MTU – 1071	73.00	20.25	4.00	64.00	1.25	2.04	6	5

S. No.	Genotype	Plant height (cm)	Panicle length (cm)	Ear bearing tillers per plant	Filled grains per panicle	Grain yield per plant (g)	100 Seed weight (g)	Initial score (10 days)	Final score (16 days)
51	MTU – 1075	71.15	19.40	4.00	54.00	1.12	2.09	5	5
52	MTU – 1078	72.50	21.15	4.00	85.00	1.44	1.70	7	5
53	MTU – 1112	85.00	21.40	3.50	59.00	0.95	1.64	7	7
54	MTU – 1121	60.00	20.00	4.00	64.00	1.40	2.19	9	7
55	MTU – 1140	76.90	20.95	4.00	43.50	1.11	2.56	9	7
56	MTU – 1153	72.00	20.50	4.00	54.00	1.22	2.20	7	5
57	MTU – 1156	76.00	19.50	4.00	46.00	1.08	2.45	9	7
58	MTU – 1166	81.00	20.75	4.00	84.00	1.10	1.32	9	7
59	MTU – 1184	74.00	19.75	4.00	61.50	1.04	1.80	9	7
60	MTU – 1187	67.00	18.00	4.00	70.00	1.35	1.92	9	7
61	MTU – 1194	70.50	18.50	4.00	61.50	1.15	1.90	7	7
62	MTU - 1210	64.40	18.40	3.00	55.50	0.89	1.62	9	9
63	MTU – 1224	59.00	17.75	4.00	63.50	1.16	1.81	7	7
64	MTU – 1226	75.50	22.25	4.50	74.50	1.99	2.70	7	7
65	MTU – 1229	67.00	18.50	4.00	64.50	1.06	1.70	5	7
66	MTU – 2067	60.50	19.00	4.00	63.00	1.40	2.20	7	7
67	MTU – 2077	63.40	20.75	4.00	74.50	1.47	2.09	7	9
68	MTU – 2716	59.75	18.55	4.00	49.50	1.08	2.28	7	9

S. No.	Genotype	Plant height (cm)	Panicle length (cm)	Ear bearing tillers per plant	Filled grains per panicle	Grain yield per plant (g)	100 Seed weight (g)	Initial score (10 days)	Final score (16 days)
69	MTU – 3626	73.50	19.75	5.00	61.00	1.90	3.19	7	7
70	MTU – 4870	66.00	14.80	4.50	63.00	1.46	2.39	9	9
71	MTU – 5182	92.50	26.02	5.00	72.00	1.92	2.53	7	9
72	MTU – 5249	72.20	21.20	4.00	65.50	1.43	2.17	7	7
73	MTU – 5293	71.50	21.00	4.50	81.00	1.60	2.09	9	7
74	MTU – 7029	64.50	18.25	4.00	60.00	1.20	1.91	7	5
75	NONABOKRA	75.00	17.25	6.00	95.00	2.77	3.08	3	3
76	PLA - 1100	78.00	19.35	4.50	59.00	1.45	2.70	7	5
77	POKKALI	109.75	22.85	6.00	96.50	2.69	2.91	3	3
78	STBN – 12 – 1	72.50	19.75	3.00	64.50	0.95	1.50	5	5
79	STBN – 12 – 7	63.00	21.75	3.00	66.50	0.98	1.47	3	5
80	STBN - 12 – 10	46.85	16.40	4.00	75.00	1.16	1.51	3	3
	MEAN	71.26	19.66	4.16	59.11	1.32	2.29	6	6.25
	C.D (5%)	6.91	3.65	0.62	3.80	0.12	0.13	0.22	0.20
	C.V (%)	4.87	9.35	7.54	3.23	4.84	2.93	6.46	6.67

The variety, Pokkali recorded maximum plant height (109.75 cm), while STBN-12-10 recorded minimum plant height (46.85 cm). Wide range of variation was observed for plant height among the genotypes and the mean value was 71.26 cm. Further, ear bearing tillers ranged from 2.00 (CST-2 and MCM-27) to 6.00 (AST-66, MLT-5, MTU 1010, MTU 1032, Nonabokra and Pokkali) with a mean of 4.16.

Panicle length ranged from 12.10cm (CST-9) to 30.68cm (MCM-225) with a mean of 19.66cm, while filled grains per panicle ranged from 32.55 (AST-62) to 110.50 (MTU 1032) with a mean of 59.11. 100-seed weight ranged from 1.30g (MLT-8) to 3.27 (CST-5) with a mean of 2.29.

Grain yield per plant ranged from 0.66g (BPT 2231) to 2.77g (Nonabokra) with a mean of 1.32g. Further, Pokkali with a grain yield of 2.69g was observed to be on par with Nonabokra. These genotypes had also recorded maximum ear bearing tillers per plant (6.0) in addition to relatively higher filled grains per panicle (>95) and test weight (>2.90g). Further, 24 genotypes, namely, AST-57, AST-64, CSR-27, CST-5, CST-7, E-456, FL-478, IR-64, IR-76393-2B-7-1-1-3-1, MCM-27, MCM-41, MTU 1001, MTU 1006, MTU 1010, MTU 1031, MTU 1032, MTU 1064, MTU 1226, MTU 2077, MTU 3626, MTU 4870, MTU 5182, MTU 5293 and PLA 1100 had recorded significantly higher grain yield per plant, compared to the mean value indicating reproductive salinity tolerance for these genotypes.

A perusal of the results on initial seedling salinity score recorded at 10 days after initial salinization revealed a range from 3.0 to 9.0. Classification of the genotypes based on the score as per SES (IRRI, 1997) is presented in Table 4.3. None of the genotypes studied recorded high tolerance (1.0) for salinity at seedling stage 10 days after initial salinization. However, 18 genotypes had recorded tolerance (3.0), while 24 genotypes were observed to be moderately tolerant (5.0). Among these, the released ANGRAU rice varieties, namely, MCM-100 had recorded tolerance, while the varieties, MTU 1075, MTU 1061, MTU 7029, PLA 1100, MTU 1153, MTU 4870 and MTU 1001 had recorded moderate tolerance. In contrast, 25 genotypes were observed to be susceptible (7.0), while 13 genotypes were noticed to be highly susceptible (9.0).

Table 4.3 Performance of 80 genotypes at initial salinity screening (10 Days) at seedling stage

S.No.	Score	Resistant reaction	No.of genotypes	Details of genotypes
1.	1	Highly tolerant	0	--
2.	3	Tolerant	18	AST-60, AST-62, AST 64, CST- 2, CST-7, CST-8, CST-9, FL-478, MCM-27, MCM-41, MCM-100, MCM-225, IR76393-23-7-1-1-3-1, IR-64, NONABOKRA, POKKALI, STBN-12-7, STBN-12-10
3.	5	Moderately tolerant	23	STBN-12-1, MLT-10, MTU -1229, MTU-1075, MTU – 1071, MTU-1061, MTU -1001, MTU 1078, MTU 1153, MTU 7029, PLA 1100, MCM-223(S), MCM-48, CST-10, CST-6, CST-5, CST-4, CST-3, CST-1, CSR-27, AST-66, AST-57, AST-56
4.	7	Susceptible	25	BPT-2231, BPT-2270, BPT-3291, BPT-5204, E-456, E-467, MLT-1, MLT-2, MLT-5, MLT-6, MLT-8, MTU-1010, MTU-1031, MTU-1032, MTU-1064, MTU-1112, MTU-1194, MTU-1224, MTU-1226, MTU-2067, MTU-2077, MTU-2716, MTU-3626, MTU-5182, MTU-5249
5.	9	Highly susceptible	14	MTU -5293, MTU -4870, MTU -1210, MTU -1187, MTU -1184, MTU -1166, MTU -1156, MTU-1140, MTU-1121, MTU-1006, MLT-7, MLT-4, E-460, MLT-11

*Scoring of genotypes was done as per standard evaluation score of visual salt injury at seedling stage IRRI (1997)

The results on final seedling salinity score recorded at 16 days after initial salinization also revealed a range from 3.0 to 9.0. Further, their classification based on the score as per SES (IRRI, 1997) is presented in Table 4.4. None of the genotypes studied had exhibited high tolerance (1.0) for salinity at seedling stage 16 days after initial salinization. However, 11 genotypes had recorded tolerance (3.0), while 27 genotypes were observed to be moderately tolerant (5.0). Among these, the released ANGRAU rice varieties, namely, MCM-100 had recorded tolerance, while PLA, 1100, MTU 7029, MTU 1075, MTU 1061, MTU 1153 and MTU 4870 had recorded moderate tolerance. In contrast, 27 genotypes were observed to be susceptible (7.0), while 15 genotypes were noticed to be highly susceptible (9.0).

A perusal of the above results revealed seedling salinity tolerance both at initial seedling salinity score and also final seedling salinity score for Nonabokra, Pokkali, MCM-41, MCM-100 and STBN 12-10. Among these, Nonabokra had recorded highest grain yield per plant (2.77g) followed by Pokkali (2.69g). These genotypes had also recorded high number of ear bearing tillers per plant, filled grains per panicle and 100-seed weight, compared to other genotypes studied in the present investigation. Hence, these genotypes are identified as promising salinity tolerant lines with both seedling and reproductive salinity tolerance. Sexcion *et al*. (2009) also classified Pokkali as salt tolerant variety due to consistent expression of high vigour, low standard evaluation score, high shoot/root biomass, lower shoot Na+ accumulation and lower shoot Na+/K+ ratio compared to sensitive genotypes. Dr. Kwon (unpublished data) also suggested that Pokkali had a higher water flux and a higher ability of water uptake and transpiration resulting in salinity tolerance.

The genotypes, MTU 1075, PLA 1100, MTU 7029, MTU 1078, MTU 1061, MTU 1153, MTU 4870, STBN 12-1, MTU 1071, MCM-48, MCM-223(S), CST-10, CST-6, CST-5, CST-4, CST-3, CSR-27, AST-66, AST-57 and MLT-10 had recorded moderate seedling salinity tolerance both for initial and final seedling salinity score. Among these, PLA-1100, MTU-4870, CST-5, CSR-27 and AST-57 had recorded relatively higher grain yield, compared to the experimental mean yield and hence, were inferred to possess moderate seedling tolerance coupled with reproductive salinity tolerance.

Table 4.4 Performance of 80 genotypes at final salinity screening (16 days) at seedling stage

S.No	Score	Resistant Reaction	No. of Genotypes	Details of Genotypes
1.	1	Highly tolerant	0	--
2.	3	Tolerant	11	AST-60, CST-9, CST -8, CST-7, MCM-41, MCM-100, NONABOKRA, POKKALI, STBN-12-10, IR76393-23-7-1-1-3-1, IR -64.
3.	5	Moderately tolerant	26	STBN-12-1, STBN-12-7, PLA-1100, MTU -7029, MTU-1075, MTU – 1071, MTU- 1061, MTU -1078, MTU – 1153, MCM-223(S), MCM-225, MCM-48, MCM-27, FL-478, CST-10, CST-6, CST-5, CST-4, CST-3, CST-2, CSR-27, AST-66, AST-64, AST-57, AST-62, MLT-10
4.	7	Susceptible	27	AST -56, BPT-3291, BPT-5204, MLT-1, MLT-11, MLT-7, MLT-6, MLT-8, MTU-1031, MTU-1001, MTU-1010, MTU-1064, MTU-1121, MTU-1112, MTU-1156, MTU-1194, MTU-1224, MTU-1226, MTU-2067, MTU-1166, MTU-3626, MTU-5293, MTU-5249,MTU-1184, MTU -1187, MTU -1140, MTU -1229
5.	9	Highly susceptible	16	MTU -5182, MTU -4870, MTU -1210, MTU -2716, MTU -2077, MTU -1032, MTU -1031, MTU-1006, MLT-5, MLT-4, MLT -2, E-460, E – 467, E -456, BPT -2270, BPT -2231

*Scoring of genotypes was done as per standard evaluation score of visual salt injury at seedling stage IRRI (1997)

4.3 CORRELATION ANALYSIS

The results on phenotypic and genotypic character associations between grain yield, yield components and seedling salinity scores of the 80 genotypes under saline conditions are presented in Table 4.5. The results revealed lower magnitude of phenotypic correlation, compared to genotypic correlation for all the characters under study, indicating the masking effect of environment. However, both genotypic and phenotypic correlations were observed to be of similar direction.

4.3.1 Plant Height

Plant height recorded positive and significant association with panicle length (0.744**), ear bearing tillers (0.199**) and test weight (0.199**) at genotypic level. However, it recorded non-significant association with number of filled grains (0.082) and also with grain yield (0.113). These results are in conformity with the findings of Vijayadurga (2015).

4.3.2 Panicle Length

It recorded positive and significant correlation with number of filled grains (0.214**), ear bearing tillers (0.206*) and grain yield (0.214*). However, panicle length exhibited non-significant association with salinity final score (0.050). Similar findings were reported by Mansuri *et al.,* (2012), Khan *et al.,* (2014) and Vijayadurga (2015).

4.3.3 Number of Filled Grains

The results recorded positive and significant association with ear bearing tillers (0.500**) and grain yield (0.684**). These results are in agreement with Mansuri *et al.* (2012) and Vijayadurga (2015).

Table 4.5 Estimates of genotypic and phenotypic correlation coefficients among yield parameters and salinity score at seedling stage

Character		Plant height	Panicle length	Number of filled grains	Ear bearing tillers	100 Seed weight	Salinity Initial score (10 days)	Salinity Final score (16 days)	Grain yield
Plant height	G	1.000	0.744**	0.082	0.199*	0.199*	-0.079	0.032	0.113
	P	1.000	0.606**	0.082	0.174*	0.181*	-0.072	0.030	0.104
Panicle length	G		1.000	0.214**	0.206*	-0.002	-0.079	0.050	0.214*
	P		1.000	0.168*	0.168*	-0.003	-0.058	0.039	0.181*
Number of filled grains	G			1.000	0.500**	-0.138	0.048	0.005	0.684**
	P			1.000	0.465**	-0.135	0.048	0.005	0.684**
Ear bearing tillers	G				1.000	0.347**	0.049	-0.020	0.591**
	P				1.000	0.309**	0.044	-0.018	0.554**
Test weight	G					1.000	-0.333**	-0.272**	0.521**
	P					1.000	-0.328**	-0.270**	0.521**
Salinity Initial score (10 days)	G						1.000	0.705**	-0.055
	P						1.000	0.703**	-0.052
Salinity Final score (16 days)	G							1.000	-0.024
	P							1.000	-0.024
Grain yield	G								1.000
	P								1.000

*indicates significant at 5 % level ; ** indicates significant at 1 % level ; P = phenotypic correlation, G= genotypic correlation

4.3.4 Ear Bearing Tillers

In the present study, the trait revealed positive and significant correlation with test weight (0.347**) and grain yield (0.591**). These results are in conformity with Khan *et al.* (2014).

4.3.5 100 Seed Weight

Test weight showed positive and significant correlation coefficients with grain yield (0.521**) and negative and significant correlation with salinity initial score (-0.333**) and salinity final score (-0.272**). These results are in agreement with Vijayadurga (2015).

4.3.6 Salinity Initial Score (10 Days)

This trait exhibited positive and significant association with salinity final score (0.705**) indicating that genotypes which exhibits tolerance at initial stress (10 days after stress) would also exhibit tolerance at final stress (16 days after stress) at seedling stage. However, non-significant association was recorded with grain yield (-0.055). The findings are in conformity with the reports of Mahmood *et al.* (2009).

4.3.7 Salinity Final Score (16 Days)

The results revealed non-significant association of the trait with grain yield (-0.024). However, it had significant and positive association with salinity initial score. Similar findings were reported by Mahmood *et al.* (2009).

In general, the results on character association revealed positive and significant association of grain yield with panicle length, number of filled grains per panicle, ear bearing tillers per plant and 100-seed weight under saline conditions. Similar results were reported by Mansuri *et al*. (2012), Khan *et al*. (2014) and Vijayadurga (2015).

4.4 MOLECULAR DIVERSITY

Identification of markers associated with salinity tolerance trait would help in the selection of salinity tolerant genotypes. In this direction, 14 SSR markers were used in the present study to assess 80 genotypes for their molecular diversity. Polymorphic information content (PIC) value of markers reveals allelic diversity and frequency among the entries. In the present study, the PIC values ranged from 0.182 to 0.838 with a mean value of 0.503 indicating the efficiency of markers for assessing diversity among the genotypes, genetically. The PIC values and the number of alleles of these markers are presented in Table 4.6. Among the markers used for study of diversity, marker RM 10793 reported highest PIC value of 0.838 followed by RM 10694 (0.763), RM 20224 (0.637) and RM 518 (0.603). In general, marker with PIC value of 0.50 or more are used at specific locus for distinguishing polymorphism rate and for genetic studies. Number of alleles for all the 14 SSR markers used in the investigation ranged from two to four with a mean value of 2.64. RM 10793 showed maximum number of four alleles along with RM 10694 (4) followed by RM 20224 (3), RM 492 (3).

Table 4.6 Number of alleles and polymorphic information content (PIC) value of the 14 SSR markers assayed in 80 genotypes

S.No.	Markers	Number of alleles	PIC
1.	RM 10793	4	0.838
2.	RM 10694	4	0.763
3.	RM 562	2	0.205
4.	RM 493	3	0.510
5.	RM 1287	2	0.182
6.	RM 10711	2	0.488
7.	RM 174	2	0.494
8.	RM 423	2	0.551
9.	RM 518	3	0.603
10.	RM 551	3	0.525
11.	RM 253	3	0.278
12.	RM 528	2	0.512
13.	RM 20224	3	0.637
14.	RM 210	2	0.462
	Mean	2.64	0.503

The amplification pattern of RM10793 on chromosome 1 which is near "*SALTOL*" locus responsible for salinity tolerance revealed that banding pattern of MCM-41, MCM-48, MCM-100, MCM-223, MLT-5, MLT- 7, MTU-1064, MTU-2077, MTU-3626, MTU-1078, MTU-1153, MTU-1156, FL-478 is analogous to Pokkali (wild donor) and Nonabokra (salinity tolerant variety) at 180bp. The genotypes, MCM-41, MCM-48, MCM-100, MCM-223, MTU-1078, MTU-1153 and FL-478 also exhibited salinity tolerance to moderate tolerance at 10 days after stress in hydroponics. Similar results were reported earlier by Aliyu *et al.* (2011) with the marker RM 10793 which amplified four alleles in a set of 150 germplasm lines studied. This marker also recorded high PIC value of 0.73 indicating that this marker is highly informative and capable of distinguishing between the genotypes for salinity tolerance. The banding pattern of RM 10793 for the 80 genotypes studied in the present investigation is presented in Plate 4.1.

The amplification pattern with primer RM10964 on chromosome 1 exhibiting polymorphism for the 80 genotypes studied is presented in Plate 4.2. Genotypes, MCM-41, FL-478, CST-9, MTU-1078, Pokkali and Nonabokra showed similar amplicon size of 210 bp which are distinct from banding pattern of remaining genotypes. During phenotypic evaluation these genotypes exhibited tolerance to moderate tolerance at 10 days and 16 days after stress. Kumari *et al.* (2018) also reported that the marker, RM 10694 is highly polymorphic and comparatively more informative as greater number of allelic variants were generated by this primer among all the SSR markers used for the characterization of germplasm for seedling stage salt stress response. The amplification pattern of RM 518 on chromosome 4 in the present study also showed significant polymorphism for the genotypes studied. This marker had recorded high PIC value of 0.603 and amplified a total of three alleles. Further, it showed similar banding pattern in salt tolerant genotypes Nonabokra, pokkali, PLA -1100, STBN-12-7 indicating its probable utility in identification of salt tolerant genotypes.

Cluster analysis and construction of dendrogram for the 80 genotypes was done by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) using Software Darwin 6.0.14 (Perrier and Jacquemond, 2006) and the results are presented in Fig 4.1 and 4.2. Based on UPGMA, the 80 genotypes studied in the present investigation were grouped into two major clusters (Table 4.7). Cluster I comprising of 29 genotypes was further ramified into three sub clusters. Sub cluster IA comprised of one genotype, cluster IB comprised of two genotypes; and sub cluster IC consisted of 26 genotypes.

Cluster II comprises of 51 genotypes and this was also further divided into two sub clusters. Sub cluster IIA contained only one genotype, while sub cluster IIB comprised of 50 genotypes. Most of the salt tolerant varieties *viz.,*Pokkali, Nonabokra, CSR -27 were grouped in sub cluster II B.

Table 4.7 Grouping of genotypes into clusters based on molecular diversity

S.No	Number of genotypes	Name of the cluster	Genotypes
1	1	Cluster I A	MLT -7
2	2	Cluster I B	MLT -11, AST - 57
3	26	Cluster I C	AST -56, AST- 60,AST-62, AST-64, AST-66, BPT -2230, BPT-2270, BPT-3291, BPT-5204, CST-1, CST-2, CST-3, CST-4, CST-5, CST-6, CST-8, CST-9, CST-10, E-460, FL-478, MTU-1075,MTU-1153, MTU-1156, MTU-2716, MTU-1140,MCM-100
4	1	Cluster II A	IR -64
5	50	Cluster II B	CST-7,E-467,E-456,MC-27,MCM-41, MCM-48, MCM-223 (S), MCM-225, MLT-1, MLT-2, MLT-4, MLT-5, MLT-6, MLT-8, MLT-10, IR – 76393 – 2B – 7 – 1 – 1 – 3 – 1,CSR-27,MTU-1001,MTU -1006,MTU-1031, MTU -1032, MTU-1061, MTU – 1064, MTU- 1071, MTU – 1078, MTU – 1112, MTU – 1121, MTU – 1166, MTU- 1184, MTU- 1187,MTU – 1194, MTU -1210, MTU – 1224, MTU – 1226, MTU – 1229, MTU – 2067, MTU – 2077, MTU -3626, MTU -4870, MTU – 5182, MTU- 5249, MTU – 5293, MTU -7029, NONABOKRA, PLA-1100, POKKALI, STBN – 12-1, STBN-12-7,STBN-12-10

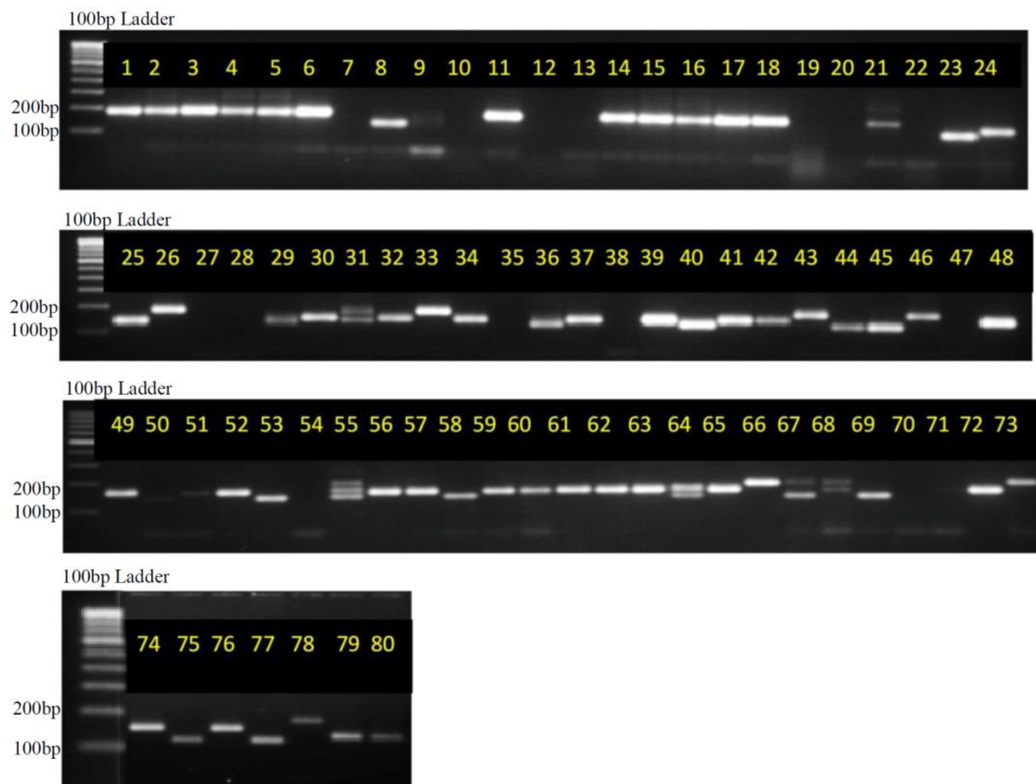

Plate 4.1 Amplification Pattern of genotypes using the primer RM 10793

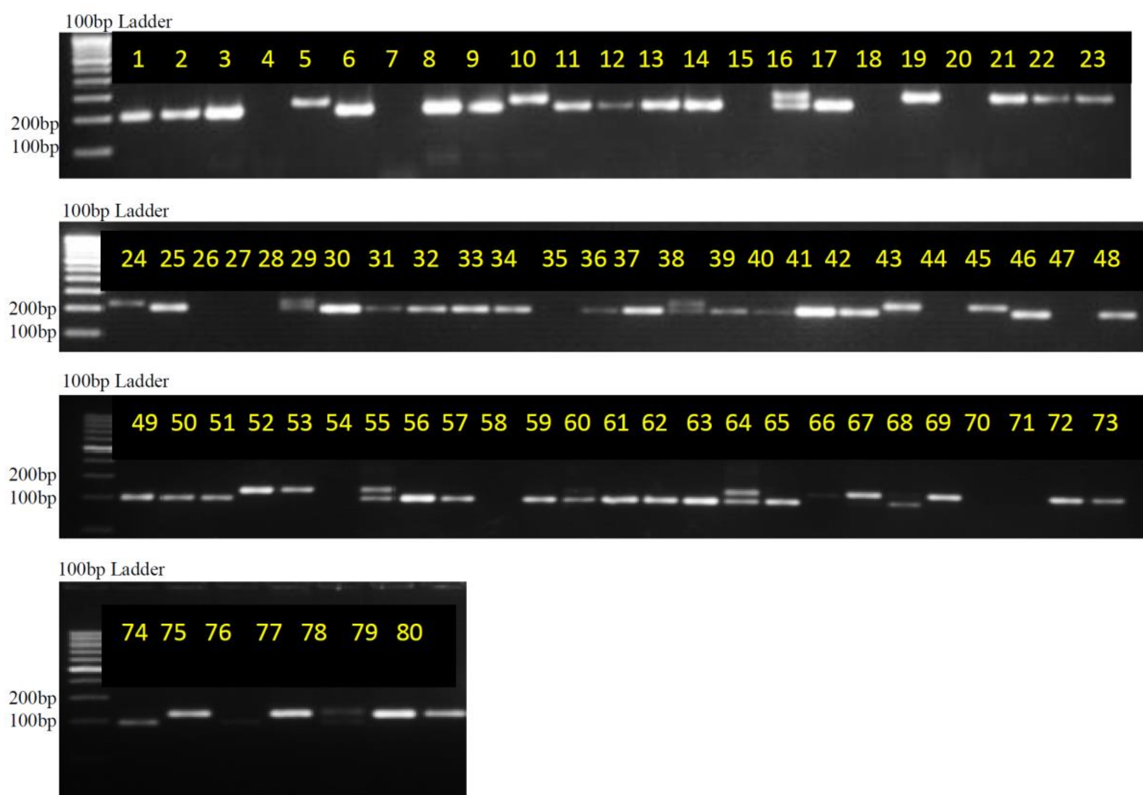

Plate 4.2 Amplification Pattern of RM 10694 among 80 genotypes

Out of the 80 genotypes studied, Pokkali, Nonabokra, MCM-41, MCM-100 displayed alike outcome while phenotyping was done and it certainly indicates that these genotypes may have the gene/QTL responsible for salinity tolerance and these can be used as donor for varietal improvement or for introgression of salinity tolerance gene into a susceptible variety.

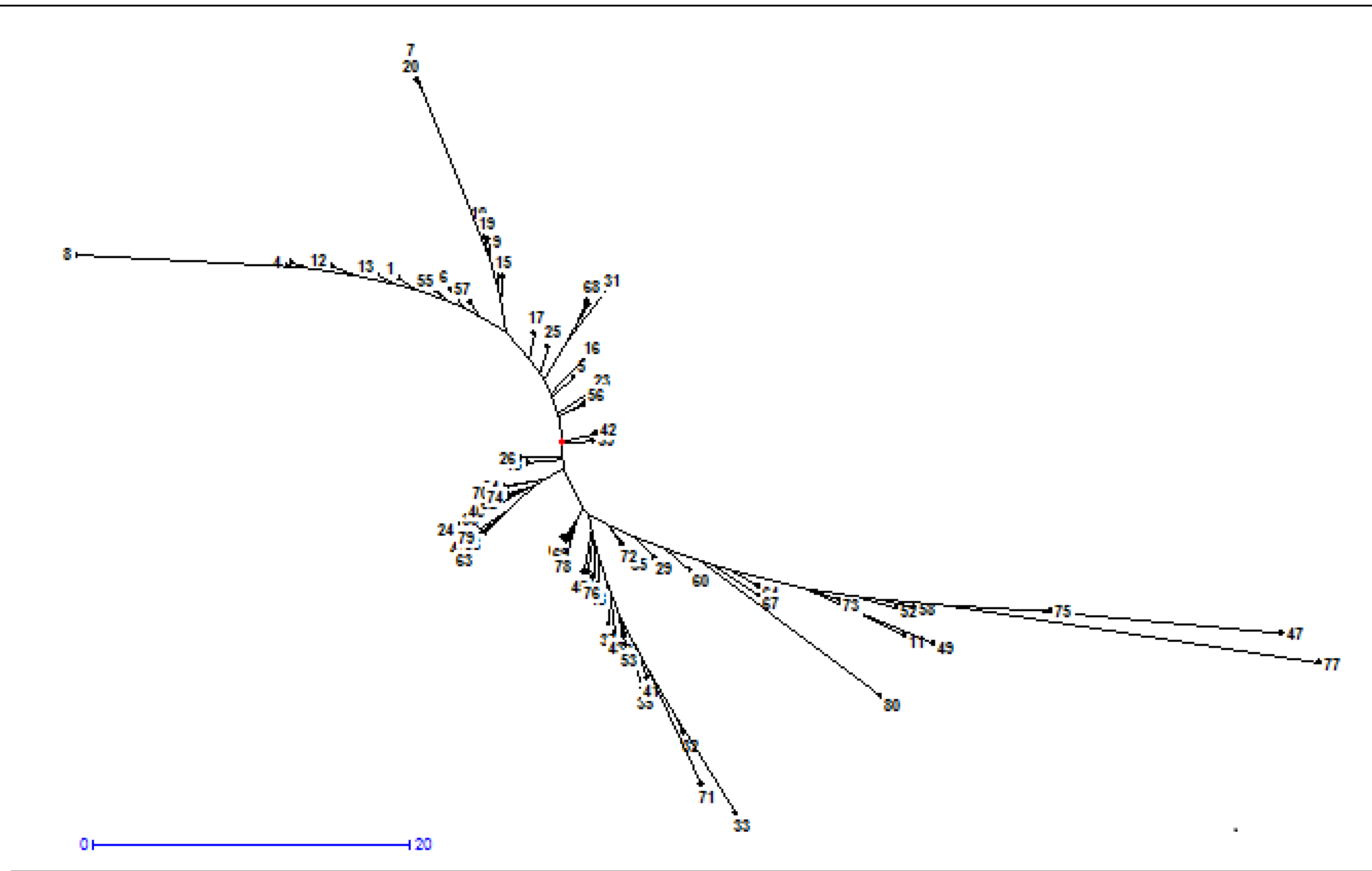

Fig 4.1 Dendrogram (Neighbour joining tree) showing genetic diversity among 80 rice genotypes using molecular markers using Darwin 6.0

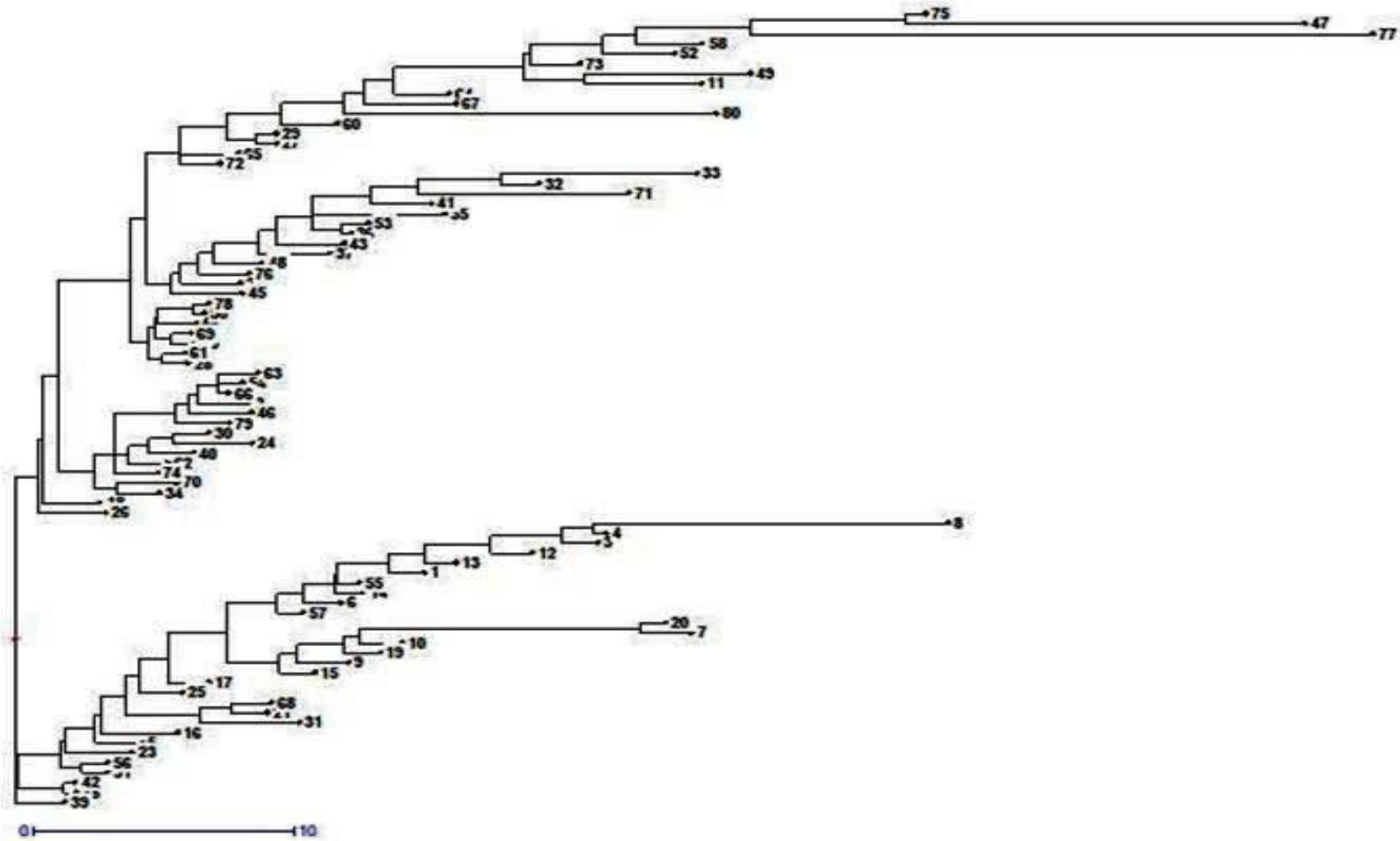

Fig. 4.2 Dendrogram (Phylogenic tree) showing genetic diversity among 80 rice genotypes using molecular markers using Darwin 6.0

Chapter – V

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

Rice (*Oryza* spp.) is staple food for more than three billion people worldwide. Asia alone contributes >90% of the world's rice production (Mitin 2009). Abiotic factors like drought, flood and salinity affect rice production adversely in more than 50% of the area cultivated. In recent era, soil salinity has become a major threat for achieving higher yields in rice. To overcome the situation, genotypes which grow normally and give higher yields even in saline soils are to be identified. Present study was conducted to identify such salinity tolerant genotypes. During the study both conventional and molecular approaches were followed to select genotypes. In addition to this association studies were carried out for yield parameters and salinity score which helps in indirect selection of genotypes with high grain yield.

Phenotyping has resulted in the classification 80 genotypes into different classes based on the performance under stress conditions. Out of 80 genotypes, the initial salinity scoring revealed that 12 genotypes were tolerant, 24 genotypes were moderately tolerant, 29 genotypes were susceptible and 15 genotypes were highly susceptible while the final salinity scoring has indicaed only 11 were tolerant, 26 were moderately tolerant, 26 were susceptible and 17 genotypes were highly susceptible.

Out of 11 genotypes which exhibited tolerance at final salinity scoring Pokkali, Nonabokra, MCM-41 and MCM-100 displayed alike outcome while genotyping these genotypes can be considered as tolerant for salinity stress.

Analysis of variance revealed that wide variation is present among the genotypes for the characters studied. Among the genotypes taken for study Pokkali (wild donor) marked higher plant height and number of ear bearing tillers while Nonabokra noted higher yield and low initial salinity score and final salinity score in addition to this MCM-225 exhibited longer panicle. Finally outcome of the investigation indicates that these varieties perform better both at reproductive and at seedling stage under saline conditions.

Association studies revealed that plant height, number of ear bearing tillers, number of filled grains, panicle length and test weight unveiled positive and significant collaboration with grain yield. It indicates that the characters taken for study assist in selecting the genotypes which can grow normally and induce grain yield under saline conditions.

The amplification motifs with primer RM10964 on chromosome 1 among 80 genotypes under study.Genotypes MCM-41, FL-478, CST-9, MTU-1078, Pokkali, Nonabokra, FL-478 showed similar amplicon size of 210 bp which are distinct from banding pattern of remaining genotypes. During phenotypic evaluation these genotypes exhibited tolerance to moderately tolerance at 10 days and 16 days after stress.

Marker RM10793 reported highest PIC value of 0.838 succeeded by RM10694 (0.763), RM20224 (0.637) and RM518 (0.603).Number of alleles ranged from 2 to 4 with a mean of 2.64. RM10793 connated maximum of 4 alleles along with RM10694 (4) succeeded by RM20224 (3), RM492 (3).

In Darwin cluster analysis 80 genotypes was assembled into two major clusters and grouping of genotypes was shown in table 4.6. Cluster I comprise of twenty nine genotypes which was futher divided into 3 sub clusters. Sub cluster I A comprises of one genotype cluster I B comprises of two genotypes and sub cluster I C consists of twenty six genotypes.

Cluster II comprises of 51 genotypes and this also dissociated into two sub clusters. Sub cluster II A contain only one genotype andsubcluster II B is a massive one with 50 genotypes. Most of the salt tolerant varieties are grouped in subcluster II B *viz.,* Pokkali, Nonabokra, CSR-27.

Out of 80 genotypes Pokkali, Nonabokra, MCM-41, MCM-100 displayed alike out come while phenotyping was done and it certainly indicates that these genotypes has gene/QTL responsible for salinity tolerance and these can be used for varietal improvement or for introgression of gene into a susceptible variety.

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*Originals not seen

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