MOLECULAR ANALYSIS OF RICE (*Oryza sativa* L.) GERMPLASM

By

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MAY, 2019

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A thesis submitted to the

DR. BALASAHEB SAWANT KONKAN KRISHI

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(AGRICULTURAL UNIVERSITY)

DIST. RATNAGIRI (MAHARASHTRA STATE), INDIA

In partial fulfillment of the requirements for the degree of

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CERTIFICATE

This is to certify that the thesis entitled, "**MOLECULAR ANALYSIS OF RICE *(Oryza sativa* L.*)* GERMPLASM.**" submitted to the Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri, Maharashtra State, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) in AGRICULTURE BIOTECHNOLOGY,** embodies the results of a piece of bona-fide research carried out by **Mr. PATIL VISHAL NAVAL** (Regd. No. 0028) under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma or published in other form. All the assistance and help received during the course of investigation and the sources of literature have been duly acknowledged by him.

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CONTENTS

CHAPTER	PARTICULARS	PAGE
I	INTRODUCTION	1-5
II	REVIEW OF LITERATURE	6-38
III	MATERIAL AND METHODS	39-50
IV	EXPERIMENTAL RESULTS	51-74
V	DISCUSSION	75-86
VI	SUMMARY AND CONCLUSION	87-90
	LITERATURE CITED	i-xv
	APPENDICES	I-III

LIST OF PLATES

Plate No.	Title	After Page No.
1.	Gel photograph of DNA isolated from 46 rice genotypes	51
2.	Gel photograph of SSR primer RM-5926	52
3.	Gel photograph of SSR primer RM-8225	52
4.	Gel photograph of SSR primer RM-122	53
5.	Gel photograph of SSR primer RM-6775	53
6.	Gel photograph of SSR primer RM-22709	53
7.	Gel photograph of SSR primer RM-3412	54
8.	Gel photograph of SSR primer RM-3825	54

LIST OF FIGURES

Fig. No.	Title	After Page No.
1.	Dendrogram constructed using Jaccard's Similarity Coefficient.	74

LIST OF APPENDICES

Appendix No.	Title	Page No.
1.	Abbreviations	I-II
2.	Composition of chemicals	III

LIST OF TABLES

Table No.	Title	Page No.
3.1.	Details of genotypes used in the study	39
3.2.	Preparation of solutions for DNA extraction	40-41
3.3.	PCR Stock Solutions and their Sources	43
3.4.	List of SSR Primers with their Sequences and linked gene	44
3.5.	Master mixture for polymerase chain reaction (PCR).	45
3.6	Thermo Profile	46
3.7.	Annealing temperature of SSR markers used in the study.	47
4.1.	Amplification of gene specific alleles with linked SSR markers for Blast resistance	55
4.2.	Amplification of gene specific alleles with linked SSR markers for Bacterial Blight resistance	56
4.3.	Amplification of gene specific alleles with linked SSR markers for Brown Planthopper resistance	57
4.4.	Amplification of gene specific alleles with linked SSR markers for Gall Midge resistance	58
4.5.	Amplification of gene specific alleles with linked SSR markers for Salt tolerance	59
4.6.	Amplification of gene specific alleles with linked SSR markers for Drought resistance	60
4.7.	Genotypes with resistance linked alleles for biotic and abiotic stresses detected by respective SSR markers.	61
4.8.	Primers with their allelic descriptions.	63-68
4.9.	Molecular Polymorphism, PIC Values, No. of alleles and Size of Loci revealed by SSR Primers in Rice Genotypes.	69
4.10.	Average genetic distance estimates of 46 rice genotypes by SSR Marker analysis based on Jaccard's dis-similarity coefficient.	71
4.11.	Distribution of 46 rice accessions into different clusters based on SSR analysis.	74
4.12.	Genetic distance values based on Jaccard's similarity index values between 46 rice accessions.	74
5.1	Studies on Genetic diversity of different rice germplasms	83

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is the world's second most important cereal crop following corn. Nearly 482 million metric tons of husked rice was produced in the last harvesting year throughout the world. Traditionally, countries in Asia have the largest share in world rice production. According to the most recent official data, with a production volume of over 210 million metric tons in 2017, China was the world's leading paddy rice producer, followed by India. The United States was ranked among the leading global rice producers in that year. The nation's rice production value amounted to some 2.37 billion U.S. dollars in 2016. The United States was also ranked among the leading five rice exporters worldwide, primarily shipping this commodity to Mexico, Japan and Haiti. Other major rice exporting nations included India, Thailand, and Vietnam with around 10.3, 10 and 5.8 million metric tons, respectively. According to the United Nation's Food and Agriculture Organization, the average rice price index remained relatively stable over the past few years, before it declined in 2015. Total global consumption of milled rice amounted to approximately 477.77 million metric tons in 2016/2017. China consumed around 146 million metric tons of milled rice per year, and was by far the world's leading rice consumer in that year. In comparison, the U.S. consumed some 3.85 million metric tons (Anonymous, 2018a). For 2018-19, the Indian agriculture ministry has fixed the production target for rice at 113 million tons, against 112.9 million tons last year (Anonymous, 2018b).

India's rice productivity is less in comparison to asia and world. Some of the major factors for low productivity of rice are

continuous use of traditional varieties due to the non-availability of new variety seeds in adequate quantities, lack of awareness of farmers regard to high yielding varieties, exposure of rice to various environmental stresses such as abiotic (salinity, heat, drought, cold, submergence, radiation, and heavy metals) and biotic (pathogens and herbivore) factors which cause a rigorous yield loss (Gomez 2013). The emergence of new diseases and pests and the changing climate are the major issues that address the requirement for sustainable crop development and resistance to biotic and abiotic stresses. For precise genetic manipulation of complex quantitative traits like, yield, tolerance against biotic/abiotic stresses, quality etc., understanding the genetic/molecular basis of target traits needs to be investigated thoroughly.

In the recent times, technological innovations and development of DNA based molecular markers has facilitated the transfer of genes that confer resistance to different biotic stresses (Bacterial Blight, blast, and gall midge etc.) and abiotic stresses (submergence, drought, salinity etc.). With the advances made in the area of molecular markers, the tracking of the genes for resistance is possible by following the path of markers that are linked/tagged to each gene for resistance, thus making the identification of plants with two and more genes possible.

A large number of rice varieties are released and notified every year in India with higher yields, tolerance to biotic and abiotic stresses and to meet the requirement of changing farming systems based on user demands. Different rice varieties of distinct genetic background are a good promise for the future of rice crop improvement programmes as genetic diversity helps in estimating and establishing of genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating

progenies with maximum genetic variability and superior recombinations for further selection and introgressing desirable genes from diverse germplasm (Thompson *et al.*, 1998; Islam *et al.*, 2012). Genetic diversity analysis guides breeders for rapid progress of breeding program. This has contributed to a large extent to the major increases in agricultural productivity in the twentieth century (Dudley, 1994). It is generally thought that continuous selection among the crosses of genetically related cultivars has led to a narrowing of the genetic base of the crops on which modern agriculture is based, thus contributing to the genetic erosion of the crop gene pools (Plucknett *et al.*, 1987).

Development of disease-resistant or stress tolerant plant is an important objective in rice breeding programs, because the production of rice can be constantly interfered by several major biotic and abiotic stresses. Rice and other crops have their own mechanisms to tolerate stress at molecular level. The knowledge of physiology and molecular biology of stress tolerance in rice are helpful for biotechnological improvement of rice productivity (Gomez, 2013). One useful biotechnological tool that was developed throughout the years is DNA based markers. From conventional breeding that takes around 10 years to select a stable and desirable phenotype to marker assisted selection (MAS), genetic functional markers were able to shorten the rice varietal development. Another use of DNA based markers is overcoming the barrier of 'linkage drag' which refers to the presence of undesirable genes in the chromosomal region of the target gene thereby making it difficult to avoid such traits when using conventional breeding (Kottearachchi, 2013). The economic analysis has also shown the potential impacts of using marker assisted breeding (MAB) by overcoming drawbacks of conventional breeding in rice that

ultimately reduce the cost of production and promote economic growth (Kottearachchi, 2013).

Traditional breeding approaches that rely on extensive phenotypic screening methods are effective but delay production of climate-resilient germplasm and also are not suitable for making rapid improvement in tolerance to multiple stresses. Hence, molecular breeding offers the opportunity to increase the speed and efficiency of plant breeding (Whitford *et al.*, 2010). Molecular breeding has led to development of plants resilient to various biotic and abiotic stresses (Roswarne *et al.*, 2012; Yang *et al.*, 2013) and abiotic stresses (Gosal *et al.*, 2009).

A robust and reliable method of fingerprinting is required for identification and purity testing of these varieties (Singh *et al.*, 1999), as well as to study the genetic relationships among different cultivars (Kibria *et al.*, 2009; Sivaranjani *et al.*, 2010). Genetic diversity can be evaluated with morphological traits, biochemical and DNA markers. In contrast to morphological traits, molecular markers can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation, and management and untouched by environmental influence. Molecular markers are promising and effective tools for measuring genetic diversity in germplasm collection and elucidating their evolutionary relationships. Using molecular marker technology, it is now feasible to analyze the quantitative traits and identify the chromosomal regions associating with such characters known as quantitative trait loci (QTLs) (Choudhary *et al.*, 2008). Identifying such regions will help to increase the selection efficiency in the breeding program.

Among various PCR based markers, SSR markers are more popular because they are highly informative, mostly monolocus,

codominant, easily analysed, highly reproducible and cost effective (Gracia *et al.*, 2004). Microsatellites or SSR are sequences of a few repeated and adjacent base pairs and abundance throughout the eukaryotic genome (Powell *et al.*, 1996). SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies, evaluate genetic diversity among rice genotypes for salinity tolerance and abiotic stresses (Zeng *et al.*, 2004; Islam *et al.*, 2012). screening of rice germplasm for several biotic and abiotic stresses (Chungada *et al.*, 2016). SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes from germplasms of various sources, even they can detect finer level of variation among closely related breeding lines within a same variety (Lapitan *et al.*, 2007).

The major objective to study biotic and abiotic stresses using SSR markers is for developing unique fingerprint for each genotype, and to identify SSR markers linked with tolerant traits or QTLs, and to identify the best genotype to be used as donors for particular stress tolerance in breeding program in the future for development of new rice varieties that are equally beneficial for farmers and the scientific community.

Thus, the present research entitled 'Molecular analysis of Rice (*Oryza sativa* L.) germplasm' was undertaken with the following objectives:

1) To establish rice genotype profiles using SSR markers.
2) To characterize the different rice genotypes for biotic and abiotic stresses through SSR markers.
3) To analyze genetic diversity among rice germplasms through SSR markers.

CHAPTER II

REVIEW OF LITERATURE

Recent development in the field of DNA technology has resulted in the development of several molecular markers, which are linked to many traits that are used in screening of different rice varieties for biotic and abiotic stresses. Out of all the known DNA markers, microsatellites are regarded as the markers of choice. Some of the recent applications of microsatellite markers in rice and different crops are reviewed briefly in this chapter.

2.1. Molecular Screening for Biotic Stress.

2.1.1 Blast Resistance

Rice blast, which is caused by *Pyricularia grisea* (Cooke) Sacc., the anamorphous state of *Magnaporthe grisea* (T.T. Hebert) Barr (Rossman *et al.*, 1990) is the primary limiting biotic factor for rice production throughout the world. The use of resistant cultivars is the most effective and economical way to control rice blast disease, and therefore, breeding efforts to develop new resistant cultivars continue to be a priority for rice breeding programs. One of the challenges facing breeders during the development of improved rice cultivars, be they for conventional or organic agriculture, is the incorporation of disease resistance. In the last few decades, new technologies have emerged that allow breeders to more easily select changes at the DNA level. Many Pi genes confer resistance to overlapping spectra of blast pathotypes, and it is often difficult to monitor for the presence of individual resistance genes and pyramid these in breeding lines usingtraditional phenotypic screening. Therefore, DNA markers provide a straightforward and rapid means to select for multiple blast resistance genes without performing

extensive progeny testing or disease screening. DNA markers linked to several of the *Pi* genes have been localized on rice chromosomes, as well as markers for *Pi-ta*(Jia *et al.*, 2002; Hittalmani *et al.*, 2000; Nakamura *et al.*, 1997) and *Pi-b* (Monna *et al.*, 1997). Markers that can be analyzed by PCR are more amenable for breeding purposes, such as the ones developed for *Pi-2* (Hittalmani *et al.*, 2000) and *Pi-ta* (Jia *et al.*, 2002). These markers may also be useful to map new genes for resistance to Blast isolates. Closely linked molecular markers are likely to enhance the efficiency of selection of resistant genotypes in rice breeding programs (Mehla *et al.*, 2011).

Fjellstrom *et al.*, (2004) mapped markersnearthe blast resistance genes *Pi-b*, *Pi-k*, and *Pi-ta2* on rice chromosomes 2, 11, and 12 respectively, using segregation information from hundreds of progeny in several crosses. Two microsatellite markers, RM208 and RM224, were found to co-segregate with the *Pi-b* and *Pi-k* genesrespectively, while additional microsatellites were found to closelyflank these two genes and the *Pi-ta2* gene.

Ashkani *et al.*, (2011) evaluated the 23 best polymorphic markers on 320 F2 progenies derived from Pongsu Seribu 2 × Mahsuri out of 120 SSR markers, about 40% showed clear polymorphism between blast susceptible and resistant parents. The patterns of all the markers varied in the segregating population. The individuals of the F2 population (derived from Pongsu Seribu 2 × Mahsuri) that had the alleles RM168 (116 bp), RM8225 (221 bp), RM1233 (175 bp), RM6836 (240 bp), RM5961 (129 bp), and RM413 (79 bp) were resistant to pathotype P7.2. The blast resistant plants had the alleles of these six SSR markers. This finding has potential for use in marker assisted selection programs and confirmation of blast resistance genes to develop rice cultivars with durable blast resistance in Malaysian rice breeding programs.

Mishra *et al.*, (2011) utilized twenty three SSR markers in the study which targeted nine different genes; *Pi*27 located on chromosome 6, *Pi*29 and *Pi*33 on chromosome 8, Pita, *Pi*20 and *Pi*31 on chromosome 12, *Pi*b and *Pi*25 on chromosome 2, *Pi*30 on chromosome 11 for 35 genotypes. Within the thirty five genotypes chosen for screening, IR-64 was selected as resistant check and GR-11 and Pankhali-203 wereselected as susceptible checks. The remaining 32 genotypes were used for comparison with the above mentioned checks.

Sadegh *et al.*, (2012) studied F3 population derived from the cross of Pongsuseribu 2 (Resistant) and Mahsuri (Susceptible) rice cultivars using 11 polymorphic simple sequence repeat (SSR) markers to investigate association with Pi gene conferring resistance to M. *Oryzae* pathotype. Four SSR markers (RM413, RM5961, RM1233 and RM8225) were found to be significantly associated with blast resistance to pathotype 7.2.

Kumar *et al.*, (2013) used GPP 2 as donor parent for *xa13*, *Xa21*, *Gm4* resistance to bacterial blight, gall midge and NLR 145 as another donor parent for *Pi-kh* gene resistance to blast and JGL 1798 as recurrent parent was investigated using 128 simple sequence repeat (SSR) primers covered on chromosome number 1-12. The results reveal that 36 HRM primers showed distinct polymorphism among the donor and recurrent parents studied indicating the robust nature of microsatellites in revealing polymorphism.

Kumar *et al.*, (2013) Rice blast caused by *Magnaporthe oryzae*, spread in more than 85 countries and has caused great yield loss. Development and growing of rice varieties would be the most effective way to control blast disease. Molecular analysis and major rice blast resistance genes for genetic diversities were

determined and molecular characterization (or) screening of major rice blast resistance genes was determined with molecular markers, which showed close set linkage to 10 major rice blast resistance genes (Pi-54, Pi-1, Pi-2, Pi-9, Pik, Pikm, Pitp, Pi-38, Pizt and Pi 7t), in a collection of 15 accession (including some varieties). Out of the 15, the Pikm, Pizt and Pi7t appeared to be widely present in all the varieties with respect their resistant allele size and gave positive express. For the gene, Pi-54, Pi-1, Pi-2, Pi-9, Pik, Pitp and Pi-38 gene frequencies were 33.33%, 40.01%, 86.67%, 6.67%, 60.07%, 33.33% and 33.33% respectively. Among the 15 accessions, 5 were positive for Pi-54, Pitp and Pi-38 genes and six accessions were positive for Pi-1 gene, thirteen for Pi-2, nine for Pikm and one for Pi-9 gene. Four accessions viz., BR 2655, JAYA, RAKSHA and BASUMATHI-370 were positive for two major and broad spectrum genes i.e Pi-54 and Pi-2. Out of 15, only three accessions namely BR-2655, JAYA and BASUMATHI-370 were detected with maximum number (> 7) of genes. Less number of genes (<4) harboured in KMP -201 and IR – 64. These results are useful in identification and incorporation of functional resistance genes from these evaluated varieties into elite cultivars through marker – assisted selection for improved blast resistance in India and worldwide.

Jayawardana *et al.,* (2014) studied detection of blast resistance genes in phenotypically evaluated, selected rice cultivars in Sri Lanka based on simple sequence repeats (SSR) DNA markers. In the present study blast pathogen was identified by morphological characters, molecular techniques which confirmed the identity as M. grisea. Hence, resulted polymorphism findings can be used in future studies to differentiate the resistant and susceptible varieties.

Singh *et al.*, (2015) carried out molecular screening and genetic diversity analysis of major rice blast resistance genes in 192

rice germplasm accessions using simple sequence repeat (SSR) markers. The genetic frequencies of the 10 major rice blast resistance genes varied from 19.79% to 54.69%. Seven accessions had maximum eight blast resistance gene, while 11 accessions had seven blast resistance genes. Twenty accessions possessed six genes, 36 accessions had five genes, 41 accessions had four genes, 38 accessions had three genes, 26 accessions had two genes, 13 accessions had single R gene and only one accession IC438644 does not possess any one blast resistant gene. Out of 192 accessions only 17 accessions harboured 7 to 8 blast resistance genes.

Thippeswamy *et al.*, (2015) evaluated 312 indigenous and 65 exotic germplasm lines against blast resistance at RARS, Jagtial. More percentage (83%) of exotic germplasm showed resistance to rice blast disease compared to indigenous germplasm (46%). Three genotypes (JGL23710, JGL23713 and JGL23714) in indigenous germplasm and two genotypes (IR09N500 and IR12M101) in exotic germplasm were immune to rice blast disease. "These can be used as donor genetic stock for development of highly resistant rice cultivars with high yields". Among five linked markers studied for *Pi*-1 gene, one marker RM6094 was able to identify resistant genotypes at allelic level and for *Pi*-2 gene; RM527 was validated in four genotypes out of six genotypes used.

Turaidar *et al.*, (2017) performed molecular screening and genetic diversity study of major rice blast resistance genes in 32 rice germplasms which included 30 traditional rice varieties (TRVs), a resistance (Tetep) and susceptible (Susceptible) checks using five simple sequence repeat (SSR) markers. A TRV, Mugadsuganda had maximum of five resistance genes, nine TRVs possess four blast resistance genes, while four TRVs like, Naweli, ThornadaBatta, Adribatta and Mullubatta had only one blast resistance gene.

Tetepand HR 12 possess three and two resistance genes respectively. Most of the TRVs were shown to be rich source R genes for rice blast, could be utilized for future breeding works to develop blast resistance varieties.

2.1.2 Bacterial leaf Blight (*Xanthomonas oryzae pv. oryzae*)

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most devastating diseases affecting entire rice acreages and causes severe yield losses of up to 80% depending on the stage of the crop, cultivar susceptibility and the environmental conditions (Srinivasan and Gnanamanickam, 2005). Exploitation of host plant resistance is considered the most effective, economical and environmentally safe measure for controlling BLB in combination with management practices. To tackle this problem, several attempts have been made to identify and characterize BLB resistance genes. Till date, approximately 34 genes (23 dominant and 11 recessive) conferring resistance against various strains of X. oryzae, have been identified (Chen *et al.*, 2011). Major resistance genes, including Xa4, Xa5, Xa7, Xa13 and Xa21 have been incorporated into rice cultivars, in order to develop new resistant varieties (Perumalsamy *et al.*, 2010). However, the cultivars containing a single major resistance gene proved susceptible due to pathogen mutation. Recently, pyramiding of more than one major resistance gene has been proven to deliver durable resistance against Xoo (Rajpurohit *et al.*, 2010).

Abbasi *et al.*, (2011) used molecular and conventional approaches for identify rice germplasm for the presence of Xa5, a bacterial blight resistance gene. Polymerase chain reaction (PCR) with primers specific for Xa5 resistances gene were used in the study. During this polymorphic study, out of 60 rice lines, 31 were observed with Xa5 gene, while 29 showed the absence of Xa5 gene.

Pakistani Basmati varieties were also surveyed. Out of the ten Pakistani Basmati varieties, Kashmir Basmati, Basmati Pak, Shahley Basmati and Basmati-622 had the Xa5 gene, while Basmati-385, Basmati-2000, Basmati-370, Basmati-198, Super Basmati and Dokri Basmati showed the absence of Xa5 gene. Identification of Xa5 gene in Pakistani rice germplasm will help in accelerating the breeding program including pyramiding of different disease resistant genes in basmati and other cultivated varieties.

Ullah *et al.*, (2012) observed that the genes conferring resistance to bacterial blight in coarse rice. They identified bacterial blight resistance genes Xa4, Xa5, Xa7, and xa13 in 52 basmati landraces and five basmati cultivars using PCR markers. The Xa7 gene was found to be the most prevalent among the cultivars and landraces. The cultivars Basmati-385 and Basmati-2000 also contained the Xa4 gene; however, xa5 and xa13 were confined to landraces only. Ten landraces were found to have multiple resistance genes. Landraces Basmati106, Basmati-189 and Basmati-208 contained Xa4 and Xa7 genes. Whereas, landraces Basmati-122, Basmati-427, Basmati 433 were observed to have Xa5 and Xa7 genes. Landraces Basmati-48, Basmati-51A, Basmati-334, and Basmati-370A possessed Xa7 and Xa13 genes.

Kumar *et al.*, (2013) used GPP 2 as donor parent for xa13, Xa21, Gm4 resistance to bacterial blight, gall midge and NLR 145 as another donor parent for Pi-kh gene resistance to blast and JGL 1798 as recurrent parent was investigated using 128 simple sequence repeat (SSR) primers covered on chromosome number 1-12 so as to provide ready to use markers for back ground selection in marker assisted breeding of rice.The results revealed that 36 HRM primers showed distinct polymorphism among the donor and recurrent parents studied indicating the robust nature of

microsatellites in revealing polymorphism. Based on this study, the large range of similarity values for related cultivars using microsatellites provides greater confidence for the assessment of simple sequence repeats (SSR) polymorphism.

Islam *et al.*, (2015) conducted the study to screen 12 aromatic rice cultivars for BLB resistant gene Xa5. The genotypes were analyzed using two genetic markers (RM 122 and RM 390) by Polymerase Chain Reaction (PCR). Where, BR 14 that was resistant to BLB was used as control. Both primers generated different banding patterns. Primer RM 122 produced 6 bands whereas primer RM 390 produced 10 bands, respectively. The highest level of gene diversity value (0.8889) was observed in locus RM 390 and the lowest level of gene diversity value (0.7361) was observed in locus RM122 with a mean diversity of 0.8125. The PIC values ranged from a low of 0.7007 (RM 122) to a high of 0.8785 (RM 390) with an average of 0.7896. Using the linked primer RM 122, the bands of Xa5 gene were standardized by the amplified DNAs. The DNA band of 246 bp was considered as resistance line and the DNA band of 230 bp was considered as a susceptible line.

Singh *et al.*, (2015) screened thirty four rice cultivars against *Xanthomonas oryzae pv.* and identify the presence of bacterial leaf blight resistance genes Xa21, Xa13 and Xa5. During the polymorphic survey of thirty four rice cultivars, using three primers (pTA-248, xa-13prom and RM-13) no amplicons specific to Xa21 and Xa13 allele were detected, showing the absence of these two genes in all the cultivars evaluated, while twenty cultivars along with resistant checks amplified 219 bp size fragments indicating the presence of Xa5. Therefore, the resistant cultivars could be used for the transfer of bacterial leaf blight resistance gene to well adapted high yielding rice cultivars.

Amgai *et al.*, (2015) screened ninety six Nepalese rice accessions using eight SSR markers RM21, RM167, RM206, RM 224, RM230, RM263, RM390 RM251 and one Sequence Tagged Sites (STS) marker pTA248 for presence and absence of BLB resistance gene. BLB resistance gene Xa-10 detected on five accessions, Xa-13 on six accessions, Xa-7 on 23 accessions, Xa-3 and Xa-4 on 52 accessions, Xa-5 on 25 accessions, Xa-8 on 30 rice accessions. No any rice accessions tested have Xa-21. Similarly, 17 rice accessions showed three and more than three BLB resistance genes.

Sabar *et al.*, (2016) conducted study to explore the genetic resources for BLB resistance genes Xa4, xa5 and Xa21 using gene specific markers MP1, RM122 and pTA 248 respectively. Eighty (80) rice genotypes comprising of diverse origin including three isogenic lines viz., IRBB4 (Xa4), IRBB5 (xa5) and IRBB21 (Xa21) as positive resistant gene checks and IR24 (carrying none of these) as negative gene check were genotyped. DNA fingerprinting results indicated the presence of Xa4 gene in 41 entries, while 14 lines were positive for xa5 gene. Only one local line was carrying Xa21 gene along with Xa4.

Sindhumole and Soumya, (2016) did marker assisted selection of rice germplasm accessions for Xa5 gene, using RM122, a closely linked microsatellite marker. Forty traditional rice genotypes from the germplasm of Division of Plant Breeding and Genetics, Regional Agricultural Research Station, Pattambi, were initially screened in field for BLB resistance. Out of them, only twenty genotypes which had either no or less symptoms of BLB disease, were utilized for marker assisted selection. The PCR products were subjected to gel Agarose electrophoresis (1.5%). Among the thirteen genotypes which displayed marker specific

bands during gel electrophoresis, five genotypes viz., Karuthakuruka, Kuruva, Kokkankoli, Kochuvithu (Thamarakulam) and Punjaparathu had no symptoms of BLB, indicating that BLB resistance in these genotypes is due to the gene Xa5. Hence these five resistant genotypes can be utilised as donors for Marker Assisted Breeding, to develop BLB resistant rice varieties.

2.1.3 Brown plant hopper tolerance (*Nilaparvata lugens*)

Brown planthopper (BPH) is the most devastating pest of rice. BPH populations on rice have been categorized in to four biotypes (Khush *et al.*, 1985). Host-plant resistance is the most desirable and economic strategy in the management of BPH. To date, 26 BPH resistance genes have been identified in wild species and Indian cultivars (Fujita *et al.*, 2008) and more than ten genes have been fine mapped to chromosome regions of less than 200 kb. Four genes (*Bph14*, *Bph26*, *Bph17* and *bph29*) have been cloned.

Development of resistant rice cultivars through host plant resistance is generally considered to be the most economic and effective way for controlling BPH damage. In contrast to morphological traits, molecular markers can reveal differences among genotypes at DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management.

Myint *et al.*, (2012) identified BPH-resistance (antibiosis) gene loci from ADR52 by linkage analysis using backcross populations. BPH25 co-segregated with marker S00310 on the distal end of the short arm of chromosome 6, and BPH26 co-segregated with marker RM5479 on the long arm of chromosome12. To characterize the virulence of the most recently migrated BPH strain in Japan,

preliminary near-isogenic lines (pre-NILs) and a preliminary pyramided line (pre-PYL) carrying BPH25 and BPH26 were evaluated.

Harini *et al.*, (2013) used twenty eight (28) rice genotypes to evaluate the genetic variability based on known BPH resistant loci spread through most of the genome (chromosomes 2, 3, 4, 5, 6, 8, 10, 11 and 12), using closely linked simple sequence repeat (SSR) markers and by different phenotypic screening methods. A total number of 155 alleles were detected by 30 polymorphic markers with an average of 4.6 per locus. The genetic diversity, polymorphic information content (PIC) ranged from 0.15 to 0.89 and 0.13 to 0.88, respectively and the allele frequency ranged from 0.21 to 0.89. These microsatellite markers linked to BPH resistance loci classified rice genotypes into three clusters with additional sub groups and sub groups.

Carsono *et al.*, (2015) analyzed twenty genotypes, and two of them were used as check varieties. Simple Sequence Repeat (SSR) markers were applied to detect *Bph3*, *Bph4, Qbph3,* and *Qbph4* genes. Polymorphic levels were analyzed by calculating PIC (*Polymorphic Information Content*). The grouping of rice genotypes were done based on principal components analysis (PCA) of SSR data, and the genetic relationship based on the presence of Bph genes was estimated using UPGMA (*Unweighted Pair Group With Arithmetic mean*). Results showed that RM313, RM8072, RM8213, RM5953, RM586, and RM589 markers were polymorphic. UPGMA clustering resulted in two main clusters, in which the first cluster consisted of 2 subclusters.

Mahmoodreza *et al.,* (2015) studied 28 polymorphic simple sequence repeat (SSR) markers were analyzed in 108 F_3 progenies derived from the cross of Rathu Heenati and MR276 rice cultivars to

investigate the association with BPH resistance against biotypes 2 and 3. Association of SSR markers with phenotypic traits in F_3 progenies were identified. Six SSR markers (RM 401, RM 5953, RM 217, RM 210, RM 242, and RM 1103) were significantly associated with BPH resistance to biotypes 2 and 3 of BPH. Out of these 6 markers, RM 401, RM 5953, and RM 217 accounted for about 17% of total phenotypic variation and RM 210, RM 242, and RM 1103 accounted for 20% of total phenotypic variation against biotypes 2 and 3, respectively. Therefore, the above 6 markers were confirmed for MAS in rice breeding programs to develop BPH-resistant rice cultivars.

Bhogadhi *et al.*, (2015) screened twenty rice genotypes using 24 SSR gene linked markers of major BPH resistance and found more than one BPH resistance genes in each resistant genotype. These lines were used as donors in rice breeding programme to develop BPH resistant varieties.The genotypes BM71 showed donor allele with only one marker each, RM589 for Bph3 and RM3180 for Bph6 and ACC2398, ACC5098 (RM17008, RM3180), having Bph6 gene.

Laksminarayana and Gurdev., (2015) studied in 28 rice (*Oryza sativa* L.) cultivars resistance to brown plant hoppers (*Nilaparvata lugens* Stal.) in the greenhouse Seven-day-old seedlings were infested with second and third-instar nymphs of brown plant hoppers and seedling injury was recorded at 7 to 8 days after infestation. Single dominant genes that are allelic to Bph 1 condition the nine genotypes resistance to BPH. A single dominant gene also conveys resistance in 'Rathu Heenati', but it segregates independently of Bph 1 and is designated as Bph 3. Similarly, a single recessive gene conveys resistance in 'Babawee' but it segregates independently of bph 2 and is designated as bph 4. The

resistance in 'Ptb 21' is controlled by one dominant and one recessive gene. The allelic relationships of these two genes to other genes are not known.

Shabanimofrad *et al.*, (2015a) analysed 110 simple sequence repeat (SSR) markers associated with Bph resistance genes. Out of primers used, thirty-five SSR markers, including RM544, RM547, and RM8213, showed a good fit to the expected segregation ratio (1:2:1) for the single gene model. Fifteen SSR markers exhibited significant deviation from the normal distribution pattern, and seven (RM208, RM348, RM163, RM455, RM160, RM228 and RM496) displayed highly significant deviation from the normal distribution pattern.

Shabanimofrad *et al.*, (2015b) studied 28 polymorphic simple sequence repeat (SSR) markers in 108 F3 progenies derived from the cross of Rathu Heenati and RM 276 rice cultivars to investigate the association with BPH resistance against biotypes 2 and 3. Association of SSR markers with phenotypic traits in F3 progenies revealed that six SSR markers (RM401, RM5953, RM217, RM210, RM242, and RM1103) were significantly associated with BPH resistance to biotypes 2 and 3 of BPH. Out of these 6 markers, RM401, RM5953, and RM217 accounted for about 17 per cent of total phenotypic variation and RM210, RM242, and RM1103 accounted for 20 per cent of total phenotypic variation against biotypes 2 and 3, respectively.

2.1.4 Gall midge tolerance (*Orseolia oryzae)*

The Asian rice Gall Midge *Orseolia oryzae* Wood-Mason is a serious pest of rice in South and Southeast Asia. In India too, the pest is widely distributed and is considered as a significant

constraint to rice production. The affected tillers do not bear panicle, thus causing significant yield losses. Chemical control is inefficient due to the internal feeding habit of the pest and the prevailing hydrological and edaphological conditions during the wet season. Use of resistant varieties has been the most feasible alternative to manage the pest and several sources of resistance are available in cultivated rice. While there is a long term need to deploy new breeding strategies to develop superior rice genotypes having durable resistance to GM across different biotypes, it is also necessary to incorporate specific resistance genes against specific biotype for the most suitable and popular varieties of a particular regions. So far, 11 gall midge resistance genes have been characterized in rice (Himabindu *et al.*, 2010), and seven biotypes of the pest have been reported (Vijayalakshmi *et al.*, 2006). Of the 11 gall midge resistance genes reported so far, eight genes viz., *Gm1*, *Gm2*, *Gm4*, *Gm5*, *Gm6*, *Gm7*, *Gm8* and *Gm11* have been tagged and mapped (Yasala *et al.*, 2012). Molecular markers linked to the resistance genes can be helpful in accelerating breeding programmes, since they facilitate selection of desirable genes without the need for initial phenotype based selection.

Nanda *et al.*, (2010) used a set of F9 recombinant inbred lines (RILs) of the cross TN1/PTB10 and identified microsatellite markers for the gall midge resistance gene in cv. PTB10 on short arm of rice chromosome 8. Markers RM22550 and RM547 flank the gene at a distance of 0.9 and 1.9 cM, respectively. Amplification of the markers in gall midge resistant and susceptible cultivars showed that these markers can be successfully used in MAB for development of gall midge resistant varieties.

Bentur *et al.,* (2011) used simple sequence repeat (SSR) markers represented di (72%), tri (15.3%), and complex repeats (12.7%).

Three biotypes of gall midge (20 individuals for each biotype) were screened using these SSRs. The results revealed that 15 loci were hyper variable and showed polymorphism among different biotypes of this pest. The number of alleles ranged from two to 11 and expected heterozygosity was above 0.5. Inheritance studies with three markers revealed sex linked inheritance of two SSRs and autosomal inheritance of one marker.

Sama *et al.*, (2012) identified simple sequence repeat (SSR) markers that are tightly linked to pest resistance genes. Based on conventional and molecular allelism tests, *Gm8* gene was identified in rice genotype Aganni conferring hyper sensitive independent (HR–type) resistance to gall midge biotypes GMB1, GMB2, GMB3, GMB4, and GMB4M. The gene *Gm8* was mapped to chromosome 8 within a 400-kbp region, and the SSR markers RM 22685 and RM 22709 flanks the gene closely. Using these closely linked flanking markers, nine other gall midge-resistant genotypes were identified as carrying the same gene Gm8. Through marker-assisted selection, *Gm8* has been introgressed into an elite bacterial blight-resistant cultivar, Improved Samba Mahsuri.

Kumar *et al.*, (2013) provided ready to use markers for back ground selection in marker assisted breeding of rice, they used GPP 2 as donor parent for xa13, Xa21, Gm4 resistance to gall midge and NLR 145 as another donor parent for Pi-kh gene resistance to blast and JGL 1798 as recurrent parent was investigated using 128 simple sequence repeat (SSR) primers covered on chromosome number 1-12. The results reveal that 36 HRM primers showed distinct polymorphism among the donor and recurrent parents. 92 per cent of markers showed clear amplification, while 31 per cent of markers showed polymorphism. The study further revealed that the

selected primers belong to Class I microsatellites that are highly polymorphic.

Dutta *et al.*, (2014) genotyped 100 gall midge resistant rice entries by using five gene specific markers which can detect the presence of Gm1, gm3, Gm4, Gm8 or Gm11 genes with high degree of success. Based on both phenotypic and genotypic analysis, these genotypes were grouped into five phenotypic sets.

Suvendhu *et al.,* (2014) studied about 100 gall midge resistant rice genotypes which were screened under field condition against gall midge biotype GMB4M at Warangal and GMB1 in greenhouse at DRR. Furthermore, these entries were genotyped by using five gene specific markers which can detect the presence of *Gm1*, *gm3, Gm4, Gm8* or *Gm11* genes with high degree of success. Based on both phenotypic and genotypic analysis, these genotypes were grouped into five phenotypic sets. In Set 1, 17 genotypes showed nil damage at both the locations and amplified one or multiple gene specific alleles. In Set 2, 31 genotypes showed low level of damage (< 20%) at DRR and or Warangal, of which two breeding lines and a land race are likely carry new gene(s). In set 3, 18 genotypes displayed resistance against GMB1 but were susceptible against GMB4M. Set 4 had 6 genotypes that were found resistant against GMB4M but recorded up to 50% plant damage in greenhouse against GMB1. Set 5 had 22 genotypes that were susceptible against both the biotypes and not carried R genes.

Yao *et al.*, (2015) identified the quantitative trait loci (QTL) associated with African Gall midge (AfRGM) pest incidence and resistance in three independent bi-parental rice populations (ITA306xBW348-1, ITA306xTOG7106 and ITA306xTOS14519) and mapped first time the genomic regions associated with the AfRGM resistance. Composite interval mapping (CIM) conducted on the

three populations independently uncovered a total of 28 QTLs associated with pest incidence (12) and pest severity (16). The number of QTLs per population associated with AfRGM resistance varied from three in the ITA306xBW348-1 population to eight in the ITA306xTOG7106 population. The major genomic region for AfRGM resistance mapped at 111 cM on chromosome 4 (qAfrGM4) in the ITA306x-TOS14519 population.

Mohapatra *et al.*, (2016) evaluated forty-eight rice genotypes comprising eighteen donors and thirty high yielding varieties against biotype 2 of gall midge under glass house conditions at seedling stage. These 48 rice genotypes were amplified with Gm4 resistance gene linked four markers RM22550, RM22551, RM547 and RM22555.

2.2. Molecular Screening for Abiotic Stress

2.2.1 Drought Resistance

Among the abiotic stresses drought is recognized as a major abiotic stress that limits rice productivity and adversely affects grain quality in rain-fed and upland ecosystems (Bimpong *et al.*, 2011). Conventional breeding for drought resistance is slow in attaining progress due to poor understanding of genetic control of drought resistance. Complex responses to drought coupled with often unreliable and labour-intensive conventional phenotyping have made it difficult to breed rice varieties with improved drought tolerance (Ingram *et al.*, 1994). To overcome this problem, molecular markers have been utilized to identify genotypes having traits directly related to drought tolerance and the strategy is already well developed and known to be more efficient than conventional variety improvement. Development of molecular markers and their use for the genetic dissection of agronomically important traits has become a powerful approach for studying the inheritance of complex plant

traits such as drought tolerance (Suji *et al.*, 2011). The use of molecular markers for the selection of complex breeding traits offers greater selection accuracy with less labor and time inputs, and enables assemblage of different target traits into a single cultivar. Hence, use of molecular markers to detect QTLs controlling drought tolerance related traits has the potential to accelerate breeding for drought tolerance and will ultimately contribute to reducing the problem of food security aggravated by changing climatic conditions.

Thanh *et al.,* (2006) used QTLs for root traits related to drought resistance (maximum root length, root thickness, root weight to shoot and deep root weight to shoot ratios) in upland rice using a recombinant inbreed (RI) population derived from a cross between Vietnamese upland rice accessions. The first molecular linked of Vietnamese upland rice were constructed. The map consists of 239 markers (36 SSR and 203 AFLP markers) mapped to all 12 rice chromosomes. This map covered 3973.1 cM of rice genome with an average distance of 16.62 cM between the markers. Twenty three putative QTLs were detected. There are several SSR markers such as RM250, RM270, RM263, RM242, RM221 linked to QTL regions. Some common QTLs for maximum root length and deep root weight to shoot ratio were observed in different genetic background (RDB09 × R2021 and IR64 × Azorean populations) and ecological locations (IRRI and Vietnam). These QTLs could be very useful for precise locating drought resistant gene(s) and marker-assisted selection.

Kanagaraj *et al.*, (2010) identified quantitative trait loci (QTLs) associated with drought resistance traits and their indirect selection using marker assisted selection through Bulked segregant analysis (BSA) using 23 recombinant inbred (RI) lines of IR20/Nootripathu, two indica ecotypes with extreme drought response. The parents

were screened for polymorphism using 1206 rice microsatellite primer pairs. Out of 134 SSR polymorphic primers between parents, three primers (RM212, RM302 and RM3825) co-segregated among the individual RI lines constituting the respective bulks. The genomic regions flanked by these markers have been reported to be associated with several drought resistance component traits.

Ballani *et al.*, (2013) studied the SSR analysis of forty genotypes with 27 microsatellite markers showed polymorphism for all markers producing 195 alleles. The clustering pattern was in line with geographical origin and parental gene pool of the genotypes. SSR analysis of genotypes validated the use of few SSR markers for marker assisted selection viz., RM205 for panicle length, RM206 for panicle number, RM212 for maximum root length and RM263 for grain yield. The marker RM3825 was found efficient in distinguishing the genotypes for grain yield under stress.

Niksiar *et al.,* (2013) studied allelic variations of microsatellite markers related to QTL controlling drought-tolerance in rice, twenty two genotypes and sixteen pairs markers were used. Results showed that the largest of alleles and highest genes variation as well as PIC were related to RM 8030 and RM 3302 respectively. Genotypes fell into two groups by genotype clustering based on SSR data. The genotypes classified with tolerant cultivar Bala in the same class showed much more drought tolerance than those with Azucena.

Ashfaq *et al.*, (2014) analyzed twenty different rice lines based on various genotypic and phenotypic seedling traits. Different SSR markers were used to study the genetic diversity and screening of the rice genotypes for developing new breeding lines. The mean number of alleles per locus was 3.70, showing that the average polymorphism information content (PIC) was 0.500. A total of 63 alleles were also identified from the microsatellite marker loci. The

results revealed that markers RM315, RM212 and RM302 on chromosome 1 may be useful for evaluation of diverse germplasms and also linked with root traits due to strong linkage disequilibrium association between them.

Ramadan *et al.*, (2015) evaluated the genetic diversity for seven rice genotypes using 46 SSR markers related to drought tolerance. Out of the seven genotypes, three genotypes were drought tolerant (Azuciena, Moroberekan and IRAT170), two were moderately tolerant (IET1444 and Giza178) and two were drought susceptible (IR64 and Giza177). Among SSR markers used, 43 SSR loci were polymorphic and produced 127 alleles. The number of alleles per locus generated by each marker variedfrom 2 to 6 alleles with an average of 2.76 alleles per locus. The polymorphic information content (PIC) values ranged from 0.21 to 0.79 with an average of 0.46. Out of the used polymorphic SSR markers, 19 markers were highly informative (PIC > 0.50), 21 markers were informative (50 < PIC < 0.25) and three markers were slightly informative (PIC < 0.25). The SSR markers RM260, RM219, RM3805 and RM72 were the highest polymorphic markers in current study. On the other side, the Jaccard's similarity coefficients among the studied genotypes ranged from 0.07 (for Giza177 and both genotypes IR64 and IET1444) to 0.804 for Azuciena and IRAT170. RM472 marker elucidated the possibility to use it in MAS for drought tolerance.

Freeg *et al.,* (2016) studied a number of 41 rice genotypes with different drought tolerance from different geographic locations were evaluated for genetic diversity by using 15 SSR markers. A total of 68 alleles were detected of which 61(89.79%) were polymorphic. The number of alleles detected by a single marker varied from 2 to 8 alleles with an average of 4.71 alleles per locus. The polymorphic

information content (PIC) values ranged from 0.07 (RM219) to 0.80 (RM263) with an average of 0.52. Genetic similarity coefficients of pair wise comparisons were estimated on the basis of the polymorphic microsatellite loci ranged from 0.23 to 0.91 indicating a wide range of genetic variation present among the studied genotypes. It was determined that the primers RM20A, RM302, RM212 and RM286 could be useful for selecting drought tolerant lines through MAS approach. The results indicated the ability of SSR markers to identify the allelic diversity and genetic variation among the studied rice genotypes. These results recommended for using this material in future breeding programs to provide important source of genetic diversity for drought tolerance in rice.

Anupam *et al.*, (2017) genotyped 74 rice germplasms including Tripura's local landraces, improved varieties, cultivars and breeding lines and other rice varieties using molecular markers for genetic diversity, drought QTLs, and blast resistance genes. The number of alleles per locus ranged from 2 to 5 with an average of 2.9. The polymorphic information content value per locus ranged from 0.059 (RM537) to 0.755 (RM252) with an average of 0.475. Cluster analysis based on 30 simple sequence repeat markers revealed 5 clusters and also indicated the presence of variability within the rice accessions.The drought QTL qDTY2.1 was found in 56.0% of germplasms and qDTY1.1 was detected in only 6.8% of the germplasms.

Sindhumole *et al.*, (2017) selected only fourteen genotypes out of the 21 rice genotypes, based on seedling vigour index and used them for molecular characterization. DNA extraction of samples was performed by CTAB method followed by PCR amplification with two specific SSR markers, viz., RM103 and RM212. Monomorphic bands were observed with RM103 for all the

fourteen genotypes; hence it cannot be considered as a suitable marker for drought tolerance in these genotypes. Polymorphic bands were observed in the studied genotypes for the marker RM212.

2.2.2 Salt Tolerance

Salinity is the secondmost widespread soil problem in rice growing countries after drought and isconsideredas a serious limitation to increase rice production worldwide. It causes yield reduction and also shrinks caloric and nutritional potential of agricultural products (Yokoi *et al.*, 2002) causing leaf injury or death of plants, thus exceeding the capacity of salt compartmentalization in cytoplasm (Munns *et al.*, 2006). Quijano-Guerta and Kirk, (2002) reported that the cheapest and easiest way to address the problem of salinity is through the development of a salt tolerant variety. For this, the foremost step is to screen the existing germplasms of paddy to identify the potential breeding materials. Screening at field level proved to be difficult due to soil heterogeneity, climatic factors and other environmental factors which may influence the physiological processes. Hence, screening under laboratory conditions is considered to be advantageous over field screening.

Islam *et al.*, (2008) used one hundred polymorphic SSR markers to characterize 21 rice genotypes. These genotypes comprised of nine salt tolerant genotypes including five Pokkali accessions. The materials also included two improved varieties with a major QTL for submergence (Sub1) in Swarna background, i.e. IR82810-407 and IR82809-237. The rest were popular or moderately salt tolerant rice varieties of Bangladesh, India, International Rice Research Institute (IRRI), or Philippines origin. The highest number of alleles (12) were found for RM418 followed

by RM10793 (11), RM3412, RM400, and RM26809 (10). The highest PIC value (0.86) was found for RM10793 followed by RM418 and RM3412 (0.85), RM26809, RM490 and RM287 (0.84), RM16 (0.83), RM493, RM562, and RM253 (0.82). Two main distinct clusters/groups were identified from cluster analysis. One cluster consisted of mostly improved and adapted genotypes while the second cluster had mostly salt tolerant donors with few exceptions.

Titov *et al.*, (2009) used three selected SSR markers already known to be polymorphic, viz., RM7075, RM336 and RM253, to evaluate rice genotypes for salt tolerance. Phenotypic and genotypic evaluation for salinity tolerance was done at the seedling stage.All the tested markers were polymorphic and were able to discriminate salt tolerant genotypes from susceptible. The genotypes having similar banding pattern with Pokkali were considered as salt tolerant. Markers RM7075, RM336 and RM253 identified eight, nine and seven salt tolerant genotypes, respectively. Through phenotypic and genotypic study, three genotypes viz., Pokkali, TNDB-100 and THDB were identified as salt tolerant rice genotypes.

Senguttuvel *et al.,* (2010) studied the genotyping through microsatellite markers for salinity tolerance in a set of twenty five genetically divergent genotypes. The polymorphic SSR markers already reported for major *saltol* QTLs were utilized in their studies found highly reproducible. The association of SSR markers *viz*; RM 23, 493 and 8053 for the trait linked to Na+/K+ ratio was regarded as the most reliable marker for marker-assisted selection to identify salinity tolerance in rice. The study revealed that the selection of genetically diverse and resistant genotypes based on association of Na+/K+ ratio with molecular markers is reliable.

Thomson *et al.*, (2010) analysed 100 SSR markers on 140 IR29/Pokkali recombinant inbred lines (RILs) confirmed the location

of the Saltol QTL on chromosome 1 and identified additional QTLs associated with tolerance.

Aliyu *et al.,* (2011) used QTLs mapping studies for diverse crop species have provided an abundance of DNA marker trait associations. Two F_2 populations with parental designation of IR88399-B and IR88317-B used in validation studies with 4 SALTOL markers on chromosome 1 failed to detect SALTOL QTL in the breeding populations, however, 2 QTLs for leaf diameter was obtained in the SALTOL region with rice Markers RM 493 and RM3412. These QTLs were significantly ($P< 0.05$) associated with salinity tolerance trait.

Islam *et al.*, (2012) genotyped a collection of 115 diverse rice genotypes using tightly linked DNA markers. These markers include five SSRs, RM1287, RM8094, RM3412, RM493 and RM140, and two EST markers, CP6224 and CP03970, on chromosome 1. Among the seven markers, the highest number of alleles (15) was found in RM8094, followed by 10 in RM187, RM3412 and RM493. The polymorphic information content (PIC) values ranged from 0.54 to 0.89. The highest PIC value (0.89) was found for RM8094 followed by RM493 and RM3412 (0.81) and RM1287 and RM140 (0.77). The marker RM8094 was useful for discriminating between tolerant and susceptible genotypes and therefore may be useful for marker-assisted selection.

Iqbal*et al.*, (2015) undertookthis study to assess the genetic diversity among saline treatment and susceptible rice lines using molecular marker (SSR). Twenty two rice lines were used for molecular analysis using three SSR markers: RM1287, RM342 and RM493 to determine salinity tolerance at reproductive stage. Using 3 SSR markers, a total of 25 alleles were detected among the 22 rice lines. The polymorphism information content (PIC) reflects the

diversity allele frequency among the lines, which ranged from 0.59 to 0.88 with an average of 0.74. RM493 was the best marker for identification of genotypes as revealed by PIC values.

Ganie *et al.*, (2016) used eight Saltol quantitative trait locus (QTL) linked simple sequence repeat (SSR) markers of rice (*Oryza sativa* L.) to study the polymorphism of this QTL in 142 diverse rice genotypes that comprised salt tolerant as well as sensitive genotypes. The SSR profiles of the eight markers generated 99 alleles including 20rare alleles and 16 null alleles. RM8094 showed the highest number (13) of alleles followed by RM3412 (12), RM562 (11), RM493 (9) and RM1287 (8) while as, RM10764 and RM10745 showed the lowest number (6) of alleles. Based on the highest number of alleles and PIC value (0.991), we identified RM8094 as suitable marker for discerning salt tolerant genotypes from the sensitive ones. Based upon the haplotype analysis using FL478 as a reference (salt tolerant genotypes containing Saltol QTL), they short listed 68 rice genotypes that may have at least one allele of FL478 haplotype. Further study may confirm that some of these genotypes might have Saltol QTL and can be used as alternative donors in salt tolerant rice breeding programmes.

Mukta *et al.*, (2017) screened 25 germplasms by 3 SSR markers which are RM510, RM585 and RM336. Data were analyzed by POPGENE (version 1.31), Power Marker (version 3.25) and NTSYS-PC (version 2.2). Results: The number of alleles/locus ranged from 10-12, with an average number of alleles of 11/locus and PIC values ranged from a low of 0.8533 (RM336) to a high of 0.8940 (RM585). The average gene diversity of overall SSR loci for the 25 genotypes was 0.8885, ranged from 0.9024-0.8672. Unweighted pair group method of arithmetic means (UPGMA) dendrogram constructed genetic distance produced five distinct

clusters of 25 rice genotypes. FL378 of IRRI was used as check variety. It is confirmed that Holde Gotal, Bazra Muri and Hamai were salt tolerant compared to FL378.They stated that this scientific information could be used for solution of suitable parents, development of salt tolerant rice varieties, gene identification for salt tolerance and genetic diversity analysis.

Hani *et al.*, (2017) screened the four indigenous rice genotypes collected at Coastal Saline Research Centre, Ramanathapuram, Tamil Nadu, India, under saline and non-saline conditions using the microsatellite markers for the identification of salt tolerant genotypes at the seedling and reproductive stages. Two selected SSR markers were used to determine salinity tolerance in rice genotypes.

2.2 Genetic Diversity Assessment

Raghunathachari *et al.,* (2000) analyzed the genetic diversity of Indian scented rice germplasm by using RAPD. According to the study, RAPD could be utilized by breeders for further improvement of scented varieties and the level of genetic variability among the local accessions would be useful for selecting parents in the development of scented rice varieties.

Chaudhury *et al.*, (2001) employed RAPD profile using 58 random decamer primers and reported that cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped all the traditional tall, photosensitive, low yielding, long grained 'basmati' aromatics together while short grained aromatic cultivars, formed a different cluster with high level of average similarity among themselves.

Ni *et al.*, (2002) evaluated the genetic diversity within a diverse collection of rice (*Oryza sativa* L.) accessions, and to determine differences in the patterns of diversity within the two rice

subspecies indica and japonica by means of 111 microsatellites and reported that japonica group showed significantly higher genetic diversity on chromosomes 6 and 7, and considerably lower diversity on chromosome 2. The results suggested that a relatively small number of microsatellite markers could be used for the estimation of genetic diversity and the identification of rice cultivars.

Zeng*et al.*, (2004) studied the genetic diversity within a subset of rice germplasm with different adaptations to saline soils using microsatellite markers.A total of 25 loci were assigned to the 23 miscrosatellite primer pairs. The number of alleles per locus ranged from 2 (in OSR12) to 9 (in OSR1). The PIC values for the microsatellite loci ranged from 0.06 to 0.85 with an average of 0.57. The low PIC values were observed for the primers of RM6 (0.06), RM16 (0.21), RM17 (0.27), RM174 (0.22), and OSR6 (0.39). The PIC valuesof the remaining microsatellite loci were all above 0.50.

Brondani *et al.*, (2005) characterized the allelic diversity of 192 traditional varieties of Brazilian rice using 12 simplesequence repeat (SSR or microsatellite) markers. The germplasm was divided into 39 groups by common namesimilarity. A total of 176 alleles were detected, 30 of which (from 23 accessions) were exclusive. The number of allelesper marker ranged from 6 to 22, with an average of 14.6 alleles per locus.The PIC varied from 0.33 (RM252) to 0.94 (OG106), with an average of 0.73.They identified 16 accessions as a mixtures of pure lines or heterozygous plants. Dendrogram analysis identified six clusters of identical accessions withdifferent common names and just one cluster with identical accessions with the same common name, indicating thatSSR markers are fundamental to determining the genetic relationship between landraces. A subset of 24 landraces, representatives of the 13 similarity groups plus the

11 accessions not grouped, was the most variable set of genotypes analyzed.

Lapitan *et al.*, (2007) assessed the genetic diversity of 24 rice cultivars using 164 SSR markers and detected average alleles of 5.89 per locus with an average PIC value of 0.68. The cluster analysis grouped the cultivars into three clusters with 40 percent similarity. The study revealed that SSR markers could facilitate the classification of cultivars according to their subspecies.

Upadhyay *et al.*, (2011) performed thisstudy with an aim to develop molecular tags for rice lines. A set of 29 accessions of Indian popular rice varieties was subjected to diversity study using simple sequence repeat (SSRs), a total of 87 alleles were produced that were 100% polymorphic. Twelve sets of SSR primers amplified specific alleles in 14 genotypes. The PIC value ranged from 0.57 (RM 313) to 0.98 (RM 442 and RM 163) with average of 0.78 and average genetic similarity of 0.38 was observed among the popular varieties. The maximum similarity of 0.82 was observed between Jayshree and Sarjoo52 and minimum similarity of 0.05, between Jaya and Pusa Basmati 1.Based on ecologies and duration groups showed a maximum similarity of 0.34 between IRM and RSL groups and a minimum similarity of 0.18 between IRE and RSL groups.

Rahman *et al.*, (2011) studied thirty-four microsatellite markers across 21 types of rice to characterize and discriminate among different varieties. The number of alleles per locus ranged from 2 to 11, with an average of 4.18 alleles across 34 loci. A total of 57 rare alleles were detected at 24 loci, whereas 42 unique alleles were detected at 20 loci. The results revealed that 14 rice varieties produced unique alleles that could be used for identification, molecular characterization, and DNA fingerprinting of these varieties. Polymorphic information content (PIC) values ranged from

0.157 to 0.838, with an average of 0.488, which revealed that much variation was present among the studied varieties. The PIC values revealed that RM401 might be the best marker for identification and diversity estimation of rice varieties, followed by RM566, RM3428, RM463, and RM8094 markers. The UPGMA cluster dendrogram created in this study identified five clusters with a similarity coefficient of 0.50. The SSR polymorphism and diversity could likely be attributed to pedigree. In this study, eight SSR markers (RM10713, RM279, RM424, RM6266, RM1155, RM289, RM20224, and RM5371) were identified that produced specific alleles only in the aromatic rice varieties and were useful for varietal identification and DNA fingerprinting of these varieties.

Nguyen *et al.*, (2012) surveyed forty–one accessions with 30 simple sequence repeat (SSR) markers revealing the genetic relationship among the varieties. A total of 192 polymorphic bands were detected. The number of alleles per locus ranged from 3 to 12, with an average of 6.4. Cluster analysis based on genetic similarities grouped the rice accessions into two major groups. These groups were divided into five subgroups. These clusters agree with origin information available on the accessions.

Karmarkar *et al.*, (2012) genotyped 16 common rice lines and one wild rice relative grown in rarh south Bengal were using six polymorphic microsatellite markers associated with saltol QTL mapped on rice chromosome 1. DNA fingerprint profiles identified each of the 17 rice genotypes unequivocally and the pair-wise polymorphism data for the studied genotypes were used to analyze the genetic diversity present within the studied rice lines. The number of alleles per SSLP marker, the size range of the PCR products and the polymorphism information content (PIC) values of each marker were calculated out. A dendrogram was constructed

using the average linkage (within group) and similarity coefficient among the studied genotypes which indicated that a considerable amount of genetic diversity is present and classify the studied genotypes into two major clusters and one minor cluster.

Choudhary *et al.*, (2013) studied genetic diversity in representative sets of high yielding varieties of rice released in India between 1970 and 2010 at molecular level employing hyper variable microsatellite markers. Of 64 rice SSR primer pairs studied, 52 showed polymorphism, when screened in 100 rice genotypes. A total of 184 alleles were identified averaging 3.63 alleles per locus. Cluster analysis clearly grouped the 100 genotypes into their respective decadal periods i.e., 1970s, 1980s, 1990s and 2000s.

Yadav*et al.*, (2013) evaluated a set of 88 rice accessions that included landraces, farmer's varieties and popular Basmati lines for agronomic traits and molecular diversity. The random set of SSR markers included 50 diversity panel markers developed under IRRI's Generation Challenge Programme (GCP) and the trait-linked/gene based markers comprised of 50 SSR markers reportedly linked to yield and related components. The molecular diversity based grouping indicated that varieties from a common centre were genetically similar, with few exceptions. The trait-linked markers gave an average genetic dissimilarity of 0.45 as against that of 0.37 by random markers, along with an average polymorphic information constant value of 0.48 and 0.41 respectively.

Gholizadeh and Navabpour (2014) evaluated 29 genotypes consisting land races, pure and improved lines using simple sequence repeat (SSR) markers. A total of 30 SSR primers were used to amplify some part of rice genome in germplasms, the PIC values ranged from 0.07 (RM 340) to 0.71 (RM 7426) with an average of 0.45. The results showed a total number of 106 amplified bands.

Among them, the primer RM7426 showed the highest number alleles while the lowest was observed for RM340 primer. Average number of observed alleles in total genotypes was 3.53. The lowest PIC value was observed in RM445, RM466, RM3345, and RM7424 primers and the highest PIC value was observed in RM7426, RM1337, RM47, and RM5430 primers. PCA components explained 84.40% of variation. The clustering patterns of the genotypes were assigned into three clusters based on their response to salinity and morpho-physiological characteristics.

Hoque *et al.*, (2014) evaluated diversity at molecular level among thirty rice genotypes, selected based on earliness and morphometric diversity through five SSR markers associated with days to heading. Three primers viz., RM147, RM167 and RM215 showed polymorphism for growth duration related traits. A total of 17 alleles were detected among the 30 rice genotypes with an average of 5.66 alleles per locus. Polymorphism Information Content (PIC) ranged from0.356 to 0.798 with an average of 0.543. A dendrogram based on total microsatellite polymorphism grouped 30 genotypes into four major clusters at 0.39 similarity coefficient differentiating early maturing genotypes from others.

Mohiuddin *et al.*, (2014) assessedgenetic diversity using a set of 27 SSR markers which generated 321 polymorphic alleles. Polymorphism information content (PIC) values ranged between 0.6806 (RM 11) and 0.9416 (RM 474) with an average of 0.8414. Dendrogram was constructed based on the Nei's genetic distance calculated from 27 SSR markers generated from the 30 rice accessions. Genetic similarity analysis using UPGMA, all 30 accessions were grouped into 6 clusters based on SSR markers' data at a cut-off similarity coefficient 0.17%.

Nachimuthu *et al.*, (2015) genotyped a set of 192 diverse rice germplasm lines using 61 genome wide SSR markers to assess the molecular genetic diversity and genetic relatedness. Genotyping of 192 rice lines using 61 SSRs produced a total of 205 alleles with the PIC value of 0.756. Among these 205 alleles, 5 % were considered as rare (showed an allele frequency of<5 %). The number of alleles per loci varied from 2 to 7 with an average of 3 alleles per locus. The highest number of alleles were detected for the loci RM316 (7) and the lowest was detected for a group of markers viz., RM171, RM284, RM455, RM514, RM277, RM 5795, Hv SSR0247, RM 559, RM416 and RM1227. The average PIC valuewas found to be 0.468. The highest genetic diversity is explained by the landraces included in this study with the mean PIC value of 0.416. PIC values ranged between 0.146 for RM17616 to 0.756 for RM316. Population structure analysis using model based and distance based approaches revealed that the germplasm lines were grouped into two distinct subgroups.

Chungada *et al.*, (2016) studied genetic diversity among 55 rice genotypes using SSR markers, which showed the higher level of polymorphism in rice germplasm. A total of 231 loci were generated by 18 primers. Each primer thus produced on an average 12.83 loci in the size ranging from 169.5 bp to 317.22 bp in the 55 rice genotypes in relation to diversity assessment. UPGMA grouped 55 rice genotypes into two main clusters which were further divided into two sub-clusters.

Singh *et al.*, (2016) genotyped a set of 729 Indian rice varieties using 36 Hv SSR markers to assess the genetic diversity and genetic relationship. A total of 112 alleles were amplified with an average of 3.11 alleles per locus with mean Polymorphic Information Content (PIC) value of 0.29. Cluster analysis grouped

these varieties into two clusters whereas the model based population structure divided them into three populations. PIC showed increasing trend from 1940 to 2005, thereafter values for both the parameters showed decreasing trend between years 2006-2013.

Krupa *et al.*, (2017) evaluated five rice genotypes for genetic diversity. Upon PCR amplification the alleles were separated on Agarose Gel Electrophoresis system. Initial polymorphism detection was conducted using twenty primer pairs distributed on five rice chromosomes. A total of 65 alleles were detected with an average of 3.25 alleles per locus. The polymorphism information content (PIC) reflections of alleles diversity frequency among the varieties, which is ranged from 0.215 to 0.791, with an average of 0.493. RM 260 was found as the best marker for identification of genotypes as revealed by PIC values.

Bhagwat *et al.*, (2017) evaluated genetic diversity among 50 rice genotypes using total 18 SSR primers, out of which, 14 primers amplified and showed the polymorphism with 357 loci. Each primer thus produced on an average 25.5 loci in the size ranging from 164.28 bp to 292.85 bp in the 50 rice genotypes in relation to diversity assessment. The PIC values of primers ranged from 0.14 in SSR primer RM6775 to 0.63 in SSR primer RM302 with an average PIC value of 0.41. UPGMA grouped 50 rice genotypes into two main clusters, which were further divided into two subclusters.

CHAPTER III

MATERIALS AND METHODS

The detailed information regarding the materials used and methods followed during the course of the present investigation is mentioned in this chapter. The investigations were carried out in the laboratory of Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.) during the period of 2017-2019.

3.1 Source of genotypes

For the present study, the seeds of 46 rice genotypes were obtained from Regional Agricultural Research Station, Karjat Dist-Raigad. The DNA was extracted from the 10-15 days old seedling of the genotypes which were grown in the pots and used for further study. The list of Genotypes is given in Table 3.1.

Table No. 3.1. Details of genotypes used in the study.

Sr.no.	Genotypes	Sr.no.	Genotypes
1.	RP Bio 189	24.	IR-22896-225
2.	IET-23557-135	25.	SLR 51214
3.	IET 13840-RP-66-67	26.	IR 664
4.	IR-44	27.	IR-32
5.	IET-8866	28.	IET-23537-1351
6.	IET-8580	29.	Gujrat-102
7.	IR 56456-4-2-3	30.	IR-60819-34-2-1
8.	IRAT-360	31.	IR 625-17-11-107
9.	CR-57 MP-1523	32.	Narmada
10.	HS 47	33.	JGl-2855
11.	IR-88-30-21-2	34.	IR 56381-139-2-2
12.	IR-1544-238-2-3	35.	Karjat-2-32
13.	HUR FG-78	36.	KJT 11-1-26-25-23
14.	IR 665575-56-1-3-19	37.	HKR 2002-81
15.	IR-60919-150-3-3	38.	DBS-13-3-47-A9
16.	IR-62036-223-3-3	39.	GVT-3514
17.	IR-35	40.	IR-50
18.	IR60997-16-2-3-22R	41.	CN-127
19.	IR-663879-195-2-2	42.	IR-293-41-41-1
20.	Kon-23	43.	Paras Sona
21.	KJT-23	44.	KJT-1-1-21-3-19
22.	IR 61614-38-19-3-2	45.	Gujrat-103
23.	Kasturi	46.	CN-1383-1-50

3.2. Extraction of genomic DNA

3.2.1. Plant material

For the present experimental study, all 46 genotypes of rice were sown in the pots and kept in greenhouse. The leaf samples were collected from 10 days old seedlings for the extraction of genomic DNA.

3.2.2 Preparation of stock solutions for reagents and buffers for DNA extraction.

The reagents and buffers for DNA isolation were prepared as per Sambrook Maniatis (1989). The composition and procedure for preparation of various stock solutions and buffers are given in the Table 3.2.

Table No. 3.2: Preparation of solutions for DNA extraction

a) Extraction buffer			
Extraction buffer (EB) stock solutions:	**Sr. No.**	**Chemicals**	**Quantity required for 100 ml.(g)**
	1.	200 mMTris-HCl,	2.423
	2.	25 mM EDTA	0.831
	3.	250 mM NaCl	1.461
Other Components of Extraction buffer:	**Sr. No**.	**Chemicals**	**Quantity required for 10 ml of EB(g)**
	1.	0.5M Glucose	0.9
	2.	0.5% SDS	0.05
	3.	3% PVP	0.3
	4.	0.4% Sodium bisulphate	0.04

	5.	5% Sarcosyl	500 µl
b) 70% Ethanol	**Sr. No.**	**Chemicals**	**Quantity 100ml**
	1.	Absolute ethyl alcohol	70ml
	2.	Distilled water	30ml
c) Chloroform: Isoamyl alcohol (24:1)	**Sr. No.**	**Chemicals**	**Quantity 100ml**
	1.	Chloroform	96ml
	2.	Isoamyl alcohol	4ml
d) TAE Buffer (50X) Add 50 times double distilled water to 50X Buffer to make 1X- TAE buffer.	**Sr. No.**	**Chemicals**	**Quantity 1000ml**
	1.	TrisHCl	242.5 g
	2.	Glacial acetic acid	57.1 ml
	3.	0.5 M EDTA (pH 8.0)	200ml
e) Ethidium bromide Final concentration was thus made 10 mg/ml. It was then stored in dark bottle at room temperature.	**Sr. No.**	**Chemicals**	**Quantity**
	1.	Ethidium bromide	0.1 g
	2.	Distilled water	10 ml

3.2.3 Procedure for extraction of genomic DNA

The DNA was isolated by following the protocol of Edwards *et al.*, 1991 with the slight modifications of buffer composition and concentration. The young newly flushing 10 days old leaves were collected and sterilized with 70% ethanol to avoid the

contamination. The extraction of genomic DNA was done using the following protocol.

- Leaf tissue (100 mg) were collected and kept in 1.5 ml eppendorf tube which leads to ensure uniform size of sample.
- Collected tissue was macerated by micro pestle at room temperature without buffer for 15 sec.
- Extraction buffer (500 µl) was added and leaf tissue macerated gently for few seconds and kept in hot water bath for 45 minutes at 65^0C.
- The sample was cooled down at room temperature and centrifuged at 10000 rpm for 10 minutes.
- Aqueous layer was transferred to fresh eppendorf tube and 200 µl of CIA i.e Chloroform-Isoamyl Alcohol (24:1) was added and mixed by gentle inversion for 5-6 times. The contents were then centrifuged at 8000 rpm for 10 minutes.
- Supernatant was mixed with double volume of chilled Iso-propanol and incubated at -20^0C for overnight.
- On the next day the solution was centrifuged at 8000 rpm for 10 minutes and pellet was collected.
- Pellet was washed with 100 µl of 70 per cent ethanol followed by centrifugation at 8000 rpm for 10 minutes.
- Pellet was dried and re-suspended in 50 µl of 1x TE buffer.
- Incubated at 37°C in a water bath for 30 minutes and stored at -20°C till further use.

3.2.4 DNA purification

Purification of DNA in samples was done to remove RNA, proteins and polysaccharides which were the major contaminants.

RNA was removed by RNase treatment. RNase was added to the DNA sample @100 µg ml^{-1} and incubated at 37°C for 1 hour.

3.2.5 DNA quantification through agarose gel electrophoresis

Concentration of DNA in the sample was determined after agarose gel electrophoresis with standard DNA i.e. uncut lambda DNA on 0.8 per cent agarose gel and by comparison of the intensity of staining with Ethidium Bromide.

3.3 Screening of rice genotypes using SSR molecular markers:

The rice genotypes were screened for biotic and abiotic resistance using 18 SSR primers over different chromosomes for different biotic and abiotic stress resistance genes. The SSR primers were obtained from the GRAMENE database (Release #39, http://archive.gramene.org/markers/microsat/all-ssr.tab, McCouch *et al.*, 2002). Details of the primers used are appended in Table 3.4.

3.3.1 Requirements

The standard requirements for the PCR amplification are given in detail in Table 3.3.

Table 3.3: PCR Stock Solutions and their Sources

Stock solutions	Source
Taq. assay buffer	M/s Bangalore Genei Pvt. Ltd., Bangalore
$MgCl_2$	M/s Bangalore Genei Pvt. Ltd., Bangalore
dNTPs	M/s Bangalore Genei Pvt. Ltd., Bangalore
Primers (Fw./Rev.)	Bio-resource Biotech Pvt. Ltd., Pune.
Taq. DNA Polymerase	M/s Bangalore Genei Pvt. Ltd., Bangalore

Thermal cycler: Eppendorf, Master cycler gradient supplied by Eppendorf gradient, 2231, Hamburg Germany was used for cyclic amplification of DNA.

Table No. 3.4: List of SSR primers with their sequences and linked gene.

Sr. No	Primer	Sequence of primers	Chromosome No.	Linked gene
1.	RM 140	Forward TGCCTCTTCCCTGGCTCCCCTG Reverse GGCATGCCGAATGAAATGCATG	1	*Saltol*
2.	RM 1287	Forward CCATTTGCAGTATGAACCATGC Reverse ATCATGCAATAGCCGGTAGAGG	1	*Saltol*
3.	RM 562	Forward GGAAAGGAAGAATCAGACACAGAGC Reverse GTACCGTTCCTTTCGTCACTTCC	1	*Saltol*
4.	RM 3412	Forward AAAGCAGGTTTTCCTCCTCC Reverse CCCATGTGCAATGTGTCTTC	1	*Saltol*
5.	RM 6775	Forward AATTGATGCAGGTTCAGCAAGC Reverse GGAAATGTGGTTGAGAGTTGAGAGC	6	*Bph25*
6.	RM 309	Forward CACGCACCTTTCTGGCTTTCAGC Reverse AGCAACCTCCGACGGGAGAAGG	12	*Bph25*
7.	RM 5479	Forward CTCACCATAGCAATCTCCTGTGC Reverse ACTTCGTTCACTTGCATCATGG	12	*Bph25*
8.	RM 5926	Forward ATATACTGTAGGTCCATCCA Reverse AGATAGTATAGCGTAGCAGC	11	*Pi1*
9.	RM 8225	Forward GCGTGTTCAGAAATTAGGATACGG Reverse GATCTCGCCACGTAATTGTTGC	6	*Pi-z*
10.	RM 206	Forward ATCGATCCGTATGGGTTCTAGC Reverse GTCCATGTAGCCAATCTTATGTGG	11	*Pi-kh*
11.	RM 212	Forward AAGGTCAAGGAAACAGGGACTGG Reverse AGCCACGAATTCCACTTTCAGC	1	*Dr*
12.	RM 302	Forward TGCAGGTAGAAACTTGAAGC Reverse AGTGGATGTTAGGTGTAACAGG	1	*Dr*
13.	RM 3825	Forward CCACTAGCAGATGATCACAGACG Reverse GAGCACCTCATAAGGGTTTCAGC	1	*Dr*
14.	RM 1233	Forward ATGGGCACGTGTAATTCATTCG Reverse ATCCTCGAAAGTAGGAGTAGGAAAG	11	*Pi-1*
15.	pTA2 48	Forward AGACGCGGAAGGGTGGTTCCCGGA Reverse AGACCGGGTAAT CGAAAGATGAAA	11	*Xa21*
16.	RM 122	Forward GAGTCGATGTAATGTCATCAGTGC Reverse GAAGGAGGTATCGCTTTGTTGGAC	5	*Xa5*
17.	RM 22709	Forward CGCGTGGGCGAGACTAATCG Reverse CCTTGACTCCGAGGATTCATTGTCC	8	*Gm8*
18.	RM 547	Forward TTGTCAAGATCATCCTCGTAGC Reverse GTCATTCTGCAACCTGAGATCC	8	*Gm4*

3.3.2. Preparation of master mixture

Since the pipetting of small volumes is difficult and often inaccurate, a master mix was prepared where constituents common to all the reactions were combined in one tube multiplying the volume for one reaction with total number of samples. Later, the appropriate amount of master mix was dispensed to each tube and template DNA was added separately in each tube.

Table No. 3.5: Master mixture for polymerase chain reaction (PCR).

Components	Stock concentration	Vol. for one reaction/10 µl
Taq buffer	10X	1.25µl
$MgCl_2$	25 mM	0.5µl
dNTP mix	10 mM	0.5µl
Primer- F	25picomole/µl	0.5µl
Primer- R	25picomole/µl	0.5µl
Taq DNA polymerase	3 U/µl	0.5µl
Template DNA	30-50 ng	1.0µl
Sterile Distilled water	-	5.25 µl
Total		10 µl

3.3.3 Thermal cycling

- Sterile micro centrifuge tubes were numbered from 1 to 46.
- 1.0 µl of template DNA from individual rice genotype was added to each tube.
- 9 µl of master mix was added to each tube and was given short spin to mix the contents.
- The tubes were placed in the thermal cycler for 30 cycles of PCR. Samples were held at 4^0C in the thermal cycler until the contents were loaded on to the gel for electrophoresis.

Table No. 3.6: Thermo Profile

Steps	Temp. (°C)	Period (min.)
Initial Denaturation	94°C	5 min.
Denaturation	94°C	20sec
Annealing	53°C - 63°C*	30sec
Extension	72°C	45 sec
Final extension	72°C	7 min.
Hold	4°C	-

(*Note: - Annealing temperature based on Tm value of each primer)

3.3.4. Optimization of annealing temperature for gene specific primers by PCR amplification studies

Gradient PCR amplification of gene specific primers was carried out so as to determine the annealing temperature of each primer. The PCR programme was set in Thermal Cycler Eppendorf, Master Cycler Gradient, made in Germany.

The annealing temperature of each primer was standardized based on Tm value of each forward and reverse primer and standardized temperature was used for further amplification (Table 3.7).

Table No. 3.7: Annealing temperatures of SSR markers used in the study.

Sr. No.	Primer	Tm value (°C)		Temperature range (°C)	Standardized Annealing temperature (°C)
		Reverse primer	Forward primer		
1.	RM 140	57.9	65.6	55-68	62.1
2.	RM 1287	56.7	54.9	50-60	56.7
3.	RM 562	57.6	57.5	52-64	62.5
4.	RM 3412	55.1	54.3	53-62	62.8
5.	RM 6775	57.9	56.6	52-62	58.7
6.	RM 309	63.3	61.2	58-68	66.9
7.	RM 5479	55.8	57.7	53-63	59.7

8.	RM 5926	49.3	51.0	50-60	58.0
9.	RM 8225	56.7	55.8	52-62	58.7
10.	RM 206	55.6	56.1	54-64	58.1
11.	RM 212	58.1	59.0	55-65	62.9
12.	RM 302	54.2	52.9	55-65	61.7
13.	RM 3825	57.9	57.1	53-64	62.8
14.	RM 1233	57.7	55.9	52-62	56.1
15.	pTA 248	56.1	58.2	50-60	54.0
16.	RM 122	61.0	59.6	57-68	61.5
17.	RM 22709	59.1	60.6	58-68	65.9
18.	RM 547	55.6	54.4	52-62	56.1

3.3.5. Separation of amplified product by agarose gel electrophoresis

- **Requirements:**

a) Electrophoresis unit (Gel casting tray, gel comb, power pack)
b) Gel documentation system
c) Agarose
d) Tracking dye (Bromophenol blue)
e) Ethidium Bromide (1 μg ml^{-1})
f) 50 x TAE buffer.
g) DNA ladder

- **Procedure**

The amplified products in SSR reaction were separated by electrophoresis in 2 per cent agarose gel (Merck, India) containing

ethidium bromide in 1x TAE Buffer (pH 8.0) and separation was carried out by applying constant voltage of 70 volts for 100 mins.

3.3.6 Photography and gel documentation

The gels were photographed under UV light using Pentax K 312 nm camera. The images of gel was taken by the documentation systems (Uvi-Tech, Fire reader, Cambridge, England) and saved in computer for further analysis.

3.3.7 Molecular screening of genotypes

The gels were carefully studied and amplicons which occurred only once for a particular genotype were marked which constituted the band for that particular genotype. Additionally, the band fragments present in two varieties were also marked, which in combination with other bands generated with other primers would constitute the screenings.

3.4 Scoring and Data Analysis

Each amplification product as a dominant expression was considered as SSR marker and was scored across all the samples. Bands were scored as present (+/1) or absent (-/0). The size of each allele was determined by running simultaneously a DNA ladder by using a software (Uvitec, Fire-reader software version 15.12). The data was used for similarity based analysis using the programme MVSP-A (Multivariate Statistical Package_5785 Version 3.1). Similarity coefficients were used to construct UPGMA (unweighted pair group method with average) to generate dendrogram.

Distance matrix and dendrogram was constructed based on diversity coefficient generated from pooled data by using Unweighted Pair Group Method of Arithmetic Means (UPGMA), a computer programme for distance estimation.

The polymorphism percentage of the obtained bands was calculated by using following formula,

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \text{ X } 100$$

3.5 Polymorphism Information Content

Polymorphism Information Content (PIC) value were calculated as per formula developed by Powell *et al.,* (1996)

$$\mathbf{PIC = 1-\sum P_{ij}^{2}}$$

Where,

P_{ij} is the frequency of i^{th} and j^{th} locus, summed across the entire locus over all lines.

PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency) were estimated for each profile generated across 46 rice genotypes.

CHAPTER IV

EXPERIMENTAL RESULTS

The present study entitled "Molecular analysis of rice (*Oryza sativa* L.) germplasm" was carried out with view to identify trait specific genotypes tolerant to specific stress condition. The experimental results obtained in this study are presented in this chapter under different headings.

In this study, trait specific 18 SSR markers were used to screen the set of 46 rice genotypes for various biotic and abiotic stresses. The polymorphic information produced by these markers between different rice

genotypes helps us in determining the presence or absence of resistance linked alleles in the given genotypes.

4.1 DNA isolation:

The standard procedure of DNA isolation along with slight modification of buffer components and their quality were employed by agarose gel electrophoresis. The good quality and desired quantity of DNA was obtained (Plate No.1).

4.2 Standardization of annealing temperature:

The annealing temperature standardized by earlier worker has been taken as reference for amplification of SSR primers. (Chungada *et al.*, 2016) (Table No. 3.7).

4.3 Molecular Screening:

4.3.1 Biotic stresses:

4.3.1.1 Blast (*Magnaporthe oryzae*)

In this study molecular screening and genetic diversities of major rice blast resistance genes were determined in 46 rice germplasms or accessions using simple sequence repeat (SSR) markers. Four primers i.e. RM8225, RM206, RM5926, RM1233 were used to identify blast resistance linked alleles in 46 rice genotypes. Marker RM 5926 specific to gene *Pi-z* indicated presence of resistance linked alleles (of 176bp) in the given genotypes **(Plate No. 2)**; RP Bio 189, IET-8580, IR-1544-238-2-3, IR 665575-56-1-3-19, IR-60819-34-2-1, IR-35, IR-663879-195-2-2, Kon-23, Kasturi, IET-23537-1351, IR-60919-150-3-3. **(Table No. 4.1)**. Marker RM-8225 specific to gene *Pi-1*(at 121bp), Marker RM1233 specific to gene

Pi1(at 170bp) and RM206 specific to gene *Pi-kh*(at 140bp) indicated absence of resistance linked alleles in all the genotypes **(Table No. 4.1) (Plate No. 3)**.

4.3.1.2 Bacterial Blight (*Xanthomonas oryzae pv. oryzae*)

The 46 rice accessions were screened to determine resistance status for BLB-resistance genes viz., Xa5 and Xa21 by using PCR based microsatellite markers RM 122 and pTA248 respectively. **(Table No. 4.2)**. Screening for the genotypes by the Xa5 gene using the microsatellite marker RM122, revealed presence of resistance linked alleles (of 240-250bp) in almost all the genotypes **(Plate No. 4)**; RP Bio 189, IET-23557-135, IET 13840-RP-66-67, IR-44, IET-8866, CN-1383-1-50, IR 56456-4-2-3, IRAT-360, CR-57 MP-1523, HS 47, IR-88-30-21-2, IR-1544-238-2-3, HUR FG-78, IR 665575-56-1-3-19, IR-60919-150-3-3, IR-62036-223-3-3, IR-35, IR60997-16-2-3-22R, IR-663879-195-2-2, Kon-23, KJT-23, IR 61614-38-19-3-2, Kasturi, IR-22896-225, SLR 51214, IR 664, IR-32, IET-23537-1351, Gujrat-102, IR-60819-34-2-1, IR 625-17-11-107, Narmada, JGl-2855, IR 56381-139-2-2, Karjat-2-32, KJT 11-1-26-25-23, HKR 2002-81, DBS-13-3-47-A9, GVT-3514, IR-50, CN-127, IR-293-41-41-1, Paras Sona, KJT-1-1-21-3-19, Gujrat-103, IET-8580. Marker pTA 248 (at 1040bp) specific to geneXa21 indicated absence of resistance linked alleles in all the genotypes **(Table No.4.2)**.

4.3.1.3 Brown Plant Hopper (*Nilaparvata lugens Stal.)*

The 46 germplasms of rice were evaluated for Brown Plant hopper resistance using the SSR markers viz., RM5479, RM6775, and RM309 **(Table No. 4.3)**. Marker RM 6775 specific to gene *Bph25* indicated presence of resistance linked alleles (of 192bp) in the genotype **(Plate No. 5).** KJT-1-1-21-3-19. Marker RM309 and RM5479 specific to gene *Bph26* (at 152bp) indicated absence of resistance linked alleles in all the genotypes. **(Table No. 4.3)**.

4.3.1.4 Gall Midge (*Orseolia oryzae)*

In the present study, the SSR markers RM547 and RM22709 were used to detect the absence and presence of resistance linked alleles for biotypes *Gm4*(270bp) and *Gm8*(170bp) respectively. **(Table No. 4.4)**. Marker RM547 specific to gene *Gm4*(at 270bp) indicated absence of resistance linked alleles in all genotypes; and Marker RM22709 specific to gene *Gm8*(at 170bp) indicated presence of resistance linked alleles inthe genotypes; KJT 11-1-26-25-23, IR-22896-225. **(Plate No. 6).**

4.3.2 Abiotic stresses:

4.3.2.1 Salt tolerance:

Salt tolerance linked markers used in this study were, viz., RM140, RM1287, RM562 and RM 3412 **(Table No. 4.5)**. Saltol QTL detected by the marker RM140 (at 260bp) was observed in the genotypes; RP Bio 189, IET-8866, DBS-13-3-47-A9, SLR 51214, Gujrat-102, CR-57 MP-1523. Saltol QTL detected by the marker RM1287 (at 160bp) was absent in all genotypes. Saltol QTL detected by the marker RM3412 (at 211bp) was observed in the genotypes; IET 13840-RP-66-67, HKR 2002-81, IR 664, KJT 11-1-26-25-23, IR 61614-38-19-3-2. **(Plate No.7).** Marker RM562 (at 243bp) indicated absence of resistance linked alleles in all the genotypes **(Table No. 4.5)**.

4.3.2.2Drought tolerance:

Drought tolerance linked markers used in this study were, viz., RM212, RM302 and RM3825 **(Table No.4.6)**. Resistance linked alleles detected by the marker RM 212 (at 135 bp) were absent in the genotypes **(Plate No.8)**. Markers RM302 specific to gene(at 140bp)indicated presence of resistance linked alleles in the genotype; IR 56381-139-2-2and RM3825 specific to gene(at 147bp) indicated presence of resistance linked alleles in the genotypes; IR 56381-139-2-2, Paras Sona, IR-50, IR-293-41-41-1, KJT 11-1-26-25-23. **(Table No. 4.6)**.

Table No. 4.1: Amplification of gene specific alleles with linked SSR markers for Blast resistance

Trait:		**RM- 8225**	**RM-5926**	**RM-206**	**RM-1233**
Sr. No	**Genotypes**	***piz(221bp)***	***Pi1(176bp)***	***Pikh(140bp)***	***Pi1(170bp)***
1.	RP Bio 189	-	+	-	-
2.	IET-23557-135	-	-	-	-
3.	IET 13840-RP-66-67	-	-	-	-
4.	IR-44	-	-	-	-
5.	IET-8866	-	-	-	-
6.	IET-8580	-	+	-	-
7.	IR 56456-4-2-3	-	-	-	-
8.	IRAT-360	-	-	-	-
9.	CR-57 MP-1523	-	-	-	-
10.	HS 47	-	-	-	-
11.	IR-88-30-21-2	-	-	-	-
12.	IR-1544-238-2-3	-	+	-	-
13.	HUR FG-78	-	-	-	-
14.	IR 665575-56-1-3-19	-	+	-	-
15.	IR-60919-150-3-3	-	+	-	-
16.	IR-62036-223-3-3	-	-	-	-
17.	IR-35	-	+	-	-
18.	IR60997-16-2-3-22R	-	-	-	-
19.	IR-663879-195-2-2	-	+	-	-
20.	Kon-23	-	+	-	-
21.	KJT-23	-	-	-	-
22.	IR 61614-38-19-3-2	-	-	-	-
23.	Kasturi	-	+	-	-
24.	IR-22896-225	-	-	-	-
25.	SLR 51214	-	-	-	-
26.	IR 664	-	-	-	-
27.	IR-32	-	-	-	-
28.	IET-23537-1351	-	+	-	-
29.	Gujrat-102	-	-	-	-
30.	IR-60819-34-2-1	-	+	-	-
31.	IR 625-17-11-107	-	-	-	-
32.	Narmada	-	-	-	-
33.	JGl-2855	-	-	-	-
34.	IR 56381-139-2-2	-	-	-	-
35.	Karjat-2-32	-	-	-	-
36.	KJT 11-1-26-25-23	-	-	-	-
37.	HKR 2002-81	-	-	-	-
38.	DBS-13-3-47-A9	-	-	-	-
39.	GVT-3514	-	-	-	-
40.	IR-50	-	-	-	-
41.	CN-127	-	-	-	-
42.	IR-293-41-41-1	-	-	-	-
43.	ParasSona	-	-	-	-
44.	KJT-1-1-21-3-19	-	-	-	-
45.	Gujrat-103	-	-	-	-
46.	CN-1383-1-50	-	-	-	-

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele for the marker).

Table No. 4.2: Amplification of gene specific alleles with linked SSR markers for Bacterial blight resistance

Trait		RM-122	pTA-248
Sr. No	**Genotypes**	***Xa5(240-250bp)***	***Xa21 (1040bp)***
1.	RP Bio 189	+	-
2.	IET-23557-135	+	-
3.	IET 13840-RP-66-67	+	-
4.	IR-44	+	-
5.	IET-8866	+	-
6.	IET-8580	+	-
7.	IR 56456-4-2-3	+	-
8.	IRAT-360	+	-
9.	CR-57 MP-1523	+	-
10.	HS 47	+	-
11.	IR-88-30-21-2	+	-
12.	IR-1544-238-2-3	+	-
13.	HUR FG-78	+	-
14.	IR 665575-56-1-3-19	+	-
15.	IR-60919-150-3-3	+	-
16.	IR-62036-223-3-3	+	-
17.	IR-35	+	-
18.	IR60997-16-2-3-22R	+	-
19.	IR-663879-195-2-2	+	-
20.	Kon-23	+	-
21.	KJT-23	+	-
22.	IR 61614-38-19-3-2	+	-
23.	Kasturi	+	-
24.	IR-22896-225	+	-
25.	SLR 51214	+	-
26.	IR 664	+	-
27.	IR-32	+	-
28.	IET-23537-1351	+	-
29.	Gujrat-102	+	-
30.	IR-60819-34-2-1	+	-
31.	IR 625-17-11-107	+	-
32.	Narmada	+	-
33.	JGl-2855	+	-
34.	IR 56381-139-2-2	+	-
35.	Karjat-2-32	+	-
36.	KJT 11-1-26-25-23	+	-
37.	HKR 2002-81	+	-
38.	DBS-13-3-47-A9	+	-
39.	GVT-3514	+	-
40.	IR-50	+	-
41.	CN-127	+	-
42.	IR-293-41-41-1	+	-
43.	ParasSona	+	-
44.	KJT-1-1-21-3-19	+	-
45.	Gujrat-103	+	-
46.	CN-1383-1-50	+	-

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele for the marker)

Table No. 4.3. Amplification of gene specific alleles with linked SSR markers for Brown plant hopper resistance

Trait		RM-6775	RM-309	RM-5479
Sr. No	Genotypes	*Bph25(192bp)*	*Bph26 (152bp)*	*Bph26 (152bp)*
1.	RP Bio 189	-	-	-
2.	IET-23557-135	-	-	-
3.	IET 13840-RP-66-67	-	-	-
4.	IR-44	-	-	-
5.	IET-8866	-	-	-
6.	IET-8580	-	-	-
7.	IR 56456-4-2-3	-	-	-
8.	IRAT-360	-	-	-
9.	CR-57 MP-1523	-	-	-
10.	HS 47	-	-	-
11.	IR-88-30-21-2	-	-	-
12.	IR-1544-238-2-3	-	-	-
13.	HUR FG-78	-	-	-
14.	IR 665575-56-1-3-19	-	-	-
15.	IR-60919-150-3-3	-	-	-
16.	IR-62036-223-3-3	-	-	-
17.	IR-35	-	-	-
18.	IR60997-16-2-3-22R	-	-	-
19.	IR-663879-195-2-2	-	-	-
20.	Kon-23	-	-	-
21.	KJT-23	-	-	-
22.	IR 61614-38-19-3-2	-	-	-
23.	Kasturi	-	-	-
24.	IR-22896-225	-	-	-
25.	SLR 51214	-	-	-
26.	IR 664	-	-	-
27.	IR-32	-	-	-
28.	IET-23537-1351	-	-	-
29.	Gujrat-102	-	-	-
30.	IR-60819-34-2-1	-	-	-
31.	IR 625-17-11-107	-	-	-
32.	Narmada	-	-	-
33.	JGl-2855	-	-	-
34.	IR 56381-139-2-2	-	-	-
35.	Karjat-2-32	-	-	-
36.	KJT 11-1-26-25-23	-	-	-
37.	HKR 2002-81	-	-	-
38.	DBS-13-3-47-A9	-	-	-
39.	GVT-3514	-	-	-
40.	IR-50	-	-	-
41.	CN-127	-	-	-
42.	IR-293-41-41-1	-	-	-
43.	ParasSona	-	-	-
44.	KJT-1-1-21-3-19	+	-	-
45.	Gujrat-103	-	-	-
46.	CN-1383-1-50	-	-	-

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele for the marker).

Table No. 4.4: Amplification of gene specific alleles with linked SSR markers for Gall midge resistance

Trait:		***RM-547***	***RM-22709***
Sr. No	**Genotypes**	***Gm4(270bp)***	***Gm8(170bp)***
1.	RP Bio 189	-	-
2.	IET-23557-135	-	-
3.	IET 13840-RP-66-67	-	-
4.	IR-44	-	-
5.	IET-8866	-	-
6.	IET-8580	-	-
7.	IR 56456-4-2-3	-	-
8.	IRAT-360	-	-
9.	CR-57 MP-1523	-	-
10.	HS 47	-	-
11.	IR-88-30-21-2	-	-
12.	IR-1544-238-2-3	-	-
13.	HUR FG-78	-	-
14.	IR 665575-56-1-3-19	-	-
15.	IR-60919-150-3-3	-	-
16.	IR-62036-223-3-3	-	-
17.	IR-35	-	-
18.	IR60997-16-2-3-22R	-	-
19.	IR-663879-195-2-2	-	-
20.	Kon-23	-	-
21.	KJT-23	-	-
22.	IR 61614-38-19-3-2	-	-
23.	Kasturi	-	-
24.	IR-22896-225	-	+
25.	SLR 51214	-	-
26.	IR 664	-	-
27.	IR-32	-	-
28.	IET-23537-1351	-	-
29.	Gujrat-102	-	-
30.	IR-60819-34-2-1	-	-
31.	IR 625-17-11-107	-	-
32.	Narmada	-	-
33.	JGl-2855	-	-
34.	IR 56381-139-2-2	-	-
35.	Karjat-2-32	-	-
36.	KJT 11-1-26-25-23	-	+
37.	HKR 2002-81	-	-
38.	DBS-13-3-47-A9	-	-
39.	GVT-3514	-	-
40.	IR-50	-	-
41.	CN-127	-	-
42.	IR-293-41-41-1	-	-

43.	ParasSona	-	-
44.	KJT-1-1-21-3-19	-	-
45.	Gujrat-103	-	-
46.	CN-1383-1-50	-	-

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele).

Trait:		RM-562	RM-140	RM-1287	RM 3412
Sr. No	Genotypes	*(243bp)*	*(260bp)*	*(160bp)*	(211bp)
1.	RP Bio 189	-	+	-	-
2.	IET-23557-135	-	-	-	-
3.	IET 13840-RP-66-67	-	-	-	+
4.	IR-44	-	-	-	-
5.	IET-8866	-	+	-	-
6.	IET-8580	-	-	-	-
7.	IR 56456-4-2-3	-	-	-	-
8.	IRAT-360	-	-	-	-
9.	CR-57 MP-1523	-	+	-	-
10.	HS 47	-	-	-	-
11.	IR-88-30-21-2	-	-	-	-
12.	IR-1544-238-2-3	-	-	-	-
13.	HUR FG-78	-	-	-	-
14.	IR 665575-56-1-3-19	-	-	-	-
15.	IR-60919-150-3-3	-	-	-	-
16.	IR-62036-223-3-3	-	-	-	-
17.	IR-35	-	-	-	-
18.	IR60997-16-2-3-22R	-	-	-	-
19.	IR-663879-195-2-2	-	-	-	-
20.	Kon-23	-	-	-	-
21.	KJT-23	-	-	-	-
22.	IR 61614-38-19-3-2	-	-	-	+
23.	Kasturi	-	-	-	-
24.	IR-22896-225	-	-	-	-
25.	SLR 51214	-	+	-	-
26.	IR 664	-	-	-	+
27.	IR-32	-	-	-	-
28.	IET-23537-1351	-	-	-	-
29.	Gujrat-102	-	+	-	-
30.	IR-60819-34-2-1	-	-	-	-
31.	IR 625-17-11-107	-	-	-	-
32.	Narmada	-	-	-	-
33.	JGl-2855	-	-	-	-
34.	IR 56381-139-2-2	-	-	-	-
35.	Karjat-2-32	-	-	-	-
36.	KJT 11-1-26-25-23	-	-	-	+
37.	HKR 2002-81	-	-	-	+
38.	DBS-13-3-47-A9	-	+	-	-
39.	GVT-3514	-	-	-	-
40.	IR-50	-	-	-	-
41.	CN-127	-	-	-	-
42.	IR-293-41-41-1	-	-	-	-
43.	ParasSona	-	-	-	-
44.	KJT-1-1-21-3-19	-	-	-	-
45.	Gujrat-103	-	-	-	-
46.	CN-1383-1-50	-	-	-	-

Table No. 4.5: Amplification of gene specific alleles with linked SSR markers for salt tolerance.

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele for the marker

Trait		**RM-212**	**RM-302**	**RM-3825**
Sr. No	**Genotypes**	***Dr(135bp)***	***Dr(140bp)***	***Dr(147bp)***
1.	RP Bio 189	-	-	-
2.	IET-23557-135	-	-	-
3.	IET 13840-RP-66-67	-	-	-
4.	IR-44	-	-	-
5.	IET-8866	-	-	-
6.	IET-8580	-	-	-
7.	IR 56456-4-2-3	-	-	-
8.	IRAT-360	-	-	-
9.	CR-57 MP-1523	-	-	-
10.	HS 47	-	-	-
11.	IR-88-30-21-2	-	-	-
12.	IR-1544-238-2-3	-	-	-
13.	HUR FG-78	-	-	-
14.	IR 665575-56-1-3-19	-	-	-
15.	IR-60919-150-3-3	-	-	-
16.	IR-62036-223-3-3	-	-	-
17.	IR-35	-	-	-
18.	IR60997-16-2-3-22R	-	-	-
19.	IR-663879-195-2-2	-	-	-
20.	Kon-23	-	-	-
21.	KJT-23	-	-	-
22.	IR 61614-38-19-3-2	-	-	-
23.	Kasturi	-	-	-
24.	IR-22896-225	-	-	-
25.	SLR 51214	-	-	-
26.	IR 664	-	-	-
27.	IR-32	-	-	-
28.	IET-23537-1351	-	-	-
29.	Gujrat-102	-	-	-
30.	IR-60819-34-2-1	-	-	-
31.	IR 625-17-11-107	-	-	-
32.	Narmada	-	-	-
33.	JGl-2855	-	-	-
34.	IR 56381-139-2-2	-	+	+
35.	Karjat-2-32	-	-	-
36.	KJT 11-1-26-25-23	-	-	+
37.	HKR 2002-81	-	-	-
38.	DBS-13-3-47-A9	-	-	-
39.	GVT-3514	-	-	-
40.	IR-50	-	-	+
41.	CN-127	-	-	-
42.	IR-293-41-41-1	-	-	+
43.	ParasSona	-	-	+

44.	KJT-1-1-21-3-19	-	-	-
45.	Gujrat-103	-	-	-
46.	CN-1383-1-50	-	-	-

Table No.4.6: Genotypes with resistance linked alleles for biotic and abiotic stresses detected by respective SSR markers.

Sr.No.	Traits	Markers	Resistance/ Tolerance genotypes
1.	Blast	RM 5926	RP Bio 189, IET-8580, IR-1544-238-2-3, IR 665575-56-1-3-19, IR-60819-34-2-1, IR-35, IR-663879-195-2-2, Kon-23, Kasturi, IET-23537-1351, IR-60919-150-3-3.
2.	Bacterial Blight	RM 122	RP Bio 189,IET-23557-135, IET 13840-RP-66-67, IR-44, IET-8866, CN-1383-1-50, IR 56456-4-2-3, IRAT-360, CR-57 MP-1523, HS 47, IR-88-30-21-2, IR-1544-238-2-3, HUR FG-78, IR 665575-56-1-3-19, IR-60919-150-3-3, IR-62036-223-3-3, IR-35, IR60997-16-2-3-22R, IR-663879-195-2-2, Kon-23, KJT-23, IR 61614-38-19-3-2, Kasturi, IR-22896-225, SLR 51214, IR 664, IR-32, IET-23537-1351, Gujrat-102, IR-60819-34-2-1, IR 625-17-11-107, Narmada, JGl-2855, IR 56381-139-2-2, Karjat-2-32, KJT 11-1-26-25-23, HKR 2002-81, DBS-13-3-47-A9, GVT-3514, IR-50, CN-127, IR-293-41-41-1, Paras Sona, KJT-1-1-21-3-19, Gujrat-103, IET-8580.
3.	Brown Plant Hopper	RM 6775	KJT-1-1-21-3-19.
4.	Gall Midge	RM 22709	KJT 11-1-26-25-23, IR-22896-225.
5.	Saltol	RM 140	RP Bio 189, IET-8866, DBS-13-3-47-A9, SLR 51214, Gujrat-102, CR-57 MP-1523.
		RM 3412	IET 13840-RP-66-67, HKR 2002-81, IR 664, KJT 11-1-26-25-23, IR 61614-38-19-3-2.
6.	Drought	RM 302	IR 56381-139-2-2
		RM 3825	IR 56381-139-2-2, ParasSona, IR-50, IR-293-41-41-1, KJT 11-1-26-25-23.

4.4 Genetic Diversity:

4.4.1 SSR Polymorphism:

The polymorphism percentage for individual primer was calculated by the ratio of number of polymorphic bands obtained over the total number of bands produced across the 46 rice germplasms. All the 46 rice accessions were genotyped with 18 trait linked microsatellite markers and were selected for their ability to produce amplified product and detect polymorphism level among the varieties and consistency of the pattern. Total 88 alleles were scored from these primer pairs, and 100 per cent were found polymorphic (Table No. 4.9). The overall size of amplified products ranged from 100bp to 710bp.

4.4.2 Polymorphism Information Content (PIC):

The polymorphism information content (PIC) values were calculated to find out the effectiveness of primers in distinguishing individual accession (Table 4.9). The polymorphic information content (PIC) values ranged from 0.13 to 0.82 with an average PIC value of 0.47 per primer. A total of 88 alleles were detected with an average of 4.88 alleles per locus. The markers pTA248 generated a maximum number of alleles (9). While the primers RM 122 and RM 212 produced minimum number of alleles (2). The SSR primers, RM 6775 and RM 547 revealed highest (0.82) PIC value; whereas the primer RM 122 revealed the lowest (0.13) PIC value. The higher the PIC value, the more informative is the SSR marker. Hence, primers RM 6775, RM 547, and pTA 248 were found to be highly informative (Table No.4.9). The results of individual primers, according to the amplification pattern are explained below under separate sub-headings and are presented in the Table No. 4.8. Primer wise amplification analysis is as follows:

Table No. 4.7: Primers with their allelic descriptions.

RM 8225		
The primer RM 8225 amplified a total of four alleles ranging in size from 250 to 350bp. These alleles were designated as A, B, C, and D. Out of these four alleles, allele B recorded the highest allele frequency and allele D the lowest. This marker showed a PIC value of 0.63.	Allele designation	Allele frequency
	A	0.1956
	B	0.5217
	C	0.2173
	D	0.0652

RM 206		
The primer RM 206 amplified a total of five alleles ranging in size from 420 to 530bp. These alleles were designated as A, B, C, D; E. Out of these five alleles, allele B recorded the highest allele frequency and allele E the lowest. This marker showed a PIC value of 0.66.	Allele designation	Allele frequency
	A	0.1521
	B	0.4347
	C	0.3478
	D	0.0434
	E	0.0217

RM 5926		
The primer RM 5926 amplified a total of four alleles ranging in size from 150 to 260bp. These alleles were designated as A, B, C; D. Out of these four alleles, allele D recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.65.	Allele designation	Allele frequency
	A	0.0434
	B	0.1956
	C	0.2826
	D	0.4782

RM 1233		
The primer RM 1233 amplified a total of six alleles ranging in size from 200 to 360bp. These alleles were designated as A, B, C, D, E; F Out of these six alleles, allele D recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.70.	Allele designation	Allele frequency
	A	0.0217
	B	0.0434
	C	0.0434
	D	0.3695
	E	0.03695
	F	0.1521

pTA 248		
The primer pTA 248 amplified a total of ninealleles ranging in size from 450 to 710bp. These alleles were designated as A, B, C, D, E, F,G, F, H, G, I. Out of these nine alleles, allele F recorded the highest allele frequency and allele B the lowest. This marker showed a PIC value of 0.80.	Allele designation	Allele frequency
	A	0.0434
	B	0
	C	0.0217
	D	0.1086
	E	0.2173
	F	0.2826
	G	0.1956
	H	0.1086
	I	0.0217

RM 122		
The primer RM 122 amplified a total of two alleles ranging in size from 220 to 280bp. These alleles were designated as A and B. Out of these two alleles, allele B recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.13.	Allele designation	Allele frequency
	A	0.1086
	B	0.8913

RM 5479

	Allele designation	Allele frequency
The primer RM 5479 amplified a total of four alleles ranging in size from 230 to 340bp. These alleles were designated as A, B, C and D. Out of these four alleles, allele C recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.67.	A	0.0652
	B	0.1956
	C	0.4347
	D	0.3043

RM 6775		
The primer RM 6775 amplified a total of seven alleles ranging in size from 170 to 360bp. These alleles were designated as A, B, C, D, E, F; G. Out of these seven alleles, allele E recorded the highest allele frequency and allele G the lowest. This marker showed a PIC value of 0.82.	Allele designation	Allele frequency
	A	0.0869
	B	0.1304
	C	0.1739
	D	0.1086
	E	0.2391
	F	0.2173
	G	0.0434

RM 309		
The primer RM 309 amplified a total of three alleles ranging in size from 170 to 260bp. These alleles were designated as A, B; C. Out of these alleles, allele B recorded the highest allele frequency and allele C the lowest. This marker showed a PIC value of 0.58.	Allele designation	Allele frequency
	A	0.2399
	B	0.5652
	C	0.1956

RM 547

The primer RM 547 amplified a total of sevenalleles ranging in size from 320 to 510bp. These alleles were designated as A, B, C, D, E, F; G. Out of these seven alleles, Both alleles B and E recorded the highest allele frequency and Both alleles C and G the lowest. This marker showed a PIC value of 0.82.	Allele designation	Allele frequency
	A	0.1304
	B	0.2173
	C	0.0434
	D	0.1739
	E	0.2173
	F	0.1739
	G	0.0434

RM 22709		
The primer RM 22709 amplified a total of seven alleles ranging in size from 120 to 310bp. These alleles were designated as A, B, C, D, E, F; G Out of these seven alleles, allele G recorded the highest allele frequency and both alleles D and E the lowest. This marker showed a PIC value of 0.75.	Allele designation	Allele frequency
	A	0.0217
	B	0.2399
	C	0.2399
	D	0
	E	0
	F	0.2173
	G	0.2826

RM 140		
The primer RM 140 amplified a total of sevenalleles ranging in size from 100 to 280bp. These alleles were designated as A, B, C, D; E. Out of these seven alleles, allele D recorded the highest allele frequency and allele B the lowest. This marker showed a PIC value of 0.74.	Allele designation	Allele frequency
	A	0.1304
	B	0
	C	0.0652
	D	0.3695
	E	0.3043
	F	0.0652
	G	0.0652

RM 1287

The primer RM 1287 amplified a total of five alleles ranging in size from 170 to 310bp. These alleles were designated as A, B, C, D; E. Out of these five alleles, allele C recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.70.

Allele designation	Allele frequency
A	0.0217
B	01521
C	0.3695
D	0.3478
E	0.1086

RM 562

The primer RM 562 amplified a total of three alleles ranging in size from 120 to 210bp. These alleles were designated as A, B; C. Out of these three alleles, allele B recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.55.

Allele designation	Allele frequency
A	0.1739
B	0.6086
C	0.2173

RM 3412

The primer RM 3412 amplified a total of three alleles ranging in size from 200 to 280bp. These alleles were designated as A, B; C. Out of these three alleles, allele B recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.36.

Allele designation	Allele frequency
A	0.0652
B	0.7826
C	0.1521

RM 3825		
The primer RM 3825 amplified a total of five alleles ranging in size from 150 to 280bp. These alleles were designated as A, B, C, D; E Out of these five alleles, allele D recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.70.	Allele designation	Allele frequency
	A	0.0434
	B	0.1086
	C	0.2826
	D	0.4347
	E	0.1304

RM 212		
The primer RM 212 amplified a total of two alleles ranging in size from 220 to 280bp. These alleles were designated as A, and B. Out of these two alleles, allele B recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.28.	Allele designation	Allele frequency
	A	0.1739
	B	0.826

RM 302		
The primer RM 302 amplified a total of five alleles ranging in size from 100 to 230bp. These alleles were designated as A, B, C, D; E. Out of these five alleles, allele D recorded the highest allele frequency and allele A the lowest. This marker showed a PIC value of 0.59.	Allele designation	Allele frequency
	A	0.0434
	B	0.0869
	C	0.2153
	D	0.5869
	E	0.0652

Table No. 4.9: Molecular Polymorphism, PIC Values, No. of alleles and Size of Loci revealed by SSR Primers in Rice Genotypes.

Sr. No.	Primer	Total no. of polymorphic Band	Average No. of bands/ genotype	% Polymorphism	No. of alleles	PIC	Range of amplified products
1	RM 8225	46	1	100	4	0.63	250-350
2	RM 206	46	1	100	5	0.66	420-520
3	RM 5926	46	1	100	4	0.65	150-260
4	RM 122	46	1	100	2	0.13	220-280
5	RM 1233	46	1	100	6	0.70	200-360
6	pTA248	46	1	100	9	0.80	450-710
7	RM 5479	46	1	100	4	0.67	230-340
8	RM 6775	46	1	100	7	0.82	170-360
9	RM 309	46	1	100	3	0.58	170-260
10	RM22709	46	1	100	7	0.75	120-310
11	RM 547	46	1	100	7	0.82	320-510
12	RM 140	46	1	100	7	0.74	100-280
13	RM 1287	46	1	100	5	0.70	170-310
14	RM 562	46	1	100	3	0.55	120-210
15	RM 3412	46	1	100	3	0.36	200-280
16	RM 212	46	1	100	2	0.28	220-280
17	RM 302	46	1	100	5	0.59	100-230
18	RM 3825	46	1	100	5	0.70	150-280
	Total	**828**	**18**	**-**	**88**		**-**
	Average	**46**	**1**	**100.00**	**4.88**	**0.61**	

4.3 Genetic distance values between germplasm accessions by combined analysis

On the basis of analysis of SSR scoring, the alleles were converted to binary score based on their presence (1) or absence (0). This data was used for similarity based analysis using the programme Multivariate Statistical Package (MVSP) to determine the Jaccard's coefficient matrices i.e. estimate of similarity among the fortysix genotypes.

Further the obtained Genetic similarity values based on Jaccard's coefficients were converted into genetic distance i.e. estimate of dissimilarity by subtracting similarity index (matrix) value from unity (1-similarity) mentioned in Table 4.10. for a set of fortysix rice accessions.

The genetic distances ranged from 0.029 to 0.636 with an average of 0.34 among these fortysix promising genotypes of rice. The lowest GD value (0.029) was found between the genotypes RP Bio 189 vs. Karjat-2-32, CN-127 vs. IET-23557-135, Karjat-2-32 vs. IR-44, CN-127 vs. IET-8580, SLR 51214 vs. IR-88-30-21-2 and KJT 11-1-26-25-23 vs. IR-663879-195-2-2 whereas highest Genetic Distance value (0.636) was found between the genotypes IR-62036-223-3-3 vs. IR60997-16-2-3-22R.

Further, the average genetic distance values per genotype from rest of the genotypes based on Jaccard's similarity index of all germplasm lines in rice were also analysed separately**.** From Table 4.10 it was revealed that an average genetic distance among the 46 rice accessions ranged from 0.583 (KJT-1-1-21-3-19) to 0.834 (IR 61614-38-19-3-2) from SSR analysis.

Table No. 4.10: Average genetic distance estimates of 46 rice genotypes by SSR Marker analysis based on Jaccard's dis-similarity coefficient.

Sr. No.	Accession	Avg. GD	Sr. No.	Accession	Avg. GD
1.	RP Bio 189	0.821	24.	IR-22896-225	0.793
2.	IET-23557-135	0.803	25.	SLR 51214	0.804
3.	IET 13840-RP-66-67	0.780	26.	IR 664	0.830
4.	IR-44	0.775	27.	IR-32	0.792
5.	IET-8866	0.781	28.	IET-23537-1351	0.760
6.	IET-8580	0.783	29.	Gujrat-102	0.792
7.	IR 56456-4-2-3	0.749	30.	IR-60819-34-2-1	0.762
8.	IRAT-360	0.747	31.	IR 625-17-11-107	0.776
9.	CR-57 MP-1523	0.773	32.	Narmada	0.780
10.	HS 47	0.780	33.	JGl-2855	0.808
11.	IR-88-30-21-2	0.824	34.	IR 56381-139-2-2	0.721
12.	IR-1544-238-2-3	0.739	35.	Karjat-2-32	0.678
13.	HUR FG-78	0.775	36.	KJT 11-1-26-25-23	0.767
14.	IR 665575-56-1-3-19	0.734	37.	HKR 2002-81	0.716
15.	IR-60919-150-3-3	0.752	38.	DBS-13-3-47-A9	0.713
16.	IR-62036-223-3-3	0.698	39.	GVT-3514	0.617
17.	IR-35	0.789	40.	IR-50	0.650
18.	IR60997-16-2-3-22R	0.727	41.	CN-127	0.634
19.	IR-663879-195-2-2	0.825	42.	IR-293-41-41-1	0.760
20.	Kon-23	0.729	43.	Paras Sona	0.713
21.	KJT-23	0.806	44.	KJT-1-1-21-3-19	0.583
22.	IR 61614-38-19-3-2	0.834	45.	Gujrat-103	0.714
23.	Kasturi	0.777	46.	CN-1383-1-50	0.708
AVERAGE GENETIC DISTANCE= 0.75					

4.4 Clustering analysis based on SSR markers

The genetic relationship among 46 rice genotypes are presented in a dendrogram, UPGMA cluster analysis was performed using Jaccard's similarity coefficient matrices calculated from SSR markers to generate a dendrogram for 46 rice genotypes (Fig No. 1) (Table No.4.11). The UPGMA based dendrogram of 46 rice genotypes generated with Multivariate Statistical Package (MVSP). Clustering pattern of dendrogram generated by using the pooled molecular data of 18 primers of 46 genotypes produced two main clusters namely I and II.

- The major cluster-I comprised 14 accessions, and was further found to be divided into two sub clusters (IA and IB).
  - Sub Cluster IA was further subdivided into two sub-sub clusters (IA (i) and IA (ii)).
  - Sub-sub cluster IA (i) consisted of one genotype i.e. IR-293-41-41-1
  - Sub-sub cluster IA (ii) consisted of two genotypes i.e. Paras Sona, IR-50.
  - Sub Cluster IB was further subdivided into two sub-sub clusters (IB (i) and IB (ii)).
    - Sub-sub cluster IB (i) consisted of six genotypes i.e. CN-1383-1-50, Gujrat-103, KJT-1-1-21-3-19, CN-127, GVT-3514, Karjat-2-32.
    - Sub-sub cluster IB (ii) consisted of five genotypes i.e. DBS-13-3-47-A9, HKR 2002-81, KJT 11-1-26-25-23, IR 56381-139-2-2, IR 625-17-11-107.
- The major cluster-IIcomprised 32 accessions, and was further found to be divided into two sub clusters (IIA and IIB).
  - Sub Cluster IIA was further subdivided into two sub-sub clusters (IIA (i) and IIA (ii)).
    - Sub-sub cluster IIA (i) consisted of two genotypes i.e. IR 61614-38-19-3-2, KJT-23
    - Sub-sub cluster IIA (ii) consisted of twenty one genotypes i.e. JGl-2855, HUR FG-78, IR-88-30-21-2, IR-35, IR-663879-195-2-2, IET-8866, HS 47, IR-60919-150-3-3, Kasturi, Narmada, IR-60819-34-2-1, Kon-23, IR60997-16-2-3-22R, IR-62036-223-3-3, IRAT-360, IR 665575-56-1-3-19, IR-1544-238-2-3, CR-57 MP-1523, IR 56456-4-2-3, IET-8580, IET 13840-RP-66-67.

- Sub Cluster IIB was further subdivided into two sub-sub clusters (IIB (i) and IIB (ii)).

  - Sub-sub cluster IIB (i) consisted of six genotypes i.e. Gujrat-102, IET-23537-1351, IR 664, IR-22896-225, IR-32, IR-44.
  - Sub-sub cluster IIB (ii) consisted of three genotypes i.e. IET-23557-135, SLR 51214, RP Bio 189.

Table No. 4.11: Distribution of 46 rice accessions into different clusters based on SSR analysis.

Cluster	Sub cluster	Sub-sub cluster	Number of genotypes	Genotypes
I	IA	IA(i)	1	IR-293-41-41-1.
		IA(ii)	2	Paras Sona, IR-50.
	IB	IB(i)	6	CN-1383-1-50, Gujrat-103, KJT-1-1-21-3-19, CN-127, GVT-3514, Karjat-2-32.
		IB(ii)	5	DBS-13-3-47-A9, HKR 2002-81, KJT 11-1-26-25-23, IR 56381-139-2-2, IR 625-17-11-107.
II	IIA	IIA(i)	2	IR 61614-38-19-3-2, KJT-23.
		IIA(ii)	21	JGl-2855, HUR FG-78, IR-88-30-21-2, IR-35, IR-663879-195-2-2, IET-8866, HS 47, IR-60919-150-3-3, Kasturi, Narmada, IR-60819-34-2-1, Kon-23, IR60997-16-2-3-22R, IR-62036-223-3-3, IRAT-360,IR 665575-56-1-3-19, IR-1544-238-2-3,CR-57 MP-1523, IR 56456-4-2-3, IET-8580, IET 13840-RP-66-67.
	IIB	IIB(i)	6	Gujrat-102, IET-23537-1351, IR 664, IR-22896-225,IR-32, IR-44.
		IIB(ii)	3	IET-23557-135, SLR 51214, RP Bio 189.

The diversity observed in the forty six rice varieties mainly attributed to the genetic dissimilarities. The Jaccard's similarity coefficient values among these rice varieties are presented in Table No. 4.12.

CHAPTER V

DISCUSSION

The present study entitled 'Molecular analysis of rice (*Oryza sativa* L.) germplasm, was carried out with a view to determine the presence or absence of gene specific, resistance linked alleles for particular traits in the given genotypes. This will help to screen rice germplasms for different races of biotic and abiotic stresses through SSR markers. The genetic diversity was analysed among the genotypes.

Presently, the integration of genomics and molecular-based breeding strategies for developing disease resistance, with gene-based marker assisted selection (MAS) being particularly effective, is a powerful method for efficient selection. It is imperative to use DNA markers identified within the gene or from the flanking region of the gene as a tool for an efficient MAS strategy in rice improvement (Fjellstrom *et al.*, 2004). Molecular markers are now widely used to track loci and genomic regions in crop breeding programs, as large numbers of molecular markers that are tightly linked to disease resistance traits are available in most major crop species. The majority of molecular markers have been isolated from genomic DNA libraries or from libraries of randomly amplified PCR fragments.

Molecular markers can be used to estimate overall genetic variability, determine the proportion of a genome that has been introgressed from a donor, identify genes that are phenotypically related to a particular analyzed trait, and select for traits during multiple rounds of introgression (Brondani *et al.*, 2006). In particular, microsatellite-based methods offer an attractive high-throughput and non-labour-intensive way to tag resistance genes in breeding programs. A number of microsatellite markers have been developed from publicly available databases (http://www.gramene.org) that are tightly linked with resistance genes.

In this study, the known microsatellite markers that are linked to various biotic and abiotic stress resistances have been evaluated for their usefulness as genetic markers in rice improvement programs and their potential use in MAS.

5.1 Molecular Screening:

5.1.1 Blast:

Rice blast disease, caused by *Magnaporthe oryzae*, is a major constraint for sustainable rice production. Though many resistant varieties to *M. oryzae* have been developed, the resistance is not long lasting, because the high pathogen plasticity in the fields makes single resistance gene break down after three to five years of the cultivar release (Bonman *et al.*, 1986; Lang *et al.*, 2009). Hence, development of broad spectrum and durable blast resistant varieties is essential for combating this disease. Breeding for broad spectrum resistance is necessary to improve blast resistance in rice. Pyramiding disease resistant genes into a single genetic background might be expected to give more durable disease resistance, as more resistant genes are incorporated into single genotypes (Koide *et al.*, 2010). Selection for quantitative trait loci (QTL) of disease resistant lines, in which numerous loci are accumulated, is referred to as gene pyramiding. This is difficult to achieve using conventional breeding approaches due to a low accuracy in the identification of desirable genotypes, and because of the laborious and time consuming process. Marker-assisted selection (MAS) allows the identification of multiple resistance genes in plants.

In this study four primers i.e. RM 5926, RM 8225, RM 1233, RM 206 were used to identify blast resistance linked genes in 46 rice genotypes. Primer RM 5926 specific to *Pi-1* resistance linked allele, conferred resistance at 175bp in the genotypes; RP Bio 189, IET-8580, IR-1544-238-2-3, IR 665575-56-1-3-19, IR-60819-34-2-1, IR-35, IR-663879-195-2-2, Kon-23, Kasturi, IET-23537-1351, IR-60919-150-3-3. Thippeswamy *et al.*, (2006), Thippeswamy *et al.*, (2015) also

evaluated rice genotypes for presence of resistance linked alleles specific to gene *Pi1* by marker RM 5926. Primer RM 8225 specific to *Pi-z* resistance linked allele, conferred absence at 221bp in all the genotypes. Fjellstrom *et al.*, (2004), Ashkani *et al.*, (2011) reported resistance linked alleles specific to gene *Pi-z* by using marker RM 8225.

The genotypes were screened for the presence of resistance linked allele for gene *Pi1* by visualization of amplicons of 170 bp fragments using SSR marker, RM 1233. The results showed that all the genotypes indicated absence of resistance linked alleles for gene *Pi1* specific marker RM 1233. Studies reported by Ashkani *et al.*, (2011) stated the use of marker RM 1233 specific to gene *Pi1* for screening blast resistance rice genotypes.

The genotypes were verified for the presence of blast resistance gene, *Pi-kh* by using the gene specific primer, RM 206 which is expected to amplify a 140bp fragment in the genotypes containing the resistance linked allele. No genotype was observed to possess the resistance linked allele for the blast resistance gene, *Pi-kh* for the primer RM 206. Kumar *et al.*, (2013) screened rice genotypes for blast resistance linked alleles specific to gene *pi-kh* by marker RM 206.

5.1.2 Bacterial Blight:

Phuc *et al.*, (2005) reported that marker assisted selection was accurate for improving the resistance of rice varieties to BLB. In this study, 46 rice accessions were screened to determine resistance status for BLB-resistance genes viz., Xa5 and Xa21 by using PCR based microsatellite markers RM 122 and pTA 248 respectively. Screening for the Xa5 resistance gene by the amplification of the microsatellite marker RM 122, which was employed to track the resistant amplicons of 240-250 bp, revealed resistance linked alleles in almost all the genotypes in this study. Studies performed by Islam *et al.*, (2015), Ullah *et al.*, (2012) and Sabar *et al.*, (2016) also revealed

presence of resistance linked alleles at 240-250bp for Xa5 gene specific marker RM 122. This indicates that bacterial blight gene Xa5 specific marker RM 122 is effective in detecting presence of resistance linked alleles in all the genotypes used in this study. No amplicons specific to resistance linked alleles for Xa21 gene were detected by marker pTA 248. This indicates that resistance linked alleles for gene Xa21 were absent in all the genotypes. Kumar *et al.*, (2013) also screened several rice entries for presence of blast resistance linked alleles (of 1040bp) by primer pTA 248 specific to gene Xa21.

5.1.3 Brown Plant Hopper:

The 46 germplasms of rice were evaluated for Brown Plant hopper resistance using the SSR markers viz., RM 6775, RM 309 and RM 5479. Among these, Marker RM 6775 was most effective in identification of the resistant linked alleles in the genotypes. Marker RM 6775 specific to gene Bph25 indicated presence of resistance linked alleles (192bp) in few genotypes. Marker RM 309 and RM 5479 specific to gene *Bph26*(at 152bp) indicated absence of resistance linked alleles in all the genotypes. These genotypes may either have resistance linked alleles for other *Bph* genes which can be detected by using corresponding gene specific markers or these genotypes are devoid of resistance linked alleles for any of the *Bph* genes. Myint *et al.*, (2012) also screened rice genotypes for *Bph25* and *Bph26* genes by the primers RM 6775, RM 309, RM 5479. Several other workers; Rahman *et al.*, (2009), Harini *et al.*, (2013), Shabanimofrad *et al.*, (2015 a and b) and Bhogadhi *et al.*, (2015) also reported similar marker study for *Bph* genes.

5.1.4 Gall midge:

The SSR markers RM 547 and RM 22709 used in this study reported the absence and presence of resistance linked alleles for biotypes *Gm4*(270bp) and *Gm8*(160 to 170bp) respectively. RM 547 at 270bp showed absence of linked alleles in allgenotypes and marker RM 22709 at 170bp showed resistance linked alleles in two genotypes. One marker used in this study was effective in detecting resistance linked

alleles. The genotypes showing presence of resistance linked alleles can further be used in various breeding programs. Using flanking SSR markers Sama *et al.*, (2012) detected *Gm8* gene in nine rice genotypes. Various reports given by, Anupam *et al.*, (2016), Mohapatra *et al.*, (2016) revealed the strong association of locus specific makers which are in agreement with this study.

5.1.5 Salt Tolerance:

Salt resistance linked markers used in this study were, viz., RM 140, RM 1287, RM 562 and RM 3412. Markers used in this study were effective in detecting saltol QTL in several genotypes screened in this study. Further study may confirm that some of these genotypes might have Saltol QTL and can be used as alternative donors in salt tolerant rice breeding programmes. Three SSR Saltol linked SSRs used in this study amplified polymorphic bands in the 46 genotypes.

Number of workers have studied the *Saltol* locus in rice specific to the salt tolerance. The SSR markers related to Salt tolerance indicated in this study were derived from a study. Thomson *et al.*, (2010) revealed that characterized Pokkali-derived quantitative trait loci (QTLs) for seedling stage salinity tolerance in preparation for use in marker-assisted breeding. Thomson *et al.*, (2010) tested several markers for robustness and polymorphism across the Saltol region in several donors and potential parents, they determined the best markers within and flanking the Saltol region and, as well, markers that can be used for negative selective above and below the region.

Several workers also reported that these markers are highly polymorphic (Zeng *et al.*, 2004; Karmakar *et al.*, 2012; Islam *et al.*, 2012; Iqbal *et al.*, 2015; Ganie *et al.*, 2016).

5.1.6 Drought Tolerance:

Molecular markers linked to drought tolerance in rice are an important tool for screening and selection of drought tolerant genotypes for use in future breeding programs. Drought resistance linked markers used in this study were, viz., RM 302, RM 3825 and

RM 212. Resistance linked alleles Dr(140bp) detected by the marker RM 302 were present in the genotype; IR 56381-139-2-2. Resistance linked alleles Dr(147bp) detected by the marker RM 3825 were present in the genotype; IR 56381-139-2-2, Paras Sona, IR-50, IR-293-41-41-1, KJT 11-1-26-25-23. Markers RM 212 (at 135bp) indicated absence of resistance linked alleles in all the genotypes. As these genotypes showed negative results for these primers, we can suggest the use of other drought related marker combinations to screen the genotypes for drought resistance.

The markers RM 302, RM 3825 and RM 212 on chromosome 1 may be useful for evaluation of diverse germplasms and on the basis of these molecular markers some genotypes of rice were identified as drought tolerant genotypes; this was also linked with the root traits, i.e. root length of the genotypes (Ashfaq *et al.*, 2014). It is evident from results that the genomic region RM 302–RM 3825–RM 212 on chromosome 1 is linked to drought resistance traits and may be useful in marker assisted breeding for drought resistance in rice (Kanagaraj *et al.*, 2010).

Various similar reports were observed in the studies done by Kanagaraj *et al.*, (2010), Ashfaq *et al.*, (2014), Ramadan *et al.*, (2015); Freeg *et al.*, (2016) and Sindhumole *et al.*, (2017).

5.2 Genetic Diversity:

5.2.1 SSR Polymorphism:

All the 46 rice accessions were genotyped with 18 trait linked microsatellite markers and were selected for their ability to produce amplified product and detect polymorphism level among the varieties and consistency of the pattern. Total 88 alleles were scored from these primer pairs, and 100 per cent were found polymorphic (Table 4.9). The overall size of amplified products ranged from 100bp to 710bp. Generally most of the SSR markers used to analyse the diversity are polymorphic in nature.

5.2.2 Polymorphic Information Content (PIC):

PIC shows how the marker can indicate the population polymorphism depending on the number and frequency of the alleles (D. Botstein *et al.*, 1980). So the PIC reflects a discriminating ability of the marker and, in fact, depends on the number of known alleles and their frequency distribution, thus being equal to genetic diversity. PIC maximal value for dominant markers is 0.7. Note, that for the markers with equal distribution in the population the PIC values are higher. They are much higher for markers with multiple alleles, and, however, also depend on the frequency distribution of the alleles.

In this study the polymorphism information content (PIC) values ranged from 0.13 to 0.82 with an average PIC value of 0.47 per primer. A total of 88 alleles were detected with an average of 4.88 alleles per locus. The markers pTA 248 generated a maximum number of alleles (9). While the primer RM 122 and RM 212 produced minimum number of alleles (2). The SSR primer, RM 6775 and RM 547 revealed highest (0.82) PIC value; whereas the primer RM 122 revealed the lowest (0.13) PIC value. The higher the PIC value, the more informative is the SSR marker. Hence, primers RM 6775, RM 547, and pTA 248 were found to be highly informative (Table 4.9).

Genetic diversity in different rice germplasms using molecular markers has been studied widely (Islam *et al.*, 2012; Nguyen *et al.*, 2012, Hoque *et al.*, 2014, Gholizadeh *et al.*, 2014, Singh *et al.*, 2016, Chungada *et al.*, 2016, Krupa *et al.*, 2017) Table 5.1.

Table 5.1 Studies on Genetic diversity of different rice germplasms

Author	No. of accessions	Total No. of markers	Total No. of alleles	Avg.No. of Alleles	Average PIC
Jain *et al.*, 2004	69	30	235	7.8	0.6
Giarrocco *et al.*, 2007	69	26	219	8.4	0.69
Thomson *et al.*, 2007	330	30	394	13.1	0.66
Islam *et al.*, 2012	14	40	168	4.2	0.57
Nguyen *et al.*, 2012	41	30	192	6.4	0.73
Hoque *et al.*, 2014	30	5	28	5.66	0.54
Gholizadeh *et al.*, 2014	29	30	106	3.53	0.45
Roy *et al.*, 2015	107	40	322	8.05	0.67
Nachimuthu *et al.*, 2015	192	61	205	3.36	0.75
Freeg *et al.*, 2016	41	15	68	4.71	0.52
Krupa *et al.*, 2017	5	20	65	3.25	0.49
This study	46	18	88	4.88	0.47

In this study a total of 88 alleles were detected with an average number of alleles of 4.88 per locus (ranged from 2 to 12 per locus). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity. The average number of alleles (4.88) obtained in this study is lower than the values reported by some of the studies performed by Islam *et al.*, 2012; Gholizadeh *et al.*, 2014; Freeg *et al.*, 2016 and Krupa *et al.*, 2017 on smaller germplasm sets and comparable to values reported by Hoque *et al.*, 2014, but quite lower than the values reported by Jain *et al.*, 2004, Giarrocco *et al.*, 2007; Thomson *et al.*, 2007 and Roy *et al.*, 2015 for other large scale collection (Table 5.1). These inconsistencies might be due to the genotypes used and selection of SSR markers. Markers that have the ability to detect high number of discernable alleles are the

suitable marker for molecular characterization and genetic diversity analysis (Islam et *al.*, 2008).

The markers showed an average PIC value of 0.47 which indicated that SSR markers used in this study were moderately informative because only PIC values higher than 0.5 indicate high polymorphism. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (DeWoody *et al.*, 1995). The overall genetic diversity (PIC=0.47) of the 46 rice germplasm accessions included in this study was comparable to the value reported in previous studies (Jain *et al.,* 2004 ;Giarrocco *et al.*, 2007; Thomson *et al.*, 2007; Roy et al.,2015), higher than the values reported by Hoque *et al.*, 2014; Gholizadeh *et al.*, 2014; Freeg *et al.*, 2016; Krupa *et al.*, 2017 who reported average PIC value equivalent to 0.54, 0.45, 0.52, 0.49 respectively and smaller than the values reported by Nguyen *et al.*, 2012 and Nachimuthu *et al.*, 2015 who reported avg. PIC value equivalent to 0.73 and 0.75 (Table 5.1).

5.2.3 Genetic distance values between germplasm accessions

On the basis of analysis of SSR scoring, the alleles were converted to binary score based on their presence (1) or absence (0). This data was used for similarity based analysis using the programme Multivariate Statistical Package (MVSP) to determine the Jaccard's coefficient matrices i.e. estimate of similarity among the fifty genotypes.

The genetic distances ranged from 0.029 to 0.636 with an average of 0.75 among these forty six promising genotypes of rice. The lowest GD value (0.029) was found between the genotypes RP Bio 189 vs. Karjat-2-32, CN-127vs. IET-23557-135, Karjat-2-32 vs. IR-44, CN-127 vs. IET-8580, SLR 51214 vs. IR-88-30-21-2 and KJT 11-1-26-25-23 vs. IR-663879-195-2-2 whereas highest Genetic Distance value (0.636) was found between the genotypes IR-62036-223-3-3 vs. IR60997-16-2-3-22R.

4.4 Clustering analysis based on SSR markers

The UPGMA based dendrogram of 46 rice genotypes was generated with Multivariate Statistical Package (MVSP). Clustering pattern of dendrogram generated by using the pooled molecular data of 18 primers of 46 genotypes produced two main clusters namely I and II. The dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered together.

The major cluster-I comprised 14 accessions, and was further found to be divided into two sub clusters (IA and IB). The major cluster-II comprised 32 accessions, and was further found to be divided into two sub clusters (IIA and IIB).

Similarly, several other workers also studied the diversity among various rice genotypes by constructing dendrogram and cluster analysis. Choudhary *et al.*, (2013) constructed the unweighted neighbour-joining (UNJ) dendrogram on the basis of genetic similarity matrix grouped the 100 genotypes into five clusters viz., landraces, 1970s, 1980s, 1990s, and 2000s. Yadav *et al.*, (2013) grouped the 88 rice accessions that included landraces, farmer's varieties and popular Basmati lines into two major clusters at the dissimilarity coefficient of 0.55 and further into four clusters at a dissimilarity coefficient of 0.58. Mohiuddin *et al.*, (2014) constructed Dendrogram based on the Nei's genetic distance calculated from 27 SSR markers generated from the 30 rice accessions. Singh *et al.*, (2016) grouped 729 rice varieties into two major clusters, 400 varieties in cluster 1 whereas; 329 varieties were grouped in cluster 2.

From this study it is revealed that, rice varieties are more divergent indicating large part of the genome may be dissimilar among themselves. However, genetic diversity detected using molecular markers in the present investigation indicates the high discrimination capacity of SSR markers.

Because of the polyallelic nature of SSR markers, they have the advantage of discriminating the individuals more precisely. The SSR

marker gave more clusters with fewer genotypes in each cluster and therefore, more variation within each cluster. In the present study, the rice varieties grouped into two major clusters. In future these genotypes can be used directly for cultivation under changing climatic condition. The evaluation of genetic similarity and cluster analysis together for stress resistance provides some useful guides for assisting plant breeders in selecting genetically diverse parents for crossing programme and also assist in broadening germplasm-based rice breeding programs in the near future.

CHAPTER VI

SUMMARY AND CONCLUSION

Molecular analysis of important biotic and abiotic stress resistance imparting traits among the genotypes, using different markers will be useful in selection of parents for hybridization and marker assisted selection to increase productivity even under unfavorable conditions.

At present, information on thousands of SSR primers is available in various rice databases. Since it is a complex phenomenon, efforts are needed to characterize and develop panel of SSR markers for quick evaluation of genotypes for biotic and abiotic stress resistance and tagging of traits with these molecular markers. DNA markers, microsatellites being co-dominant, cost effective and most reliable were used in the study. The present study was conducted with an objective to screen the 46 rice genotypes obtained from Regional Agricultural Research Station Karjat, Raigad using SSR markers.

- Blast resistance linked alleles were observed in genotypes; RP Bio 189, IET-8580, IR-1544-238-2-3, IR 665575-56-1-3-19, IR-60819-34-2-1, IR-35, IR-663879-195-2-2, Kon-23, Kasturi, IET-23537-1351, IR-60919-150-3-3.
- Bacterial Blight resistance linked alleles, for gene Xa5 detected by primer RM 122 were observed in almost all the genotypes.
- Brown Plant Hopper linked alleles were observed in genotype; KJT-1-1-21-3-19.
- Gall midge resistance linked alleles were present in the genotypes; KJT 11-1-26-25-23, IR-22896-225.

- Salt tolerant resistance linked alleles were found in genotypes; RP Bio 189, IET-8866, DBS-13-3-47-A9, SLR 51214, Gujrat-102, CR-57 MP-1523, IET 13840-RP-66-67, HKR 2002-81, IR 664, KJT 11-1-26-25-23, IR 61614-38-19-3-2.
- Drought tolerance linked alleles were present in genotypes; IR 56381-139-2-2, IR 56381-139-2-2, Paras Sona, IR-50, IR-293-41-41-1, KJT 11-1-26-25-23.
- Eighteen SSR primers were used in this study and all amplified and showed the 100 % polymorphism in rice genotypes. A total of 88 alleles were detected with an average of 4.88 alleles per locus.
- The markers pTA 248 generated a maximum number of alleles (9). While the primer RM 122 and RM 212 produced minimum number of alleles (2).
- The polymorphism information content (PIC) values ranged from 0.13 to 0.82 with an average PIC value of 0.47 per primer. The SSR primers, RM 6775 and RM 547 revealed highest (0.82) PIC value; whereas the primer RM 122 revealed the lowest (0.13) PIC value. The higher the PIC value, the more informative is the SSR marker. Hence, primers RM 6775, RM 547 and pTA 248 were found to be highly informative.
- Maximum (9) alleles were produced by the primer pTA 248 whereas the primers RM 122 and RM 212 produced minimum (2) alleles.
- The genetic distances ranged from 0.029 to 0.636 with an average of 0.34 among these forty six genotypes of rice. The lowest GD value (0.029) was found between the genotypes RP Bio 189 vs. Karjat-2-32, CN-127 vs. IET-23557-135, Karjat-2-32 vs. IR-44, CN-127 vs. IET-8580, SLR 51214 vs. IR-88-30-

21-2 and KJT 11-1-26-25-23 vs. IR-663879-195-2-2 whereas highest Genetic Distance value (0.636) was found between the genotypes IR-62036-223-3-3 vs. IR60997-16-2-3-22R.

Further, the average genetic distance values per genotype from rest of the genotypes based on Jaccard's similarity index of all

- germplasm lines in rice were also analysed separately. Average genetic distance among the 46 rice accessions ranged from 0.583 (KJT-1-1-21-3-19) to 0.834 (IR 61614-38-19-3-2) from SSR analysis.
- Clustering pattern of dendrogram generated by using the pooled molecular data of 18 primers of 46 genotypes produced two main clusters namely I and II. The first cluster contained 14 genotypes while the second cluster had 32 genotypes.

Conclusion:

Among all the screened genotypes; KJT 11-1-26-25-23 showed presence of multiple resistance traits for the Bacterial Blight, Gall Midge, Saltol, Drought. Moreover genotype; RP Bio 189 showed presence of multiple resistance for three traits. Hence these genotypes appear to be promising genotypes for presence of multiple resistance traits.

Genotype IR 56381-139-2-2 showed presence of resistance Linked Alleles for two Drought Tolerance genes Dr(140bp) and Dr(147bp). Hence it appears to be promising for Drought Tolerance trait.

Molecular screening of rice germplasms for biotic and abiotic stresses through microsatellites is of immense scope in hastening the breeding research in order to increase production and meet the needs for feeding a fast growing population.

Environmental factor of climate change and global warming presents a great challenge to future production of food grains; these factors include both biotic and abiotic stresses that are expounded by the global warming. These stresses are one of the main reasons to cause the biggest losses in global rice production.

In order to mitigate these challenges effectively, there is a need to utilize molecular markers especially SSR markers with high selection accuracy for selecting resistant plant sources which can further be used in marker-aided selection. Illustrated in this study, SSR primers showed 100% polymorphism. It also depicted that rice genotypes used in the present screening were divergent which is a very important factor for breeding programmes.

Hence, It is concluded that screening of rice genotypes for biotic and abiotic stresses through microsatellites is very important for identifying parental genotype with resistant genes that will be exploited for MAS breeding of elite lines that are more stable to stresses and this can lead to increased yields.

LITERATURE CITED

Abbasi, F. M, Rabia M., Habib A., Khan U., Afzal M., Inamullah P., Rehman M., Md. Khan T., Akbar and Md. Khan A.

(2011). Molecular screening of Pakistani rice germplasm for xa5 gene resistance to bacterial blight *Afr. J. Biotech*, **10**(15): 2833-2837.

Aliyu, R., Adamu A. K., Muazu S and Alonge S. O. (2011). Tagging and validation of ssr markers to salinity tolerance QTLs in rice (*Oryza spp*).International Conference on Biology, *Environment and Chemistry* 1: 328-323.

Amgai, R. B., Niroula R. K., Pantha S., Hamal, S. S., Tamang, B. G., Sah, B. P., and Bhatta M. R. (2015). Marker assisted screening of nepalese rice for bacterial leaf blight (BLB) resistance. *Nepal Jr of Biotech*, **3**(1): 35-39.

Anonymous. (2018a). Rice - Statistics & Facts. The Statistics Portal Statistics and Studies from more than 22,500 Sources https://www.statista.com/topics/1443/rice/

Anonymous. (2018b). The economic times/Agriculture, https://economictimes.indiatimes.com/news/economy/agriculture/india-sets-record-farm-output-target-for-2018-19/articleshow/65858058.cms

Anupam, A., Jahangir I., Syed, M. Q., Anantha, S., Das, S. P., Variar, M. and Nimai, P. M. (2017). Genetic diversity analysis of rice germplasm in tripura state of northeast India using drought and blast linked markers. *Rice Science*, **24**(1): 10:20.

Ashfaq, M., Saleem, H. M., Ali, A., Ali, M., Hanif, S. and Mubashar, U. (2014). Screening of diverse germplasms for genetic studies of drought tolerance in rice (*Oryza sativa* L.). *Caryologia*, **67**(4): 296-304.

Ashkani, S., Rafii, M. Y., Sariah, M., Akmar, A. M., Rahim, R. A., Latif, M.A. (2011). Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genetics and Molecular Research,* **10**(3): 1345-1355.

Ballani, A. (2013). M.Sc. Thesis on Molecular analysis for drought tolerance in upland rice (*Oryza sativa* L). Anand Agricultural University.

Bentur, J. S., Sinha, D. K., Padmavathy, Revathy, C., Muthulakshmi, M. and Nagaraju, J. 2011.Isolation

and Characterization of Microsatellite Loci in the Asian Rice Gall Midge (*Orseolia oryzae*) (Diptera: Cecidomyiidae). *Int. J. Mol.* Sci. **12**: 755-772.

Bhagwat, S., Gokhale, N. B., Sawardekar, S. V., Kelkar, V. G., Kambale, S.R. and Kunkarker, R. L. (2017). Evaluation of rice *(Oryza sativa* L.) germplasm for biotic and abiotic stresses and their genetic diversity using SSR markers. Oryza, **54**(3): 258-265.

Bhogadhi, S. C., Bentur, J.S., Rani, C.V.D., Thappeta, G., Yamini, K.N., Kumar, N.A.P., Jamaloddin, M., Swathi, G., Lakshmi, V.J., Bhanu, K.V. and Satynarayana, P.V., (2015). Screening of Rice Genotypes for Resistance to Brown Plant Hopper Biotype 4 and Detection of BPH Resistance Genes. *Int. J. Life Sci. Biotechnol. Pharma Res*, **4**(2): 90.

Bimpong, I. K., Serraj, R., Chin, J. H, Ramos, J., Mendoza, E., Hernandez, J., Mendioro, M. S., Brar, D. S. (2011). Identification of QTLs for drought-related traits in alien introgression lines derived from crosses of rice (*Oryza sativa* cv. IR64) × O. glaberrima under lowland moisture stress. *J. Pl. Bio.*, **54**: 237-250.

Bonman, J. M., Dedios, T. I .V., Khin, M. M. (1986). Physiological specialization of Pyricularia oryzae in the Philippines. *Plant Dis,* **70**(8): 767–769.

Brondani, R. P. V., Zucchi, M. I., Brondani, C., Rangel, P. H. N., Borba, T. C. D., Rangel, P.N., Magalhaes, M.R. and Vencovsky, R., (2006). Genetic structure of wild rice Oryza glumaepatula populations in three Brazilian biomes using microsatellite markers. *Genetica*, **125**(2): 115-123.

Carsono, N., Barus, Y. V., Sari, S., Widarmi, W. D., Dono, D., Sumekar, Y., Murdaningsih, H.K. (2015). Identification of polymorphism on simple sequence repeats markers associated with brown plant hopper resistance genes in twenty rice genotypes and their genetic relationship. Knowledge Publishing Services, **2**:98-106.

Chaudhary, P. R., Kohli S., Srinivasan K., Mohapatra T and Sharma R.P. (2001). Identification and classification of of aromatic rices based on DNA fingerprinting. *Euphytica.*,**118**: 243-251.

Chen, S., Liu, X., Zeng, L., Ouyang, D., Yang, J. and Zhu, X. (2011). Genetic analysis and molecular mapping of a novel recessive gene xa34(t) for resistance against

Xanthomonas oryzae pv. Oryzae. *Theoretical and Applied Genetics*, **122**:1331–1338.

Choudhary, G., Ranjitkumar, N., Surapaneni, M., Deborah, D. A., Vipparla, A., Anuradha, G., Siddiq, E.A. (2013). Molecular Genetic Diversity of Major Indian Rice Cultivars over Decadal Periods. *Trends in Rice Molecular Genetic Diversity*, **8**(6): e66197.

Choudhary, K., Choudhary, O. P., and Shekhawat, N. S. (2008). Marker assisted selection: a novel approach for crop improvement. *Am. Eurasian J. Agric.***1**:26–30.

Chungada, A. S., Gokhale, N. B., Patil, D. M., Sawardekar, S. V. and Patil, P. P. (2016). Molecular screening of rice germplasm for biotic and abiotic stresses and their diversity study by using SSR markers. *J. Indian Society Coastal Agriculture Research*, **34**(2): 7-14.

Devanna, N. B, Vijayan, J., Sharma, T. R. (2014). The blast resistance gene Pi54 of cloned from Oryza officinalis interacts with AvrPi54 through its novel non-LRR domains. PLoS One, **9**(8): e104840.

DeWoody, J. A., Honeycutt, R. L., and Skow, L. C. (1995). "Microsatellite markers in white-tailed deer", *J. Heredity*, **86**: 317–319.

Dudley, J., (1994). Plant breeding a vital part of improvement in crop yields, quality and production efficiency. In: Frey KJ, editor. Historical perspectives in plant sciences. Ames: Iowa State University Press; pp. 163–77.

Dutta, S. S., Divya, D., Rani, C. V. D., Reddy, T. D., Visalakshmi, V., Cheralu, C., Singh, K. I. and Bentur, J. S., (2014). Characterization of gall midge resistant rice genotypes using resistance gene specific markers. *J Experimental Biology and Agricultural Sciences*, **2**(4): 439-446.

Edwards, K., Johnstone, C., and Thompson, C. (1991). A Simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research*, **19**(6): 1349.

Fjellstrom, R., McClung, A. M., Marchetti, M. A., Shank A.R. (2004). Development of DNA Markers Suitable for Marker Assisted Selection of Three Pi Genes Conferring Resistance to Multiple Pyricularia grisea Pathotypes. *Crop Sci.***44**:1790–1798.

Freeg H. A., Anis G. B., Abo-Shousha A. A., El-Banna A.N., El-Sabagh A. (2016). Genetic diversity among some rice

genotypes with different drought tolerance based on SSR markers. *Cercetari Agronomicein,* **3**(167): 39-50.

Ganie, S. A., Borgohain M. J., Kashyap K., Talukdar A., Pani D. R., Mondal T. K. (2016). Assessment of genetic diversity of Saltol QTL among the rice (*Oryza sativa* L.) genotypes. *Physiol Mol Biol Plants*, **22**(1):107–114.

Gholizadeh, F. and Navabpour. S. (2014). Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers under salinity stress. *Iranian J. Plant Physiology*, **4**(3):1039-1046.p

Giarrocco, L. E., Marassi M. A. and Salerno L. (2007). Assessment of the genetic diversity in Argentina rice cultivars with SSR markers. *Crop Sci.*, **47**: 853–860.

Gomez, S.R., (2013). Abiotic and biotic stress in plants. Molecular stress physiology of plants, Rout, G. R., and Das, A. B., ed. New Delhi: Springer, pp. 1-20.

Gosal, S. S., Wani, S. H., Kang, M. S (2009). Biotechnology and drought tolerance. *J. Crop Improvement*, **23**: 19-54.

Gracia, A. A. F., Benchimol, L. L., Antonica, M. M., Geraldi, I. O., Deuza, A. P. (2004). Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. *Euphytica*, **108**: 53-63.

Hani, A. A., Arjunan, S., Senthilkumar, R., (2017). Detection of Salt Tolerant Rice Genotypes at the Seedling Stage Using Molecular Markers: A Genotypic Analysis. *Int. J. Curr. Res. Biosci. Plant Biol.* **4**(3):81-87.

Harini, A. S., Kumar, S. S., Balaravi, P., Sharma, R., Dass, M. A., and Shenoy, V. (2013). Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. *African J. Biotechnology,* **12**(19): 2515-2525.

Himabindu, K., Suneetha, K., Sama, VSAK., Bentur, J. S. (2010). A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. *Euphytica*, **174**:179–187.

Hittalmani, S., Parco, A., Mew, T. V., Zeigler, R. S. and Huang, N. (2000). Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theoretical and Applied Genetic*, **100**: 1121-1128.

Hoque, A., Begum, S. N., and Hassan, V. (2014). Genetic diversity assessment of rice (*Oryza sativa* L.) germplasm using SSR markers. *Res. Agric., Livest. Fish,***1**(1): 37-46.

Ingram, K. T, Bueno, F. D, Namuco, O. S, Yambao, E. B, Beyrouty, C. A. (1994).Rice root traits for drought resistance and their genetic variation. In: Kirk GJD (ed) Rice Roots: Nutrient and Water Use. Selected papers from the International Rice Research Conference. *International Rice Research Institute, Manila, Philippines*, pp. 67-77.

Iqbal, S. A, Islam M. M, Ahmed Hossain Md, Malaker, A (2015). DNA Fingerprinting of Rice Lines for Salinity Tolerance at Reproductive Stage. *Advances in Crop Science and Technology*, S1: 006.

Islam, M. R., Gregorio, G. B., Salam, M. A., Collard, B. C., Singh, R. K., and Hassan, L. (2008). Molecular diversity of stress tolerant rice genotypes using SSR markers. *SABRAO Journal of Breeding and Genetics*, **40**(2): 127-139.

Islam, M. R., Gregorio, G. B., Salam, M. A., Collard, B. C. Y., Singh. R. K., Hassan, L. (2012). Validation of SalTol linked markers and haplotype diversity on chromosome 1 of rice. *Mol Plant Breed*, **3**:103–114.

Islam, M. S., Mia, M., Khanom, S. M., Nasiruddin, K. M., Islam, M. M. (2015). Screening of Aromatic Rice Cultivars for Bacterial Leaf Blight (BLB) Disease Resistance Gene *Xa5* through Molecular Marker. *British Biotech. J.*, **9**(4): 1-13.

Jayawardana, W. D., Jayasekera, G. U., Wijesundera, R. C., Dissanayake, D. N., Sooriyapathirana, S. S., Weebadde, C.K., Perera, K. S., Gunapala, K. D. And Hettige, P. (2014). Evaluation of DNA Markers Linked to Blast Resistant Genes, Pikh, Pit (p), and Pita, for Parental Selection in Sri Lankan Rice Breeding. *Tropical Agricultural Research*. **26**(1): 82 – 93.

Jia, Y., Wang, Z. and Singh, P. (2002). Development off dominant rice blast Pita resistance gene markers. *Crop Sci.*, **42**: 2145-2149.

Kanagaraj, P., Prince, K. S., Sheeba, J. A., Biji, K. R., Paul, S. B., Senthil, A. and Babu, R. C. (2010). Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current Science*, **98**(6): 836-839.

Karmakar, J, Roychowdhury, R., Kar, R. K., Deb, D., Dey, N. (2012). Profiling of selected indigenous rice (*Oryza sativa* L.) landraces of rarh Bengal in relation to osmotic stress tolerance. *Physiol MolBiol Plants*, **18**:125–132.

Khush, G. S, Karim, A. N. M. R., Angeles, E. R. (1985). Genetics of resistance of rice cultivar ARC10550 to Bangladesh brown planthopper biotype. *J Genet*, **64**:121–125.

Kibria, K., Nur, F., Begum, S. N., Islam, M. M., Paul, S. K., Rahman, K. S., (2009.) Molecular marker based genetic diversity analysis in aromatic rice genotypes using SSR and RAPD markers. *Int J. Sustain Crop Prod*, **4**(1): 23–34.

Koide, Y., Kawasaki, A., Telebanco-Yanoria, M. J., Hairmansis, A., Nguyet, N. T. M., Bigirimana, J., Fujita, D., Kobayashi, N., Fukuta, Y. (2010). Development of pyramided lines with two resistance genes, Pish and Pib, for blast disease (Magnaporth oryzae) in rice (*Oryza sativa* L.). *Plant Breed,* **129**: 670–675.

Kottearachchi, N.S (2013). Utility of DNA markers in rice breeding. *Eur Int. J. Sci Technol.* **2**: 111-122

Krupa, K. N., Shashidhar, H. E., Dalawai, N., Reddy, M. and Swamy, H. V. V., (2017). Molecular Marker Based Genetic Diversity Analysis in Rice Genotypes (*Oryza sativa* L.) using SSR Markers, *Int. J. Pure App.*Biosci. **5**(2): 668-674.

Kumar, S. G., Kumari, K. A., Durga Rani, C. V., Sundaram, R. M., Vanisree, S., Jamaloddin, M. and Swathi, G. (2013). Study of simple sequence repeat (SSR) polymorphism for biotic stress resistance in elite rice variety JGL 1798. *African J. Biotech.* **12**(40): 5833-5838.

Laksminarayana A. and Gurdev K. S. (2015). New Genes for Resistance to the Brown Planthopper in *Rice. J. Allia. Crop, Soil, Env. Sci. Soc.,* **17**: 96-100.

Lang, N. T., Luy, T. T., Ha, P. T. T., Buu, B. C. (2009). Monogenic lines resistance to blast disease in rice (*Oryza sativa* L.) in Vietnam. *Int J Genet* Mol Biol, **1**(7): 127–136.

Lapitan, V. C., Brar, D. S., Abe, T., Redofia, E. D. (2007). Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breed. Sci.*, **57**: 263-270.

Mahmoodreza S., Rafii, M. Y., Ashkani S., Hanafi M. M., Adam N. A., Latif M. A. and Sahebi M. (2015). Analysis of SSR markers linked with brown planthopper resistance genes (Bph) using high-resolution melting (HRM) in rice. *Plant Omics*, **8**(3): 212.

Mehla, K., Chaudhary, S., Kumar, A., Kumar, V., Chauhan, P., Gupta, S. (2011). Advances in DNA sequencing:

Challenges and limitations of personal sequencing. *African J. Agril. Research*, **6**(6):1277-1280.

Mishra, A. (2011). M.Sc Thesis on Analysis for blast resistance genes of rice genotypes through molecular markers. Anand Agricultural University.

Mohapatra, S., Behera, L., Jena, M. S., Pradhan, K., Mohanty, S. K., andSahu, S. C. (2016). Molecular screening of rice genotypes using linked markers for gall midge resistance gene, Gm4. *The Ecoscan*, **10**(1&2): 349-357.

Mohiuddin, A. K. M., Nadia, I., Sultana, S., Ferdous, J. (2014). Diversity analysis of indica rice accessions (*Oryza sativa* L.) Using morphological and SSR markers. *Annals of Biological Research*, **5**(11): 20-31.

Monna, L., Miyao, A., Zhong, H. S., Yano, M., Iwamoto, M., Umehara, Y., Kurata, N., Haysaka, H., Sasaki, T. (1997). Saturation mapping with subclones of YACs: DNA marker production targeting the rice blast disease resistance gene, Pi-b. *Theor. Appl. Genet*. **94**:170–176.

Mukta, S., Hossain, S. M., Khondoker, M., Islam, M. M., (2017). Screening of Rice Landraces of Coastal Areas for Salt Tolerance at Seedling Stage Using Molecular Markers. *Asian J. Biotechnol.*, **9**(2): 71-79, 2017.

Munns, R., James, R. A., Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *J Exp Bot*, **57**: 1025–1043.

Myint, K. K. M., Fujita, D., Sonoda, T., Yoshimura A., Yasui, H (2012). Mapping and pyramiding of two major genes for resistance to the brown planthopper (Nilaparva talugens [Sta ˚l]) in the rice cultivar ADR52. *Theor Appl Genet*, **124**: 495–504.

Nachimuthu, V. V., Muthurajan, R., Duraialaguraja, R., Sivakami, R., Pandian, B. A., Ponniah, G., Gunasekaran, K., Swaminathan, M., Suji, K. K., Sabariappan, R. (2015). Analysis of Population Structure and Genetic Diversity in Rice Germplasm Using SSR Markers: An Initiative towards Association Mapping of Agronomic Traits in *Oryza Sativa*. *Rice*, **8**: 30.

Nakamura, S., Asakawa, S., Ohmido, N., Fukui, K., Shimizu, N., Kawasaki, S. (1997). Construction of an 800-kb contig in the nearcentromeric region of the rice blast resistance gene Pi-ta2 using a highly representative rice BAC library. *Mol. Gen. Genet.*, **254**: 611–620.

Nanda, A., Mohanty, S. K., Panda, R. S., Behera, L., Prakash, A., Sahu, S. C. (2010). Flanking Microsatellite Markers for Breeding Varieties Against Asian Rice Gall Midge. *Tropical Plant Biol.*, **3**:219–226.

Nguyen, T. T. T., Nguyen, N. M. T., Hoang, L.H., Furuya, N., Tsuchiya, K. (2012). Genetic diversity in Vietnamese upland rice germplasm revealed by SSR markers. *J. Fac. Agr., Kyushu Univ.*, **57**(2): 383-391.

Ni J. P. M and Mackill D. J. (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science*, **42**: 601–607.

Niksiar P., Navvabpour S., Sabouri H., Soltanlu H., Rahimi M. (2013). The study of allelic diversity of microsatellite markers related to QTL controlling drought-tolerance in rice. *Int J. Agro.and Plant Production*, **4**(5): 3719-3723.

Perumalsamy, S., Bharani, M., Sudha, M., Nagarajan, P., Arul, L., Saraswathi, R., Balasubramanian, P., Ramalingam, J. (2010). Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). *Plant Breed.***129**: 400-406.

Phuc, N. V., Lang, N. T and Buu, B. C. (2005). STS and microsatellite marker-assisted selection for bacterial blight resistance in rice, (*Oryza sativa* L.) *Omon Rice.* **13**:18-25.

Plucknett, D. L., Smith, N. J. H., Williams, J. T., Anishetty, N. M. (1987). Gene banks and the world's food. Princeton, New Jersey: *Princeton University Press*.pp. 247

Powell, W., Marchray, G. C., Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends Plant Sci* **1**:215–222.

Quijano-Guerta, C., Kirk G. J. D. (2002). Tolerance of rice Germplasm to salinity and other chemical stresses in tidal wetlands. *Field Crop Res*, **76**:111–121.

Raghunathachari P., Khanna V. K, Singhand U. S Singh N. K. (2000). RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.) *Current Science*, **79**: 7.

Rahman M. M., Hussain A., Syed M. A., Ansari A. and Mahmud M. A. A. (2011). Comparison among Clustering in Multivariate Analysis of Rice Using Morphological Traits, Physiological Traits and Simple Sequence Repeat Markers. *American-Eurasian J. Agric. and Environ. Sci.*, **11**(6): 876-882.

Rajpurohit, D., Kumar, R., Kumar, M., Paul, P., Awasthi, A. A., Basha, P.O., Puri, A., Jhang, T., Singh, K. Dhaliwal, H. S. (2010). Pyramiding of two bacterial blight resistance and a semi dwarfing gene in Type 3 Basmatiusing marker-assisted selection. *Euphytica* **178**: 111-126.

Rama Devi S. J. S., Singh, K., Prasad, M. S., Umakanth, B., Ram Kumar, G., Viraktamath, B. C., Madhav, M. S. (2015). Development and Identification of Novel Rice Blast Resistant Sources and Their Characterization Using Molecular Markers. *Rice Science*, **22**(6): 300–308.

Ramadan, E. A., Elmoghazy, A. M., and El-Mowafi, H. F. (2015). Molecular markers based genetic diversity analysis for drought tolerance in rice (*Oryza Sativa* L.) using SSR Markers. *Int. J. Sci. Res. Agric. Sci.*, **2**: 137-146.

Rossman, A. Y., Howard, R, J., Valent, B. (1990). Pyricularia grisea, the correct name for the rice blast fungus. *Mycologia*. **82**: 509–512.

Sabar, M., Bibi, T., Farooq, H. U., Haider, Z., Naseem, I., Mahmood, A., and Akhter, M. (2016). Molecular screening of rice (*Oryza sativa* L.) germplasm for Xa4, xa5 and Xa21bacterial leaf blight (BLB) resistant genes using linked marker approach. *African J. Biotech*. **15**(41): 2317-2324.

Sadegh, A., Rafii, M. Y., Rusli, I., Sariah, M., Abdullah, S. N. A., Rahim, H. A., and Latif, M. A. (2012). SSRs for marker assisted selection for blast resistance in rice (*Oryzasativa*L.). *Plant Molecular Biology Reporter*, **30**(1): 79-86.

Sama, V. K., Himabindu, K., Naik, S. B., Sundaram, R. M., Viraktamath, B. C., and Bentur, J. S. (2012). Mapping and marker-assisted breeding of a gene allelic to the major Asian rice gall midge resistance gene Gm8. *Euphytica*. **187**(3): 393-400.

Sambrook, E. F., and Maniatis. T. (1989). Molecular cloning: A laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Senguttuvel, P., Raveendran, M., Vijayalakshmi C., Thiyagarajan K., Kannan-Bapu J. R., and Viraktamath B. C., (2010). Molecular mechanism of salt tolerance for genetic diversity analysed in association with Na+/K+ ratio through SSR markers in Rice (*Oryza sativa* L.), *International J Agriculture research,* **5**(9): 708-719.

Shabanimofrad, M., Rafii, M. Y., Ashkani, S., Hanafi, M. M., Adam, N. A., Latif, M. A., Rahim, H. A. and Sahebi, M. (2015a). Analysis of SSR markers linked with brown planthopper resistance genes ('Bph') using high-resolution melting (HRM) in rice. *Plant Omics,* **8**(3): 212.

Shabanimofrad, M., Yusop, M.R., Ashkani, S., Musa, M.H., Adam, N.A., Haifa, I., Harun, A.R. and Latif, M.A., (2015b). Marker-assisted selection for rice brown plant hopper (Nilaparvata lugens) resistance using linked SSR markers. *Turkish J. Biology*, **39**(5): 666-673.

Sindhumole, P and Soumya, K.(2016).Marker assisted selection of rice (*Oryza sativa* L.) genotypes for bacterial leaf blight disease resistance. *Electronic J. Plant Breeding*, **7**(3): 799-802.

Sindhumole, P., Salam, S. A., Sajini, S., Waghmare, S. G. (2017).Molecular characterization of rice (*Oryza sativa* L.) genotypes for drought tolerance using two SSR markers. *Electronic J. Plant Breeding*, **8**(2): 474-479

Singh, A. K., Singh P. K., Madhuri Arya, Singh N. K. and Singh U. S. (2015). Molecular Screening of Blast Resistance Genes in Rice using SSR Markers. *Plant Pathology J.*, **31**(1): 12-24.

Singh, B. P., Singh, B., Mishra, S., Kumar, V., and Singh, N. K. (2016). Genetic diversity and population structure in Indian wild rice accessions. *Australian J. Crop Science*, **10**(2): 144.

Sivaranjani, A. K. P., Pandey, M. K., Sudharshan, I., Kumar, G. R., Madhav M.S., Sundaram, R. M., (2010). Assessment of genetic diversity among Basmati and non-Basmati aromatic rices of India using SSR markers. *Current Sci.*; **99**(2): 221–26.

Srinivasan, B., and Gnanamanickam, S. (2005). Identification of a new source of resistance in wild rice, Oryza rufipogon to bacterial blight of rice caused by Indian strains of Xanthomonas oryzaepv. oryzae. *Curr. Sci.* **88**:1229–1231.

Suji, K. K, Biji, K. R., Poornima, R., Prince, K. S, Amudha, K., Kavitha, S., Mankar, S., Babu, R. C. (2011). Mapping QTLs for plant phenology and production traits using indica rice (*Oryza sativa* L.) lines adapted to rainfed environment. *Molecular Biotechnology*, **52**: 151-60.

Suvendhu, D. S., Divya, D., Durga Rani C. V., Reddy, T., Visalakshmi, V., Cheralu, C., Singh, K. and Bentur, J. S. (2014). Characterization of gall midge resistant rice genotypes using resistance gene specific markers. *J. Exp. Bio. and Agri. Sci.* **42**: 440-446.

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res*, **17**: 6463–6471.

Thanh, D., Kim Lien, N. T., Chung, P. Q., Trong, T. Q., Thuy, L. T., and Nguyen, H. (2006). Mapping QTLs associated with root traits related to drought resistance in Vietnamese upland rice. *AJSTD*. **23**(4): 323-332.

Thippeswamy, S. Y., Chandramohan, S. B., Srinivas, D., Padmaja and Pravalika, K. (2015). Identification of blast resistant rice (*Oryza sativa* l.) genotypes in indigenous and exotic germplasm and validation of pi gene linked molecular markers. *Electronic J. Plant Breeding*, **10**: 5958.

Thompson, J. A., Nelson, R. L. And Vodkin, L. O. (1998). Identification of diverse soybean germplasm using RAPD markers. *Crop Sci*, **38**: 1348-1355.

Thomson, M. J., Ocampo, M., RahmanEgdane, J.,Rahman, M. A., Sajise, A. G., Dante, L. A., Raiz, E. T., Blumwald, E., Seraj, Z. I., Singh, R. K., Gregorio, G. B., Ismail, A. M. (2010). Characterizing the Saltol Quantitative Trait Locus for Salinity Tolerance in Rice. *Rice*, 2.

Titov, S., Bhowmik S. K., Islam M. M., Siddika A., Sultana S. and Haque M. S. (2009). Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *Revista UDO Agrícola*, **9**(4): 770-775.

Turaidar ,V., Kumar, H., Deepak, K. M., Rajanna, C. A., Manoj, M. P., Krupa, H. B., K. N. and Dalawai,I. (2017). Molecular Screening of Traditional Rice Varieties (TRVS) For Blast Resistance Genes Using SSR Markers.Bull. *Env. Pharmacol. Life Sci.*, **6**(1): 460-466.

Ullah, I., Jamil, S., Iqbal, M. Z., Shaheen, H. L., Hasni, S. M., Jabeen, S., Mehmood, A. and Akhter, M., (2012). Detection of bacterial blight resistance genes in basmati rice landraces. *Genetics and Molecular Research,* **11**(3): 1960-1966.

Upadhyay, P., Singh, V. K., and Neeraja, C. N. (2011). Identification of Genotype Specific Alleles and Molecular Diversity Assessment of Popular Rice

(*Oryza sativa* L.) Varieties of India. *Int J. Plant Breeding and Genetics*, **5**: 130-140.

Vijayalakshmi, P., Amudhan, S., Himabindu, K., Cheralu, C., Bentur, J. S . (2006). A new biotype of the Asian rice gall midge Orseolia oryzae (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. *Int. J. Trop Insect Sci*, **26**: 207–211.

Whitford, R., Gilbert, M., Langridge, P. (2010). Biotechnology in agriculture. In: M.P. Reynolds, ed., Climate change and crop production, CABI Series in Climate Change, **1**: 219-244, CABI, UK.

Yadav, S., Singh, A., Singh, M. R., Goel, N., Vinod, K. K., Mohapatra, T. and Singh A. K. (2013). Assessment of genetic diversity in Indian rice germplasms (*Oryza sativa* L.): use of random versus trait-linked microsatellite markers. *J. Genet*, **92**: 545–557.

Yadav, M. K., Ngangkham, U., Subudhi, H. N., Bag, M. K., (2017). Use of molecular markers in identification and characterization of resistance to rice blast in India. *Plos one*, **12**(6): e0179467.

Yang, E. N., Rosewarne, G. M., Herrera-Foessel, S. A., Huerta-Espino, J., Tang Z. X., Sun, C. F., Ren, Z. L., Singh, R. P (2013). QTL analysis of the spring wheat "Chapio" identifies stable stripe rust resistance despite inter-continental genotype × environment interactions. *Theor. Appl. Genet*, **126**: 1721-1732.

Yao N., Lee C. R., Semagn K., Sow M., N wilene F., Kolade O., and Ndjiondjop M. N. (2016). QTL Mapping in Three Rice Populations Uncovers Major Genomic Regions Associated with African Rice Gall Midge Resistance. *African J. Biotech*, **11**(8): 160 -161.

Yasala, A. K., Rawat, N., Sama, V. S. A. K., Himabindu, K., Sundaram, R. M. and Bentur, J. S. (2012). Analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. *Plant Omics J*, **5**(4): 405-413.

Yokoi, S., Bressan, R. A., Hassegawa, P. M. (2002). Salt Stress tolerance of Plant. *JIRCUS Working Report*, pp. 25–33.

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ABSTRACT

The present study was carried out to screen the rice germplasm for biotic (Blast, Bacterial Blight, Brown Plant Hopper, Gall Midge) and abiotic (Drought, Salt) stress tolerance in 46 genotypes using 18 SSR markers.

Blast resistance linked alleles, for gene *Pi-1* detected by primer RM 5926 were observed in the genotypes viz., RP Bio 189, IET-8580, IR-1544-238-2-3, IR 665575-56-1-3-19, IR-60819-34-2-1, IR-35, IR-663879-195-2-2, Kon-23, Kasturi, IET-23537-1351, IR-60919-150-3-3.

Bacterial Blight resistance linked alleles, for gene *Xa5* detected by primer RM 122 were observed in almost all the genotypes.

Brown Plant Hopper resistance linked alleles, for gene *Bph25* detected by primer RM 6775 were observed in the genotypes; KJT-1-1-21-3-19.

Gall midge resistance linked alleles, for gene *Gm8* detected by primer RM 22709 were observed in the genotypes; KJT 11-1-26-25-23, IR-22896-225.

Saltol QTL detected by primer RM 140 was observed in the genotypes; RP Bio 189, IET-8866, DBS-13-3-47-A9, SLR 51214, Gujrat-102, CR-57 MP-1523. Saltol QTL detected by the marker RM 3412 was observed in the genotypes; IET 13840-RP-66-67, HKR 2002-81, IR 664, KJT 11-1-26-25-23, IR 61614-38-19-3-2.

Drought tolerance linked alleles detected by the marker RM 302 were observed in the genotypes; IR 56381-139-2-2. And by the marker RM 3825 were observed in the genotypes; IR 56381-139-2-2, Paras Sona, IR-50, IR-293-41-41-1, KJT 11-1-26-25-23.

Among all the screened genotypes; KJT 11-1-26-25-23 showed presence of multiple resistance traits for the Bacterial Blight, Gall Midge, Saltol, Drought. Moreover genotype; RP Bio 189 showed presence of multiple resistance for three traits. Hence these genotypes appear to be promising genotypes for presence of multiple resistance traits.

Genotype IR 56381-139-2-2 showed presence of resistance Linked Alleles for two Drought Tolerance genes Dr(140bp) and Dr(147bp). Hence it appears to be promising for Drought Tolerance trait.

All the eighteen SSR primers used in this study amplified and showed the polymorphism in rice genotypes. A total of 88 alleles were detected with an average of 4.88 alleles per locus. The polymorphism information content (PIC) values ranged from 0.13 to 0.82 with an average PIC value of 0.47 per primer. UPGMA grouped 46 rice genotypes into two main clusters which were further divided into two sub-clusters.

This study will be helpful for selection of parental lines and development of new breeding population tolerant to specific traits through Marker Assisted Selection (MAS).

Keywords: Abiotic & Biotic stress, Germplasm, MAS, Marker, Polymorphism, SSR

APPENDIX I

ABBREVIATIONS

AFLP	**:**	Amplified Fragment Length Polymorphism
BSA	**:**	Bulk Segregate Analysis
Bp	**:**	Base Pair
Cv.	**:**	Cultivar
cM	**:**	Centi Morgan
CIA	**:**	Chloroform-Isoamyl Alcohol
DNA	**:**	Deoxyribose Nucleic Acid
dNTPs	**:**	Deoxyribo nucleotide tri-phosphates
EDTA	**:**	Ethylene Diamine Tetra Acetic Acid
EtBr	**:**	Ethidium Bromide
et al.,	**:**	Co-workers
GD	**:**	Genetic Distance
i.e.	**:**	That is
ISSR	**:**	Inter Simple Sequence Repeats
MVSP	**:**	Multivariate Statistical Package
mM	**:**	Milli Molar
$MgCl_2$	**:**	Magnesium Chloride
NaCl	**:**	Sodium Chloride
ng μl^{-1}	**:**	Nano gram per micro litre
nm	**:**	Nano meter
OD	**:**	Optical Density
PAGE	**:**	Polyacrylamide Gel Electrophoresis
PCR	**:**	Polymerase Chain Reaction
PIC	**:**	Polymorphic Information Contain
RM	**:**	Rice Microsatellite
RF	**:**	Resolution Factor
RAPD	**:**	Random Amplified Polymorphic DNA

RFLPs	**:**	Restriction Fragment Length Polymorphisms
RNA	**:**	Ribose Nucleic Acid
RNase	**:**	Ribonuclease enzyme
rpm	**:**	revolution per minute
SI	**:**	Similarity Index
SSLP	**:**	Single Sequence Length Polymorphism
SSRs	**:**	Simple Sequence Repeats
SDS	**:**	Sodium Dodecyl Sulphate
PVP	**:**	Polyvinyl Pyrrolidone
TAE	**:**	Tris-Acetate EDTA
TE	**:**	Tris Buffer
Tris HCl	**:**	Tris-Hydrochloride
TGMS	**:**	Temperature Sensitive Genetic male Sterility
UV	**:**	Ultra Violet
U μl^{-1}	**:**	Units per micro litre
UPGMA	**:**	Unweighted Pair Group Method with Arithmetic Mean
viz.,	**:**	Namely
µl	**:**	Micro litre
MT	**:**	Metric tonnes
MSL	**:**	Mean sea level
QTL	**:**	Quantitative Trait Loci
%	**:**	Per cent

APPENDIX II

COMPOSITION OF CHEMICALS

CIA (25 ml)

Chloroform	24 ml
Isoamyl alcohol	1 ml

Ethidium bromide (10 ml)

EtBr	0.01 mg
Distilled water	10 ml
Stored at 4^0C	

6x Gel Loading dye (50 ml)

0.25% Bromophenol blue	125 mg
40% sucrose in water	20 g
Stored at 4^0C	

5% Sarcosyl (10 ml)

Sarcosyl	0.5 g
Distilled water	10 ml

1x TE buffer (10 ml)

10x TE	1 ml
Sterile water	9 ml

50x TAE (1 lit.)

Tris Base	242 g
Glacial acetic acid	57.1 ml
0.25 M EDTA (pH 8.0)	200 ml
Final volume	1000 ml

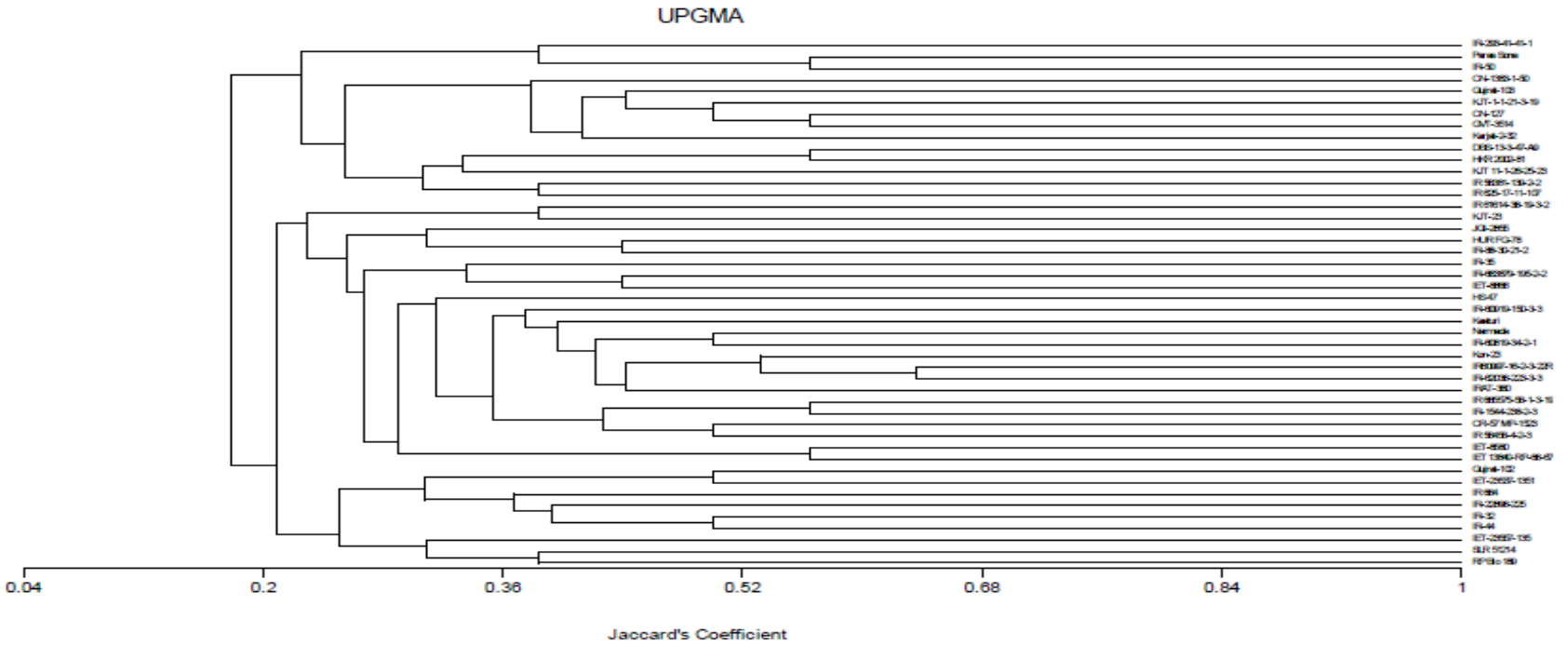

UPGMA
0.04
0.2
0.36
0.52
0.68
0.84
1
Jaccard's Coefficient

Fig. No. 1: Dendrogram constructed using Jaccard's Similarity Coefficient.

Genotypes showing multiple resistance / tolerance for different

Sr. No	Genotypes	Blast	Bacterial Blight	Brown Plant hopper	Gall Midge	Saltol	Drought
1.	RP Bio 189	+	+	–	–	+	–
2.	IET-23557-135	–	+	–	–	–	–
3.	IET 13840-RP-66-67	–	+	–	–	+	–
4.	IR-44	–	+	–	–	–	–
5.	IET-8866	–	+	–	–	+	–
6.	IET-8580	+	+	–	–	–	–
7.	IR 56456-4-2-3	–	+	–	–	–	–
8.	IRAT-360	–	+	–	–	–	–
9.	CR-57 MP-1523	–	+	–	–	+	–
10.	HS 47	–	+	–	–	–	–
11.	IR-88-30-21-2	–	+	–	–	–	–
12.	IR-1544-238-2-3	+	+	–	–	–	–
13.	HUR FG-78	–	+	–	–	–	–
14.	IR 665575-56-1-3-19	+	+	–	–	–	–
15.	IR-60919-150-3-3	+	+	–	–	–	–
16.	IR-62036-223-3-3	–	+	–	–	–	–
17.	IR-35	+	+	–	–	–	–
18.	IR60997-16-2-3-22R	–	+	–	–	–	–
19.	IR-663879-195-2-2	+	+	–	–	–	–
20.	Kon-23	+	+	–	–	–	–
21.	KJT-23	–	+	–	–	–	–
22.	IR 61614-38-19-3-2	–	+	–	–	+	–
23.	Kasturi	+	+	–	–	–	–
24.	IR-22896-225	–	+	–	+	–	–
25.	SLR 51214	–	+	–	–	+	–
26.	IR 664	–	+	–	–	+	–
27.	IR-32	–	+	–	–	–	–
28.	IET-23537-1351	+	+	–	–	–	–
29.	Gujrat-102	–	+	–	–	+	–
30.	IR-60819-34-2-1	+	+	–	–	–	–
31.	IR 625-17-11-107	–	+	–	–	–	–
32.	Narmada	–	+	–	–	–	–
33.	JGl-2855	–	+	–	–	–	–
34.	IR 56381-139-2-2	–	+	–	–	–	+
35.	Karjat-2-32	–	+	–	–	–	–
36.	KJT 11-1-26-25-23	–	+	–	+	+	+
37.	HKR 2002-81	–	+	–	–	+	–
38.	DBS-13-3-47-A9	–	+	–	–	+	–
39.	GVT-3514	–	+	–	–	–	–
40.	IR-50	–	+	–	–	–	+
41.	CN-127	–	+	–	–	–	–
42.	IR-293-41-41-1	–	+	–	–	–	+
43.	Paras Sona	–	+	–	–	–	+
44.	KJT-1-1-21-3-19	–	+	+	–	–	–
45.	Gujrat-103	–	+	–	–	–	–
46.	CN-1383-1-50	–	+	–	–	–	–

traits

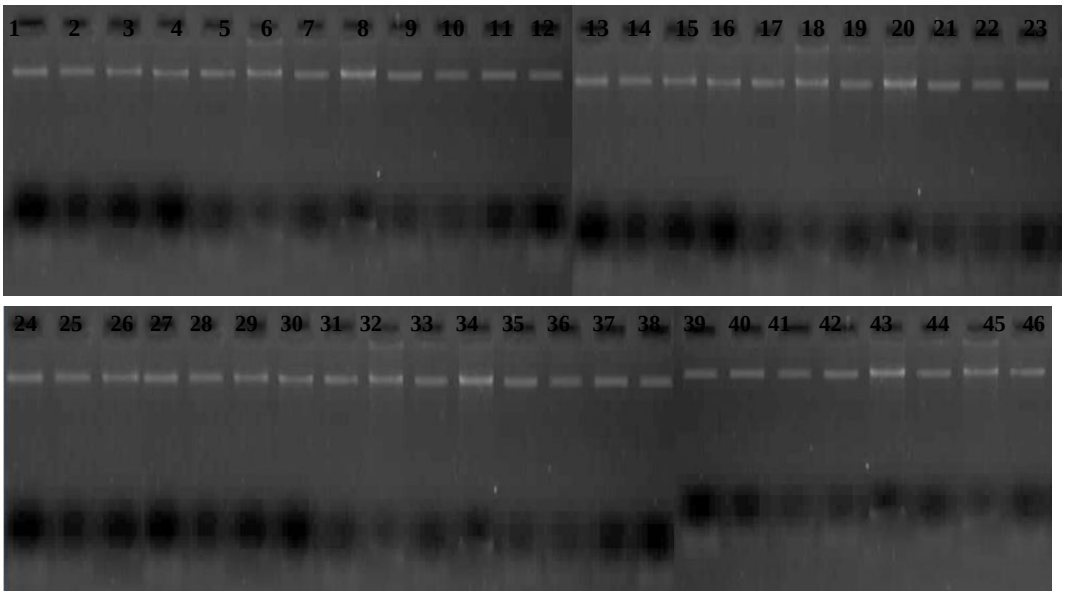


Plate No. 1: Gel photograph of DNA isolated from 46 rice genotypes

Sr. no.	Genotypes	Sr.No.	Genotypes	Sr.No.	Genotypes	Sr.No.	Genotypes	Sr.No.	Genotypes
1	RP Bio 189	11	IR-88-30-21-2	21	KJT-23	31	IR 625-17-11-107	41	CN-127
2	IET-23557-135	12	IR-1544-238-2-3	22	IR 61614-38-19-3-2	32	Narmada	42	IR-293-41-41-1
3	IET 13840-RP-66-67	13	HUR FG-78	23	Kasturi	33	JGl-2855	43	Paras Sona
4	IR-44	14	IR 665575-56-1-3-19	24	IR-22896-225	34	IR 56381-139-2-2	44	KJT-1-1-21-3-19
5	IET-8866	15	IR-60919-150-3-3	25	SLR 51214	35	Karjat-2-32	45	Gujrat-103
6	IET-8580	16	IR-62036-223-3-3	26	IR 664	36	KJT 11-1-26-25-23	46	CN-1383-1-50
7	IR 56456-4-2-3	17	IR-35	27	IR-32	37	HKR 2002-81		
8	IRAT-360	18	IR60997-16-2-3-22R	28	IET-23537-1351	38	DBS-13-3-47-A9		
9	CR-57 MP-1523	19	IR-663879-195-2-2	29	Gujrat-102	39	GVT-3514		
10	HS 47	20	Kon-23	30	IR-60819-34-2-1	40	IR-50		

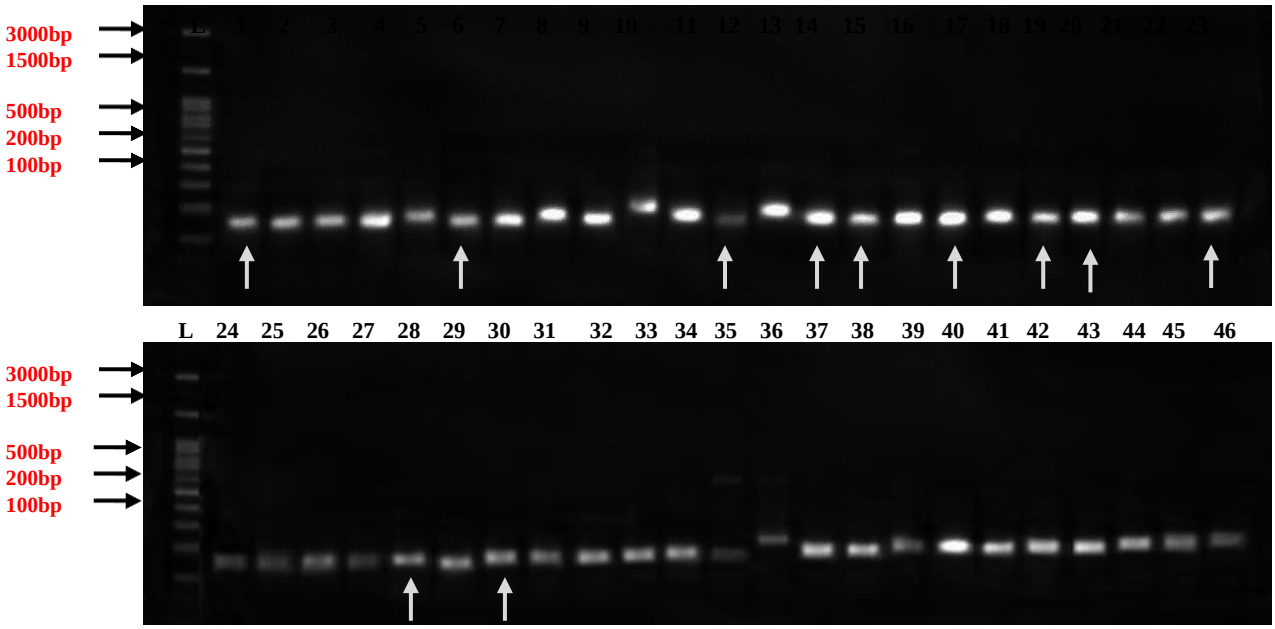


Plate No. 2: Amplified DNA bands of 46 rice genotypes using SSR marker RM 5926 linked to Blast resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).

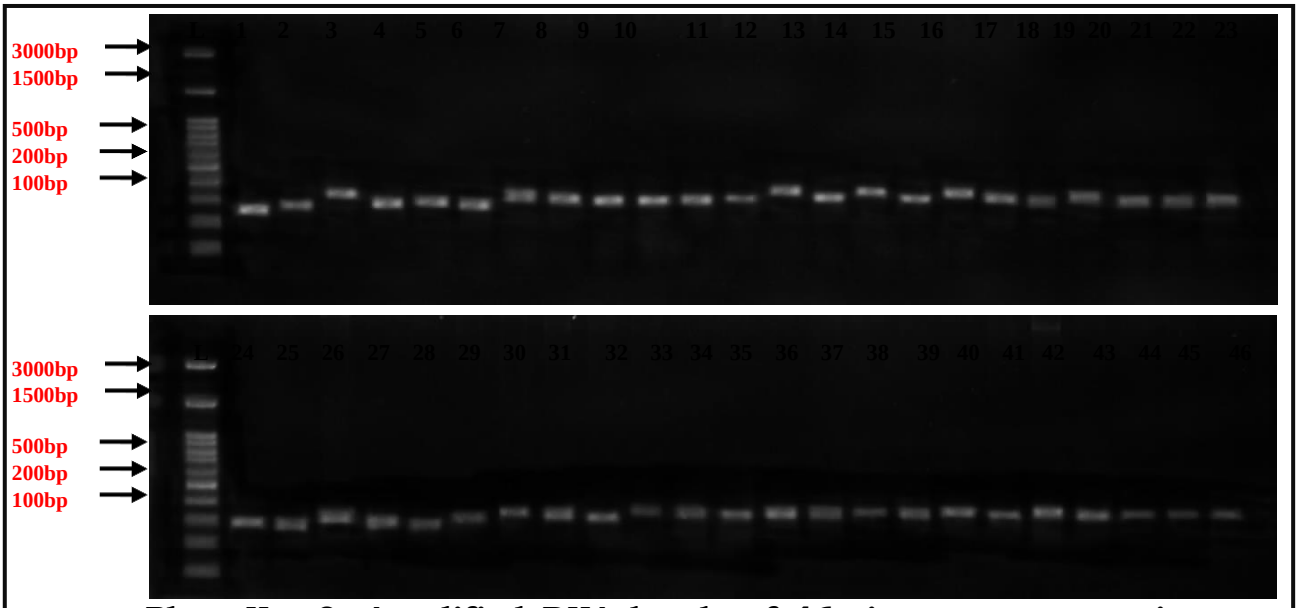


Plate No. 3: Amplified DNA bands of 46 rice genotypes using SSR marker RM 8225 linked to blast resistance trait. L= 100bp ladder. (All the genotypes showed absence of resistance linked allele).

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

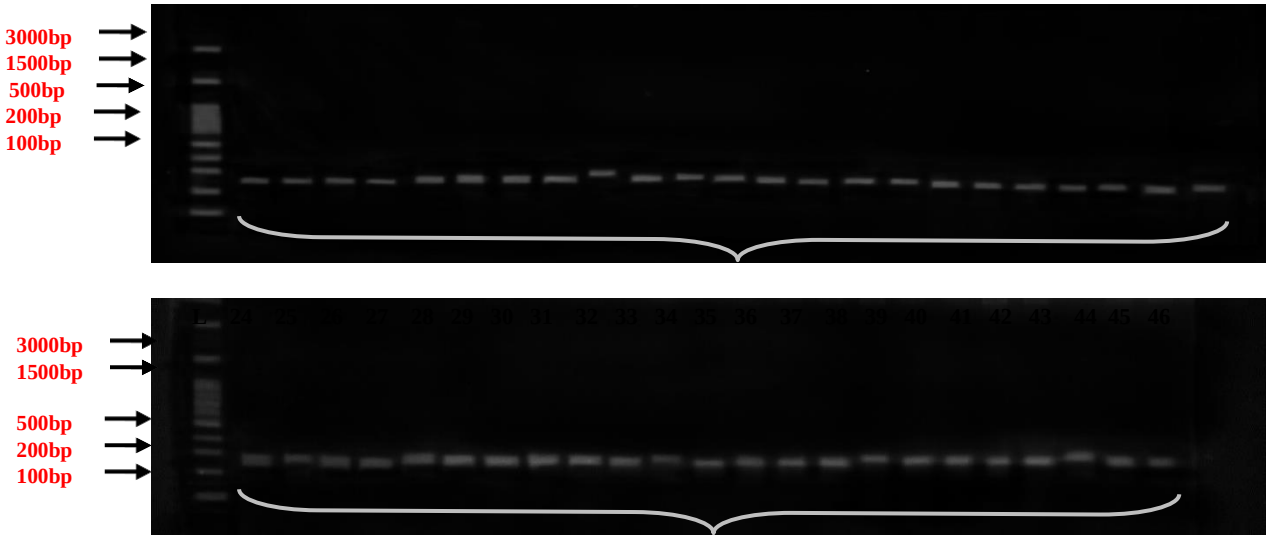


Plate No. 4: Amplified DNA bands of 46 rice genotypes using SSR marker RM 122 linked to Bacterial Blight resistance trait. L= 100bp ladder. (Curly bracket indicates presence of the resistance linked allele). Almost all the genotypes showed presence of resistance linked allele.

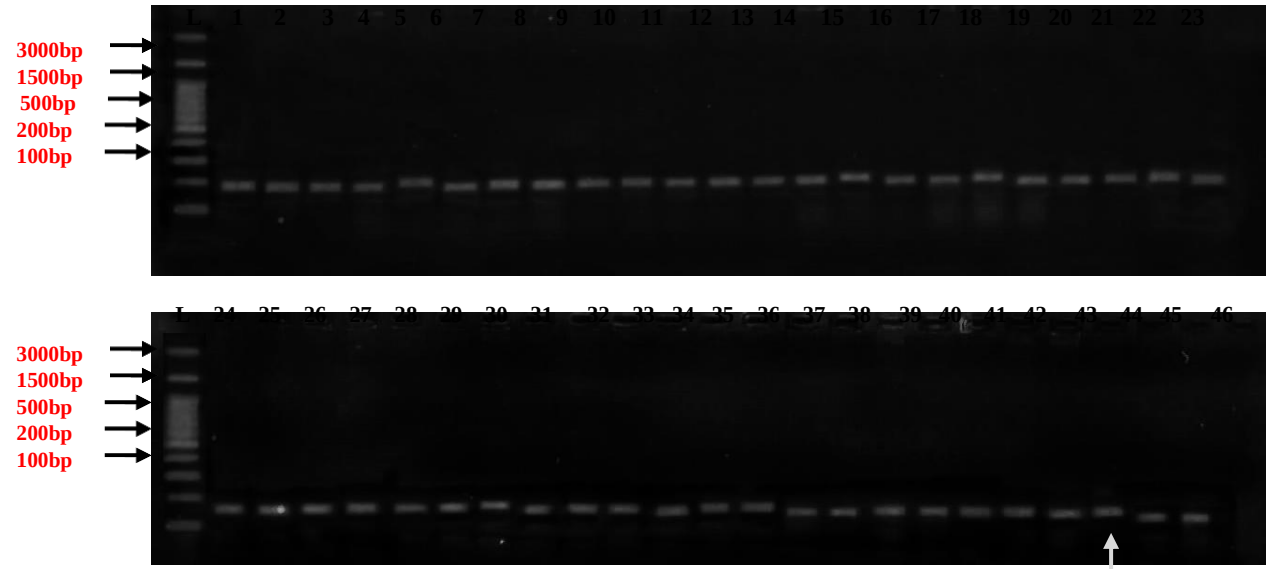
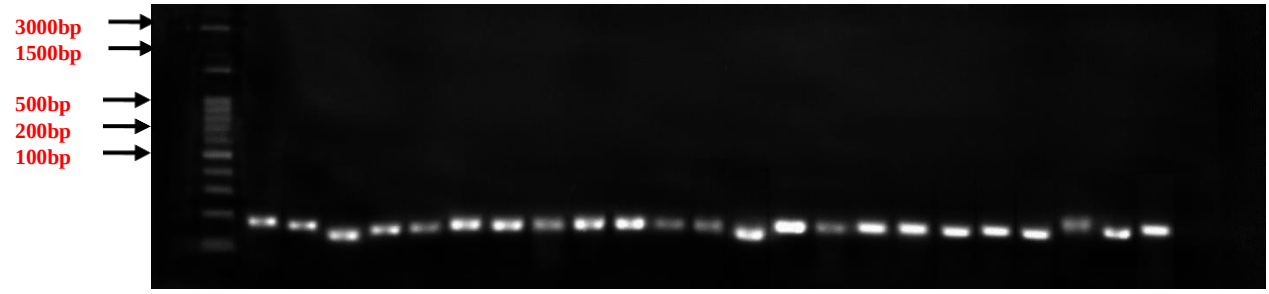


Plate No. 5: Amplified DNA bands of 46 rice genotypes using SSR marker RM 6775 linked to Brown Plant Hopper resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23



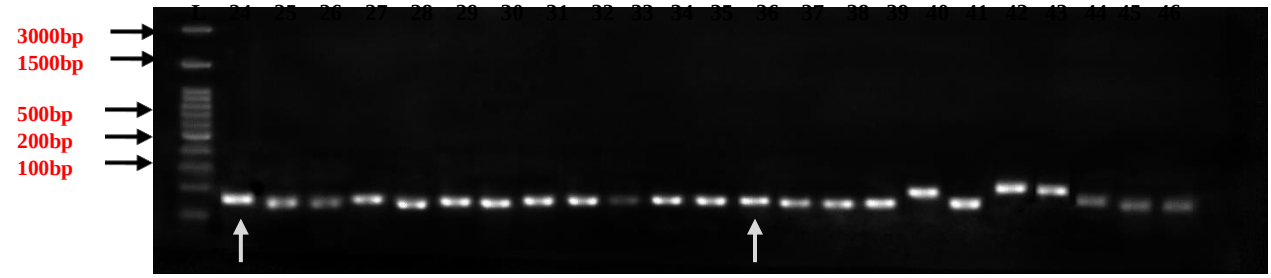
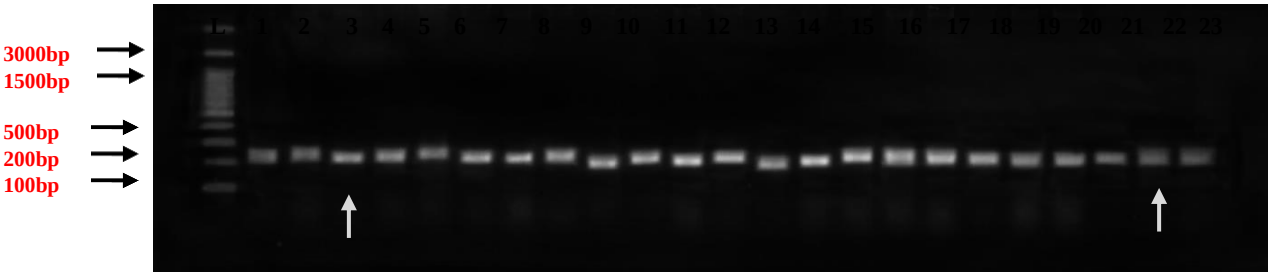


Plate No. 6: Amplified DNA bands of 46 rice genotypes using SSR marker RM 22709 linked to Gall Midge resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).



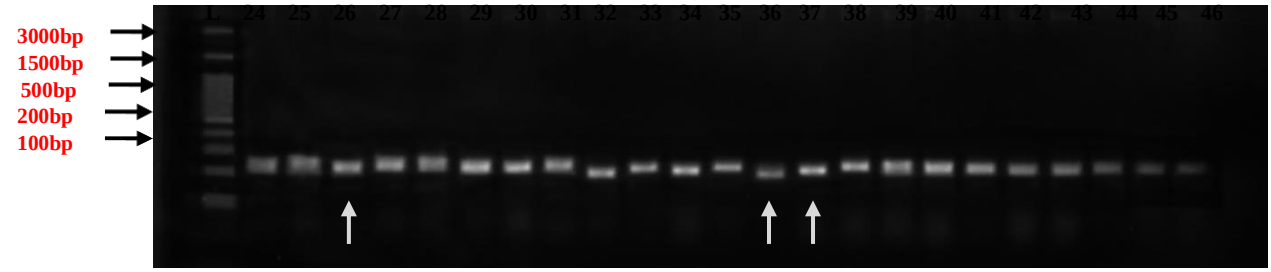
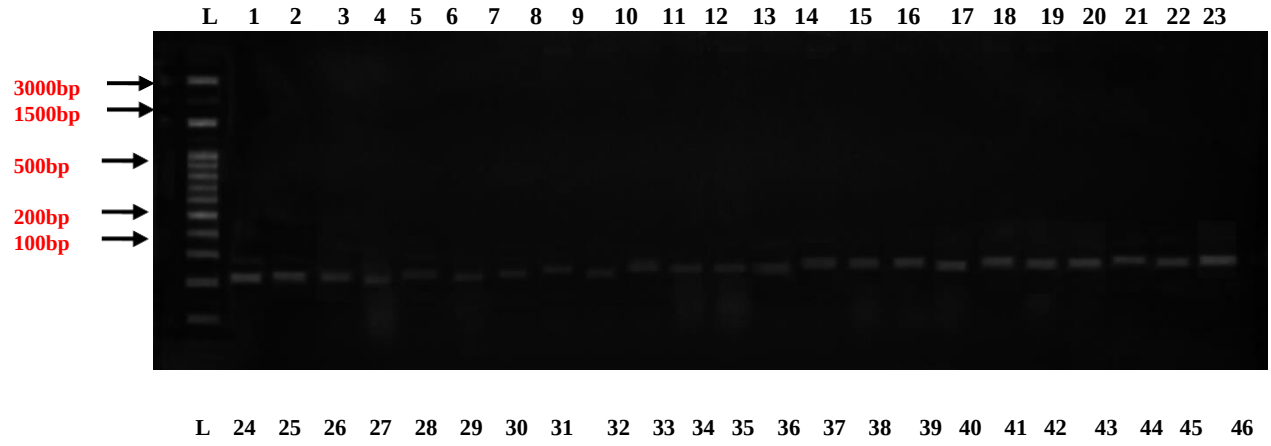


Plate No. 7: Amplified DNA bands of 46 rice genotypes using SSR marker RM 3412 linked to salt resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).



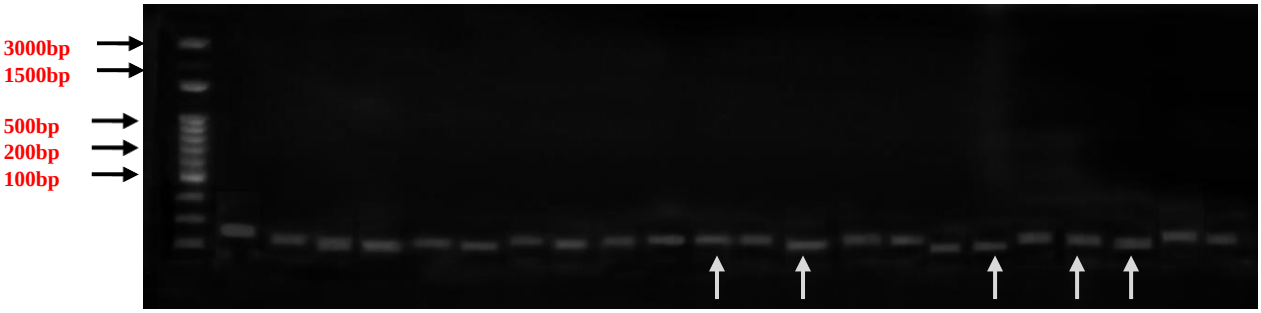


Plate No. 8: Amplified DNA bands of 46 rice genotypes using SSR marker RM 3825 linked to drought resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).

INTRODUCTION

MATERIAL METHODS

REVIEW OF LITERATURE

EXPERIMENTAL RESULTS

DISCUSSION

SUMMARY AND CONCLUSION

LITEATURE CITED

APPENDICES